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PREDICT

STANDARD OPERATING PROCEDURES FOR
ONE HEALTH SURVEILLANCE

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TABLE OF CONTENTS

4 PREFACE

6 GENERAL INFORMATION

7 Basic Laboratory Safety

37 Biosafety and PPE Use

61 Emergency Preparedness

84 Implementing Cold Chain for Safe Sample Transport and Storage

113 Packing and Shipping Biological Samples

13 QGIS User Guide

184 FIELD SAMPLING GUIDES

18 Avian Sampling Methods

19 Bat Sampling Methods

21 Bushmeat Sampling Methods

21 Livestock Sampling Methods

23 Non-Human Primate Sampling Methods

28 Rodent Sampling Methods

30 Safe Animal Capture and Sampling

32 Small Carnivore Sampling Methods

333 QUALITATIVE RESEARCH GUIDES

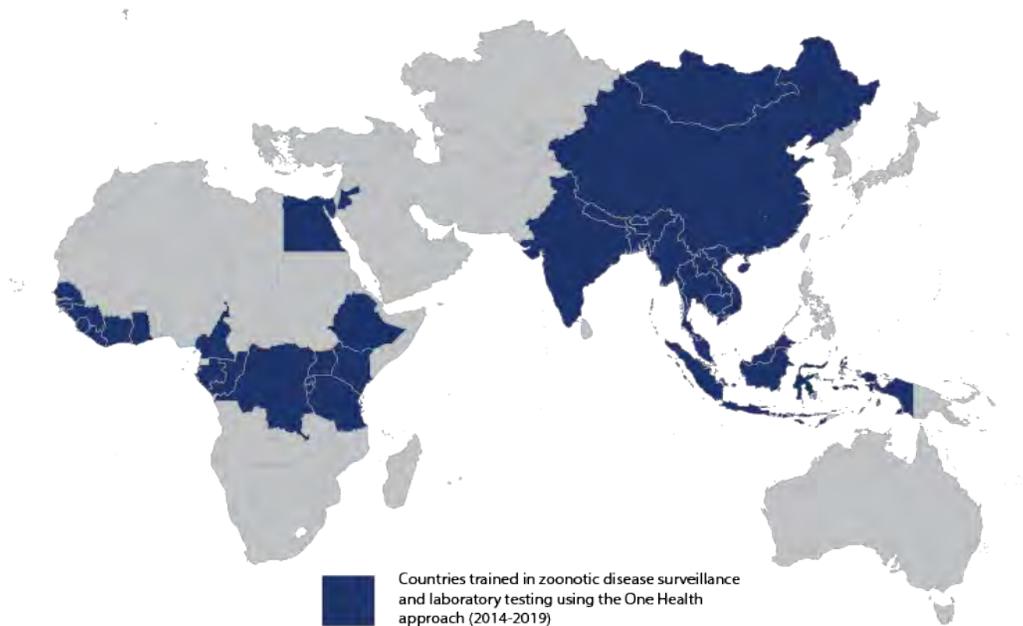
33 Qualitative Research: Introduction & Observational Research Methods

34 Qualitative Research: Focus Groups, Ethnographic Interviews, & Data Analysis

370 TRAINING QUIZZES



PREFACE



PREDICT, a project of USAID's Emerging Pandemic Threats (EPT) Program, has contributed to global early warning systems to detect and reduce the impacts of emerging diseases that move between animals and people (zoonotic diseases). PREDICT field teams sampled bats, rodents, primates (human and non-human), livestock, and other animals in zoonotic hotspots in Africa and Asia.

In order to successfully implement One Health disease surveillance in these regions, PREDICT developed One Health protocols and trained over 6,500 people in 19 unique One Health topics resulting in 20,000+ One Health training events. These training topics included safe animal surveillance by taxa, safe laboratory practices, and techniques for behavioral risk and human syndromic research. In addition, PREDICT provided training on topics such as safe handling of animal and human samples, emergency preparedness and outbreak response.

Each training topic completed by an individual was recorded and tracked in the project, information system, EIDITH, "Emerging Infectious Disease Information Technology Hub". This knowledge management system housed the training materials and quizzes that each individual needed to complete. To ensure all PREDICT personnel remained current on training relevant to their scope of work, all PREDICT staff completed regular refresher trainings accompanied by a quiz demonstrating their knowledge of the PREDICT protocols.

The protocols and guides available in this document were developed to ensure safe (for humans and animals) and efficient sampling of wild animals, livestock, and humans. They were prepared for the specific activities of the PREDICT Program.

Study of these protocols and guides does not serve as an alternative to professional training in veterinary medicine, handling of infectious pathogens, animal capture and handling, animal sampling, laboratory procedures, and other specific tasks they cover. It is assumed that only qualified professionals, with experience regarding the specific relevant activities, will use these documents.



GENERAL INFORMATION

Basic Laboratory Safety

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and the PREDICT One Health Consortium
Last updated: 29 November 2016

Objective: To provide a safe and healthy environment for staff, volunteers and all personnel involved in PREDICT activities. This Guide is to provide basic information to ensure a safe laboratory environment and to comply with environmental standards. The recommendations in this Guide are consistent with the requirements of the U.S. Occupational Safety and Health (OSHA) Act of 1970, Executive Order 12196.

Section 1. Learning Objectives

If you understand the material in this Guide, you should be able to:

- Work safely in a basic laboratory environment.
- Recognize laboratory hazards and take the appropriate measures to reduce those hazards.
- Obtain a Material Data Safety Sheet (MSDS) for a hazardous material and explain the kinds of information in an MSDS.
- Explain important precautions to avoid needlestick injuries.
- Explain how to avoid exposure to pathogens in the laboratory.
- Describe the safety measures for a BSL 2 laboratory.
- Explain why medical monitoring of laboratory personnel is important.
- Describe the proper disposal of sharps and medical waste.
- Describe safety procedures for handling chemicals in the laboratory.

Section 2. Principles

Guiding principles for PREDICT laboratory operations:

1. Prevent loss of life, personal injury or illness, property loss or damage, or environmental harm.
2. Comply with the ***PREDICT Environmental Compliance Protocol*** and local and national safety and health requirements.
3. Comply with applicable local building safety codes.
4. Ensure all PREDICT personnel understand relevant safe and healthy work practices.
5. Identify and assess hazards in the laboratory environment.
6. Establish overall safety and health guidelines that ensure employee safety and health at all times during PREDICT activities.
7. Periodically review and evaluate PREDICT plans, facilities, equipment, and activities to ensure that safety and health objectives are achieved.

Section 3. General Guidance for Laboratory Safety

This Laboratory Safety Guide describes safe work practices, personal protective equipment, and other control measures necessary for the safe use of chemicals and other hazardous materials and procedures in the basic laboratory environment. PREDICT personnel involved in laboratory activities must review and follow this Guide. Staff, interns, visiting scientists, and volunteers are to receive this Guide prior to conducting laboratory activities for the PREDICT Program. This Guide will be updated as needed to improve safety procedures.

Ensure Safe Working Conditions

- Inspect your personal protective equipment (PPE), such as goggles and gloves, to ensure that each component fits well and works properly. Examine your gloves for cracks. Nitrile and latex gloves are disposable and a new pair should be used for each task.
- If you are working with PPE kits, ensure that the kit is complete (a list with the contents of the PPE kit should be available).
- Dispose of broken glass and biohazard materials in designated sharps and hazardous waste containers in the laboratory.
- Help provide a safe work environment by keeping the workspace neat and uncluttered.
- Sinks and eye wash stations should be kept clear.
- Wash your hands and forearms after you have removed and disposed of your PPE.

Hazard Identification and Assessment

Personnel should be able to recognize the possible hazards and inherent risks associated with laboratory procedures and equipment.

Table 1: Hazards associated with laboratory procedures

Procedure	Possible hazards	Likelihood of illness or injury	Risk
Using autoclave or hotplate	High temperature	Moderate	Burns
Handling animal and human samples including body fluids, tissues, swabs	Infectious organisms	Low to moderate	Pathogen exposure zoonotic diseases
Reagent preparation	Acids or alkalines Solvents (alcohols, acetone)	Low Low	Burns Inhalation irritant
Disposal of needles and slides	Sharp objects Infectious organisms	Low to moderate	Needlesticks, cuts, zoonotic disease, pathogen exposure
Dry ice, liquid nitrogen or ultra-low freezers	Extreme cold (~-100F)	Low	Burns
Media preparation	Extreme heat	Low	Burns
Formaldehyde	Inhalation of vapors, ingestion of liquid or direct contact with the liquid or vapor (skin, eye contact)	Moderate	Cancer, skin, eye and respiratory tract irritation
TRIzol Reagent (or Tri reagent; phenol solution)	Toxic if inhaled, absorbed through skin or ingested; reacts with bleach	Moderate	Contact burns, systemic poisoning; creates toxic gas if mixed with bleach

Safe Laboratory and Operating Procedures

Personnel must understand and follow the safe operating procedures of laboratory equipment and PPE to minimize health and safety risks. **The use of the PPE for specific laboratory tasks, listed in Table 2, is mandatory** and all PREDICT personnel must follow the special precautions listed for handling highly hazardous materials.

Table 2: PPE required for laboratory tasks

Lab Task	Health or Safety Hazards	Required PPE	Precautions for Highly Hazardous Materials*
Handling all samples from animals and humans (body fluids, tissues, swabs)	Zoonotic disease potential	Lab coat, closed shoes, disposable nitrile gloves, eye protection and respirator (N95 minimum)	Use of Biosafety Cabinet Class II and eye protection for samples known to be highly infectious or use PPE kits.
Handling acids or chemicals that are irritants (i.e. formaldehyde)	Respiratory irritation, acid or alkaline burns	Lab coat, laboratory gloves, face mask, closed toe shoes.	Chemical fume hood
Operation of autoclaves	Burns	Appropriate gloves, eye protection, closed toe shoes.	Care in opening the door to avoid burns from escaping steam.
Dry ice, liquid nitrogen	Burns, asphyxiation risk	Appropriate gloves, eye protection, closed toe shoes, and use in well-ventilated room.	Dispose of any unused dry ice or liquid nitrogen in ventilated fume hood.
Centrifuges	Aerosolized fluids, zoonotic disease	Lab coat, facemask, appropriate gloves when handling samples or cleaning centrifuge.	Ensure proper balancing of centrifuge and contents. Do not open until rotor has stopped. Use closed-top swinger rotors to spin biological materials.
Hot plate	Possible burns	Appropriate gloves, closed toe shoes.	Do not leave unattended for extended periods.
Use of bleach to disinfect	Possible burns, respiratory irritation	Lab coat, gloves, closed toe shoes, and eye protection.	Use of chemical fume hood recommended when preparing bleach.
Disposing of needles, glass slides	Cuts, zoonotic disease	Gloves, sharps container, closed toe shoes.	Follow sharps safety procedures in this guide.
TRIzol Reagent (or Tri reagent; phenol solution)	Contact burns, systemic poisoning	In lab: Gloves, lab coat, close toe-shoes, eye goggles In field: Gloves, close toed-shoes, appropriate field PPE (e.g. coveralls, N95 mask, goggles per the task)	Aliquot TRIzol for sampling in the field in a ducted biosafety cabinet or fume hood; Perform RNA extraction of samples collected into TRIzol in a biosafety cabinet

Definitions

***Highly hazardous materials** are chemicals, toxics and reactives that have the potential to cause immediate and permanent harm at feasible exposure levels. Chemicals that are highly toxic, are known to cause cancer or birth defects, have very low "permissible exposure limits," are highly reactive, or that react vigorously with common materials (such as water or air) should all be considered "highly hazardous materials." Chemicals that are under pressure, that can build up pressure, that can auto-ignite at possible temperatures, that burn vigorously and energetically, or that when burning cannot be extinguished with conventional methods, should be considered highly hazardous.

For a complete and updated list of Highly Hazardous Materials, visit the following OSHA link: http://www.osha.gov/pls/oshaweb/owadisp.show_document?p_id=9761&p_table=standards

****Personal Protective Equipment (PPE)** is specialized clothing or equipment worn by an employee for protection against infectious and other hazardous materials. The warranted components of PPE vary according to the tasks being performed by personnel. A basic PPE kit may include: gloves, gowns or other protective clothing (e.g., plastic apron), shoe and head covers, mask or respirator, and face or eye protection (e.g., goggles).

Review of Material Safety Data Sheets

PREDICT personnel must verify that a Material Safety Data Sheet (MSDS) for each product to be used during PREDICT activities is readily available, complete and updated (less than three years old).

Coordinators must ensure that personnel have read and understand the MSDS BEFORE using a chemical product.

Personnel must be familiar with the name of the chemical and understand the hazards, safe handling and storage, and specific emergency procedures BEFORE using a chemical product.

Copies of MSDSs for all chemicals used in the laboratory should be kept together in a binder and placed in an accessible location known to all laboratory personnel.

What is a Material Safety Data Sheet?

A MSDS is prepared by the supplier or manufacturer of the material and contains information on the potential hazards (health, fire, reactivity and environmental) and safe use of the chemical product. It is an essential information resource for all health and safety programs. The MSDS also contains information on the safe use, storage, handling and emergency procedures for all hazardous materials. The MSDS contains much more information about the material than found on the product label including what to do if accidents occur, and how to recognize and treat overexposure to the chemical product.

What information is on the MSDS?

The information of greatest concern to workers is featured at the beginning of the data sheet, including information on chemical composition and first aid measures. More technical information that addresses topics regarding the physical and chemical properties of the material and toxicological data appears later in the document. The 16-section MSDS is now recognized internationally. Each MSDS must include:

1. Identification (name, manufacturer and supplier names, address and emergency phone numbers)
2. Hazard(s) identification
3. Composition/information on ingredients
4. First-aid measures
5. Fire-fighting measures
6. Accidental release measures
7. Handling and storage
8. Exposure controls/personal protection
9. Physical and chemical properties
10. Stability and reactivity
11. Toxicological information
12. Ecological information
13. Disposal considerations
14. Transport information
15. Regulatory information
16. Other information

MATERIAL SAFETY DATA SHEET
Metal Cleaner

Page: 1

HEALTH	3
FLAMMABILITY	1
PHYSICAL HAZ.	1
PPE	n

Revision: 11/27/1996

Printed: 12/01/2003

Date Created: 12/09/1996

1. Product and Company Identification

Product Code: DX579
Product Name: Metal Cleaner
Manufacturer Name and Address
Company Name: PPG Industries, Inc.
 4325 Rosanna Drive
 P.O. Box 9
 Allison Park, PA 15101
Emergency Contact 1 Emergency Medical/Spill Info: (304)842-1300
Information Contact Technical Information (614)363-9610
Chemical Family: ACID

2. Composition/Information on Ingredients

Hazardous Components (Chemical Name)	CAS #	Percentage	OSHA TWA	ACGIH TWA	Other Limits
1. Ethanol, 2-Butoxy-	111-76-2	10.0 - 20.0 %	(S) 25 ppm	(S) 25 ppm	No data.
2. Diethylene glycol monobutyl ether	112-34-5	10.0 - 20.0 %	Not Estab.	Not Estab.	No data.
3. Phosphoric acid	7664-38-2	30.0 - 40.0 %	1 mg/m3	1 mg/m3	No data.

3. Hazards Identification

Emergency Overview

Harmful or fatal if swallowed. May be corrosive. This product contains a material which causes skin burns. This product contains a material which causes irreversible eye damage. May be harmful if absorbed through the skin. Vapor and/or spray mist harmful if inhaled. Vapor irritates eyes, nose, and throat. Vapor generated at elevated temperatures irritates eyes, nose, and throat.

Route(s) of Entry: Inhalation? No Skin? No Eyes? No Ingestion? No

Potential Health Effects (Acute and Chronic)

INGESTION: Harmful or fatal if swallowed.

EYE CONTACT: This product contains a material which causes irreversible eye damage.

SKIN CONTACT: May be corrosive. This product contains a material which causes skin burns. May be harmful if absorbed through the skin.

INHALATION: Vapor and/or spray mist harmful if inhaled. Vapor irritates eyes, nose, and throat. Vapor generated at elevated temperatures irritates the eyes, nose, and throat. Repeated exposure to high vapor concentrations may cause irritation of the respiratory system and permanent brain and nervous system damage.

CHRONIC OVEREXPOSURE: Avoid long-term and repeated contact. This product contains an ethylene series glycol ether and/or acetate which has been shown to cause adverse effects on the kidneys, liver, blood and/or blood-forming tissue. This product contains diethylene glycol monobutyl ether (DEGBE). DEGBE consumed in drinking water at low levels by rats for 30 days caused injury to either the liver, kidney, spleen, or testes.

Different jurisdictions have different content requirements for Material Safety Data Sheets. Despite the internationally recognized standard, a MSDS prepared in accordance with the United States OSHA Hazard Communication Standard is not necessarily acceptable in other countries. Check with local health authorities to ensure that your MSDSs are in compliance with local regulations.

Where to obtain MSDSs for chemical products?

A MSDS can be requested from the manufacturer or supplier of the product; in addition several MSDS databases exist online including:

SIRI MSDS index: <http://siri.org/msds/index.php>

MSDS online: <http://www.msdonline.com> or <http://www.msdonfile.com/mctx/msds/msdonfile.jsp>

MSDS Hazard Communication Library: <http://www.setonresourcecenter.com/MSDSs/comply1.htm>

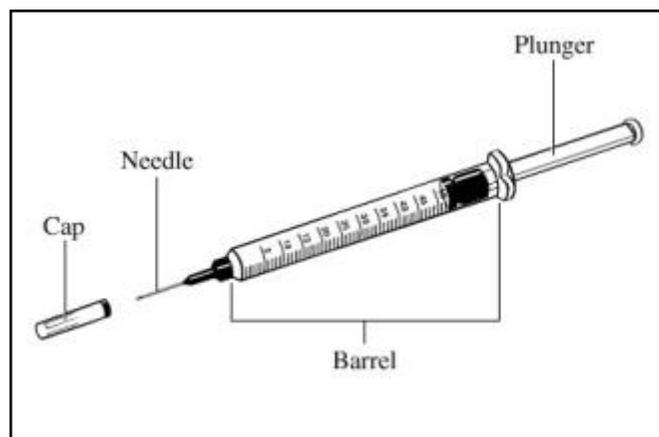
Needlestick Injury Prevention

Needlestick injuries are of concern in basic laboratory settings because they can result in the inoculation of personnel with infected materials. Additionally, skin breaks from needlesticks can act as portal of entry for environmental pathogens.

Most needlestick injuries occur during the following activities

- Recapping, bending, or breaking needles.
- Inserting a needle into a test tube or specimen container and missing the target.
- Carrying unprotected sharps.
- Leaving sharps in unexpected places, such as clothing.
- Handling or disposing of waste that contains used sharps.

Parts of a Syringe and Needle



Procedures to Prevent Needlestick Injuries

- Follow proper techniques when using needles and syringes.
- Be familiar with the different types and components of syringes and needles.
- When **uncapping a syringe needle**, pull the cap straight off to remove it and expose the needle.
- **Never leave an uncapped needle lying around.** A used syringe with the attached needle should be placed in a sharp disposal container immediately after use (a sharps disposal container is designed for safe containment of medical articles that may cause punctures or cuts to those handling them – see below).
- **Removal of the syringe needle** may be necessary for transfer of the sample to another container, or for disposal of only the needle in the sharp container. When removal of the needle is necessary:
 - Make sure not to remove the cap--twist the entire needle to take it off the syringe along with the cap. Alternatively, the needle may be removed from the syringe by use of forceps.
 - Uncapped needles should never be removed from the syringe by hand.
 - **Syringes and needles** used on humans should never be recapped. However, when working with animals and in the field, it may be necessary to carefully recap a needle to avoid accidental sticks if a sharps container is not immediately available.

If you recap a needle, use the *ONE HAND METHOD*

1. Lay the cap on a table or on a flat surface.
2. Hold the syringe by the end.
3. Tilt the end of the syringe up, so that the needle inside the cap is point down onto the surface.
4. Insert the needle on the syringe into the cap.
5. “Fish” up the cap with the needle.
6. Use the same hand to recap the needle.
7. Apply enough pressure to set the cap onto the needle.



If a needlestick occurs, it must be reported to your local PREDICT Supervisor and a medical professional immediately.

Section 4. Biohazards of Zoonotic Pathogens

Investigators working with domestic and wild animals and humans or with animal and human samples are at risk of disease due to exposure to zoonotic pathogens (pathogens transmitted between animals to humans). The zoonotic disease risk varies depending on the animal species being handled, but is generally caused by direct contact (e.g., contaminated/dirty hands), through open cuts, contact with blood and other body fluids, or inhalation of contaminated materials.

When performing tasks with risk of exposure to zoonotic pathogens (such as handling live or dead animals or samples from humans, collecting, testing, or packaging samples), PREDICT personnel should always wear the appropriate PPE as warranted by the assessed risk. It is the responsibility of the supervising veterinarian or medical specialist to determine the required PPE components for specific activities, based on an established PREDICT protocol or based on a risk assessment. (*See **Biosafety and PPE Use** for more information about determining the appropriate PPE.*)

In the event that any personnel believe they have been exposed to material from a person or animal, they should immediately report the exposure to their supervisor, and if warranted seek the appropriate medical attention and follow-up.

Species-Specific Biosafety Precautions

The PREDICT Program will conduct surveillance and sampling among several groups of species. This section discusses special biosafety considerations for some of the key groups of species (bats, rodents, and non-human primates) likely to be handled as part of PREDICT activities.

Rodents, bats, non-human primates and other wild species may harbor pathogens that are transmittable to, and highly pathogenic in, humans. When handling these rodents, bats or non-human primates, careful consideration needs to be given to conscientious use of PPE, good personal hygiene (i.e., hand washing), safety training, and application of good animal handling and sampling techniques to minimize exposure to infection or injury.

In the event of an injury while handling animals that pose risk of zoonotic pathogen exposure, appropriate first aid must be applied. The risk of infection can be significantly reduced with immediate and thorough scrubbing of the wound with soap or antiseptic.

Vaccination to prevent rabies infection: Personnel who are handling animals that are known reservoirs for rabies (e.g., bats and dogs) should be immunized against rabies virus according to World Health Organization and CDC recommendations.

Investigators should familiarize themselves with known biohazards specific to species under study and with the procedures for the isolation and control of zoonotic pathogens.

Specific considerations with regard to working with rodents, bats and non-human primates are discussed below:

Rodents

Wild rodents have the potential to carry a variety of zoonotic bacteria and viruses that can be passed on to those handling them. Because of the serious consequences of becoming infected, personnel must always follow good personal hygiene and animal handling procedures and use the provided PPE to protect against exposure.

Special Precautions:

- Wear the minimum PPE for handling rodents as specified in the PREDICT PPE Use Guide, this includes an N95 mask, eye-protection, gloves and coveralls, or clean dedicated clothing.
- Personnel who are handling animals should be immunized against rabies virus according to the World Health Organization and CDC recommendations.

Bats

Exposure to wild bat roosts (in caves or trees), handling of bats in the field or handling bat excreta (urine or feces) presents a potential for exposure to zoonotic pathogens. Rabies, Nipah virus, Ebola virus, and the fungal disease histoplasmosis are examples of zoonotic pathogens carried by some bat species. Bat bites, scratches and wound and mucous membrane exposure to bat saliva are the ways in which rabies can be transmitted. Spores of histoplasmosis can be present in soil and debris enriched with bird and bat droppings. When this dry soil is disturbed, spores can become airborne and cause infection by inhalation.

Special Precautions:

- When working around bats in enclosed spaces, such as in a cave, wear at a minimum an N95 respirator, goggles, gloves and Tyvek coveralls (or dedicated long-sleeved clothing).
- Personnel who are handling animals such as bats should be immunized against rabies virus and be aware of appropriate post exposure prophylaxis in the case of bites according to World Health Organization and CDC recommendations.

Non-Human Primates

Non-human primates may be infected with a number of potentially serious zoonoses. For example, all macaque monkeys and their fluids should be considered to be infected with **Herpes Simian B virus**. Marmosets, although they do not carry the herpes B virus, can carry other disease agents that affect humans such as lymphocytic choriomeningitis virus and *Trypanosoma cruzii*, the cause of Chagas' disease. It is critical that work with non-human primates be done while wearing the appropriate personal protective equipment and with the well-established safe protocols and procedures.

Special Precautions:

- Personnel must follow strict hygiene procedures. Frequent and thorough hand washing, although too often overlooked by the staff, is critical to physically remove bacterial contamination and prevent ingestion exposure.
- PREDICT personnel must wear the minimum PPE for handling non-human primates as specified in the PREDICT PPE Use Guide. This includes an N95 mask, eye-protection, gloves and coveralls or clean dedicated clothing.

Biosafety Levels and Practices

General

All laboratories handling biological agents must post signage indicating that the site is a potential biological hazard area, and identifying all agents in use. Supervisors shall ensure that employees are informed of biological hazards and that suitable biosafety controls are in place. Country Coordinators and lab and field supervisors managing surveillance and other field and laboratory activities should ensure that appropriate biosafety practices are implemented by personnel. Biological safety cabinets are to be certified annually.

It is important to know the biosafety level of the disease that you are working with before beginning work, so that the correct precautions can be taken.

Note: All samples collected for the PREDICT project are to be handled in a Class II Biosafety Laboratory.

Basics of Biosafety Level 1

Biosafety Level 1 (BSL1) practices represent a basic level of containment that relies on standard microbiological practices and basic safety equipment and lab design for laboratories that work with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. However, many agents not ordinarily associated with disease processes in humans are opportunistic pathogens and may cause infection in the young, the aged, and immuno-deficient or immunosuppressed individuals.

BSL-1 Standard Microbiological Practices

1. Access to work areas is limited at the discretion of the supervisor.
2. Hands must be washed after handling biological materials, removing gloves, or before leaving the laboratory.
3. No eating or drinking is allowed in the laboratory.
4. Only mechanical devices are used for pipetting.
5. Safety devices such as self-protected injection syringe or non-sharps should be used as an alternative to sharps. Sharps used should be handled and disposed of properly.
6. Activities that are likely to create splashes, sprays, or aerosols should be minimized.

7. Work surfaces should be decontaminated with 10% bleach (70% ethanol for metal surfaces) at least daily (before and after work with infectious samples) and after any spills.
8. Waste materials should be disposed of properly.
9. Secondary containment should be used when transporting bio-hazardous materials outside of the laboratory. Avoid public areas during transport.

BSL-1 Safety Equipment (Primary Barriers)

1. BUTTONED lab coats should be worn to protect street clothes.
2. Barrier (preferably non-latex) gloves should be worn, particularly if hands have broken skin or a rash.
3. Appropriate eye/face protection (safety goggles as a minimum) should be worn if splashes or sprays are anticipated, or if wearing contact lenses during lab work.

BSL-1 Laboratory Facilities (Secondary Barriers)

1. The lab should have a sink for hand washing.
2. The lab should have an eye wash station.
3. The lab should have a door for access control.
4. The lab fixtures and floors should be easily cleaned and disinfected (no carpets or rugs); bench tops are to be impervious to water and resistant to both moderate heat and the chemicals used to decontaminate the work surface and equipment.

Note: BSL-1 is NOT APPROPRIATE for PREDICT samples.

Basics of Biosafety Level 2

Biosafety Level 2 is more restrictive than BSL-1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment. All PREDICT samples are to be handled in a Biosafety level 2 laboratory. It differs in that (a) laboratory personnel have specific training in handling pathogenic agents and are directed by trained technologists, (b) access to the laboratory is limited when work is being conducted, (c) extreme precautions are taken with contaminated sharp items, and (d) certain procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment. **All PREDICT samples are to be handled in Class II biological safety cabinets, in Biosafety level 2 laboratory.**

BSL-2 Standard Microbiological Practices

1. Personnel must wash their hands after they handle viable materials, after removing gloves, and before leaving the laboratory.
2. Eating, chewing gum, drinking, smoking, handling contact lenses, and applying cosmetics should not be permitted in the laboratory. Persons who wear contact lenses in laboratories should also wear goggles or a face shield. Food should be stored outside the work area in cabinets or refrigerators designated for this purpose only.
3. Only mechanical pipetting devices are used for pipetting.

4. Policies for safe handling of sharps (when non-sharps are not available) should be instituted.
5. All procedures should be performed carefully to minimize the creation of splashes or aerosols.
6. Work surfaces should be decontaminated with 10% bleach (70% ethanol for metal surfaces) at least once a day (before and after working with infectious samples) and after any spill of viable material.
7. All cultures, stocks, and other regulated wastes are disposed of in the biohazard trash by placing them in a durable, leak-proof container, closed for transport from the laboratory, and transferred to the designated receptacle for disposal. Materials to be decontaminated at off-site locations from the laboratory should be packaged in accordance with applicable local, state, and federal regulations, before removal from the facility.

BSL-2 Special Practices

1. Access to the laboratory is limited or restricted by the laboratory supervisor when work with infectious agents is in progress. In general, persons who are at increased risk of acquiring infection, or for whom infection may be unusually hazardous are not allowed in the laboratory. Persons who are immuno-compromised, immunosuppressed, pregnant or at higher risk of acquiring infections, should not be permitted in the laboratory.
2. The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazard and meet specific entry requirements (e.g., immunization) enter the laboratory.
3. Laboratory personnel receive appropriate immunizations or tests for the agents handled or potentially present in the laboratory (e.g., hepatitis B vaccine or TB skin testing).
4. A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
 - Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials. Used disposable needles must not be bent, sheared, broken, recapped, removed from disposable syringes, or otherwise manipulated by hand before disposal; rather, they must be carefully placed in conveniently located puncture-resistant containers used for sharps disposal. Non-disposable sharps must be placed in a hard-walled container for transport to a disposal area.
 - Broken glassware must not be handled directly by hand, but must be removed by mechanical means such as a brush and dustpan, tongs, or forceps. Containers of contaminated needles, sharp equipment, and broken glass should be decontaminated with 10% bleach before disposal, according to any local, state, or federal regulations.
5. Cultures, tissues, or specimens of body fluids are placed in a container that prevents leakage during collection, handling, processing, storage, transport, or shipping.
6. Laboratory equipment and work surfaces should be decontaminated with an appropriate disinfectant (such as 10% bleach) on a routine basis, before and after work with

infectious materials is finished, and especially after overt spills, splashes, or other contamination by infectious materials. Contaminated equipment must be decontaminated according to any local, state, or federal regulations before it is sent for repair or maintenance or packaged for transport in accordance with applicable local, state, or federal regulations, before removal from the facility. Bleach (10%) can be used on all non-steel surfaces; however, 70% ethanol or other recommended disinfectant should be used when those chemicals are not available.

7. Spills and accidents that result in overt exposures to infectious materials should be reported immediately to the laboratory director. Medical evaluation, surveillance, and treatment should be provided as appropriate and written records should be maintained.

BSL-2 Safety Equipment (Primary Barriers)

1. **Properly maintained biological safety cabinets, Class II**, and other appropriate personal protective equipment or physical containment devices **should be used**.

Procedures with a potential for creating infectious aerosols or splashes are a hazard. These may include centrifuging, grinding, blending, vigorous shaking or mixing, sonic disruption, opening containers of infectious materials whose internal pressures may be different from ambient pressures, inoculating animals, and harvesting infected tissues from animals, eggs or cell cultures.

2. Face protection (goggles, mask, face-shield or other splatter guards) should be used for anticipated splashes or sprays of infectious or other hazardous materials to the face, when the microorganisms must be manipulated outside of the biosafety cabinet.
3. Protective laboratory coats, gowns, smocks, or uniforms designated for lab use should be worn while in the laboratory. This protective clothing should be removed and left in the laboratory before leaving for non-laboratory areas (e.g., cafeteria, library, administrative offices). All protective clothing should be disposed of either in the laboratory or laundered by the institution; it should never be taken home by personnel.
4. Gloves (nitrile or latex) should be worn when hands may contact infectious materials, contaminated surfaces or equipment. Wearing two pairs of gloves may be appropriate, if a spill or splatter occurs; the hand will be protected after the contaminated glove is removed. Gloves should be removed and disposed of when contaminated, removed when work with infectious materials is completed, and should not be worn outside the laboratory. Disposable gloves are not washed or reused.

BSL-2 Laboratory Facilities (Secondary Barriers)

1. Each laboratory should contain a sink for hand washing.
2. The laboratory is designed so that it can be easily cleaned and disinfected. Rugs in laboratories are not appropriate, and should not be used because proper decontamination following a spill is extremely difficult to achieve.
3. Bench tops are impervious to water and resistant to acids, alkalis, organic solvents, and moderate heat.

4. Laboratory furniture is sturdy, and spaces between benches, cabinets, and equipment are accessible for cleaning.
5. An eyewash facility is readily available.
6. The laboratory should be at negative pressure with respect to areas outside the lab. Hoods and biosafety cabinets should be positioned away from doors, supply vents and air conditioner airflow.

Biosafety Level 3

Biosafety Level 3 is applicable to working with indigenous or exotic agents, such as brucella and tuberculosis, that may cause serious or potentially lethal disease through the inhalation route of exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures. All procedures involving the manipulation of infectious materials must be conducted within a BSC (preferably Class II or Class III), or other physical containment devices. A BSL-3 laboratory has special engineering and design features.

Biosafety Level 4

Biosafety Level 4 is required for work with dangerous and exotic agents, such as Ebola, that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease that is frequently fatal, for which there are no vaccines or treatments, or a related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. The laboratory supervisor in accordance with institutional policies controls access to the laboratory.

Section 5. Medical Monitoring

The major purpose of medical monitoring is the early detection of disease or conditions for which treatment can prevent further illness. Medical monitoring is conducted to evaluate exposure to human and zoonotic diseases and unanticipated adverse health effects of exposure. It can also be a valuable tool for hazard control to monitor if initially effective control or work practice has lost effectiveness, or by detecting previously unknown exposures.

Medical consultations should take place:

- Whenever an injury occurs, such as a needlestick, or splash with contaminated material.
- Whenever an employee develops symptoms of exposure to a hazardous chemical or biological agent to which the employee may have been exposed in the laboratory.
- Whenever a spill, leak, explosion, or other occurrence results in the likelihood of an overexposure to a hazardous chemical or biological agent.
- When an employee requests a medical consultation due to health concerns related to assigned tasks and/or change in personal medical history, such as pregnancy, special medications, diagnosed hypersensitivities or other illnesses.
- When exposure monitoring results trigger medical surveillance requirements or when other regulations mandate medical consultations, such as for the use of respiratory protection.

Section 6. Medical Waste Management

Safe Sharps Disposal

The term “sharps” refers to any object that can cut or puncture the skin including, but not limited to, needles (hypodermic and suture), scalpels, lancets, broken vials or glass, broken capillary tubes, slides and coverslips, and exposed ends of contaminated wires. The primary cause of occupational exposure to blood-borne pathogens in field and laboratory personnel is injury from needlesticks or other sharp objects. At least 20 pathogens are known to have been transmitted following percutaneous exposure to blood. Infections with each of these pathogens are potentially life threatening – and preventable.

How to prevent sharp injuries:

- Do not bend, break, or cut sharps. Shearing or breaking of needles is prohibited.
- Concentrate on what you are doing and do not get distracted.
- Dispose of all sharps in an approved puncture-resistant container as soon after use as possible.
- Ensure this container is placed in the area where sharps are used.
- Ideally, needle and syringe should be disposed as one unit if possible. If a needle must be removed follow the directions on the **Removal of the syringe needle** section above.
- Do not recap needles unless absolutely necessary. If recapped, never use two hands, instead use the one-hand “scoop” technique (see **Removal of the syringe needle** section above).

- Do not overfill sharps disposal container. Seal the container and replace when it is $\frac{3}{4}$ full.
- Do not empty sharps containers. Dispose of whole container as one unit.
- Wear utility gloves when disposing of medical waste including sharps containers.
- To prevent sharp injuries during transport of medical waste, use a puncture-proof container that remain closed.

Sharps Disposal Containers

Never discard needles and sharps in waste bags, as personnel might be injured when they handle the bags.

Sharp containers are available commercially or can be adapted from some containers that comply with minimal safety standards.



Commercial sharp disposal container



Non-commercial sharp disposal containers (safety boxes)

There are four major criteria for sharps disposal container safety performance, functionality, accessibility, visibility, and accommodation:

Functionality: Containers should remain in a good state during their entire usage. They should be leak-resistant on their sides and bottoms and puncture-resistant until final disposal. Individual containers should have adequate volume and safe access to the opening.

Accessibility: Containers should be accessible to all workers who use, maintain, or dispose of sharp devices. Containers should be placed in all areas where sharps are used and, if necessary, be portable within the workplace or for fieldwork. Portable containers must have a lid to prevent spills and injuries during transport or while working in the field.

Visibility: Containers should be plainly visible to the workers who use them. Workers should be able to see the warning labels and the degree to which the container is full.

Accommodation: Container designs should be convenient, environmentally sound, and easy to store.

Medical Waste Disposal

Biological waste includes human and animal tissues, fluids and animal carcasses. These are generated along with the sharps and other biologically contaminated equipment that typically need to be discarded in all laboratories (e.g. pipette tips, gloves).

Animal carcasses should be bagged, sealed, and stored in freezers located in the facility until pick up for incineration.

All other biologically contaminated material should be placed in a red bag-lined medical waste box. When the medical waste box is full, it is the responsibility of the field and laboratory personnel to seal the bag, seal the box, and apply a label that contains information about the generating lab.



Medical waste container appropriate for storing and transporting biological waste



Inappropriate container and method for storing and transporting biological waste

Section 7. Special Chemical Storage and Handling Practices

Laboratory chemical storage and handling hazards can be effectively managed if you:

- Maintain good inventory control and purchase/use the least amount possible.
- Label all stored and in-process chemicals clearly and completely.
- Adopt safe handling practices.
- Use secondary containment and practice your spill response plan.
- Segregate incompatible chemicals and store them in separate appropriate cabinets or cold-storage.
- Develop special controls for highly hazardous materials.

Inventory Control

- Purchase chemicals only in the quantities needed and in containers of the smallest practical size. Although the cost may be higher, significant savings will be gained by reduced hazardous waste disposal or clean-up costs.
- Inventory your chemical supplies at least annually and actively share or distribute excess stocks with other departments to minimize waste. Dispose of all unused and outdated chemicals through appropriate hazardous waste programs.
- Products that could also be purchased for home use, such as soap, oil, or cleaning sprays, should be part of your chemical inventory and have an MSDS on file if the product will be used in an occupational setting and could cause a health exposure in the workplace.
- Before laboratory personnel leave the laboratory, all leftover chemicals should be inventoried and distributed or disposed of.

Labeling

Personnel should ensure that labels on containers of hazardous chemicals are not removed or altered, particularly the manufacturer's original label. Empty chemical containers must never be reused for another purpose, even if the labeling is changed as reactions with new liquid and residual chemical could be extremely dangerous. All bottles, containers, and other apparatus containing chemicals should be accurately and clearly labeled as to contents, hazards, and where practical, the appropriate precautions required when handling the chemical.

Avoid the use of grease pencils or other markers that will wear off.

There are three levels of complexity to labeling: original container, secondary transfer containers, and small container (vials, flask, beakers) for immediate, same-day use.

1. The manufacturer's original labels must contain the following information:
 - Name of chemical or solution
 - Manufacturer name and emergency telephone number
 - Hazard warning (health effect or target organs)

When opening you must add:

- Date received and opened
 - Initials
2. For laboratory-prepared solutions and when chemicals are transferred to secondary containers not intended for immediate use, labels should include:
- Name (no abbreviations) of the chemical and its concentration.
 - For prepared solutions or any secondary containers: initial and date prepared.
 - Hazard warning on the most serious health or safety hazard posed (consult MSDS). Stickers can be applied indicating "corrosive," "carcinogen," "water-reactive," "flammable," etc.
 - If special precautions are critical, expand the hazard warning to include the target organ and the required protection (e.g., "Corrosive, esp. to skin and eyes. Use gloves and goggles").
3. Containers for immediate (same-day) use should have:
- Chemical name and its concentration
 - Date
 - Initials

Safe Handling and Transfer

Hand-carried chemicals should be placed in unbreakable secondary containers such as bottle carriers or acid-carrying buckets. Wheeled carts used to transport chemicals should have side guards and lipped surfaces capable of containing a break, and sturdy wheels that move easily over uneven surfaces.

Staff should wear protective aprons, gloves, goggles and closed-toed shoes when transporting chemicals.

Class I flammable liquids (any liquid having a flash point below 37.7°C should not be stored or transferred from one vessel to another in an exit access corridor, open plan building, or in an ancillary space unprotected from the exit access corridor.

Transfer of Class I liquids to smaller containers from bulk stock containers not exceeding 5 gallons in capacity should be performed in a laboratory hood, in an area provided with ventilation adequate to prevent accumulations of flammable vapor exceeding 25% of the lower flammable limit, or within an inside liquid storage area approved for dispensing.

Class I liquids should not be transferred between conductive containers of greater than 1.1 gallons, unless the containers are bonded and grounded (the process of providing an electrically conductive pathway - usually by clipping connecting wires - between a dispensing container and a receiving container [bonding], and the receiving container and an earth ground).

Secondary Containment and Spill Control

Liquid chemicals should be stored in corrosion-resistant trays or on spill pallets or other secondary containment to contain a break or leak.

Concentrated acids and bases should be stored in acid or caustic storage cabinets. If possible, keep corrosives stored in their original (e.g. Styrofoam cubes) shipment containers.

In the event of a chemical spill, try to turn off all reaction apparatus, especially heat sources, notify supervision immediately and follow the response steps in your facility.

Cabinet and Shelf Storage – General Precautions

Cabinets and other storage areas should be marked with the general class of chemical stored, and any other pertinent warnings.

Storage areas should have good general ventilation and be well lighted.

On shelves, containers should be staggered for easy access, with labels facing out. DO NOT ALPHABETIZE STORED CHEMICALS; SEPARATE BY COMPATIBILITY (see next section).

Heavy and large containers are to be placed on bottom shelves. Chemicals, especially liquids, should be stored below eye level. Larger containers should be stored on lower shelves. Exposure to heat or direct sunlight should be avoided. Avoid storing chemicals on the floor unless in approved shipping containers. Minimize open shelf or bench top storage, except for those chemicals currently being used, to prevent accidental spills and reduce the risk of fires.

Cabinets specifically for corrosives (either acids or bases) should have corrosion-resistant paint. Flammable storage cabinets should provide an airtight seal; vent holes should be kept covered and flame-arrestor kept in place.

Oxidizers MUST be stored in separate cabinets from flammables and combustibles. Oxidizers, explosives, and organic peroxides must be separated from combustibles and placed in a metal cabinet, or in an approved dry, cool, and well-ventilated location.

If acids and bases must be stored together in the same cabinet, place each in separate secondary containers (non-reactive trays) on opposite sides of the cabinet to minimize intermingling in case of a spill or drip (in other words, do not store all the acids on one shelf, and all the bases on the shelf below).

Initially assign each chemical to broad hazard classes, for example: flammable, corrosive (acids and bases), reactive oxidizer or reducer, special hazard (air/water reactive, peroxide forming chemical, store at reduced temperature or under an inert atmosphere, highly toxic).

Chemicals that possess more than one hazard (i.e., oxidizer and corrosive) are assigned to the class that represents the greater hazard for that laboratory.

Post incompatibility lists (from your MSDSs) for reference.

Hazardous chemicals should be disposed of in clearly labeled containers, and as with storage, separated by class. For example, acids should not be disposed of with bases but should be separated. The same is true for corrosives and flammables.

Refrigerators and Freezers – Flammable Storage

All refrigerators or freezers should be distinctly marked as to whether they are suitable for the storage of flammable liquids.

Standard household-variety refrigerators should not be used to store flammable liquids.

Flammable liquids stored in refrigerated equipment should be in closed containers.

Storage of Chemicals by Class

Flammables and Combustibles

Flammables are chemicals that have a flash point less than 37°C (100°F). Combustible chemicals have flash points that are 37-93°C. If stored or used improperly, flammables and combustibles can be a fire hazard.

Examples of flammable liquids include benzene, alcohols, acetone, ethers, organic acids (i.e., glacial acetic acid).

The quantity of flammable/combustible hazardous chemicals within a laboratory unit or in a laboratory work area, that is stored in the open, shall be limited to the minimum necessary to perform required tasks.

Bulk supplies of alcohol (such as 95% EtOH in drums) should be stored in an approved flammable liquids storage room.

To the greatest degree possible, the storage of flammable liquids in a laboratory work area, outside of an approved flammable liquids cabinet, or storage room should be limited to what is needed for a single day's use. Otherwise, flammable liquids should be stored within an approved flammable liquids cabinet when not in use.

Corrosives: Acids

Acids are corrosive and react violently with bases. There are two main groups of acids: organic acids, and inorganic (mineral) acids. Some inorganic (mineral) acids are oxidizers and will react with organics, increase burning rate of combustibles and contribute an oxygen source to a

combustion reaction. Therefore, inorganic (mineral) acids should be stored separately from organic acids.

Examples of inorganic OXIDIZING acids: perchloric acid (particularly dangerous at elevated temperature), chromic acid, nitric acid, sulfuric acid (particularly dangerous at elevated temperature).

Examples of inorganic MINERAL acids: hydrochloric acid, hydrofluoric acid, phosphoric acid.

Examples of organic acids: acetic acid, formic acid, butyric acid, propionic acid, picric acid, acrylic acid.

Oxidizing inorganic acids should be segregated from organic acids, flammable and combustible materials. Most mineral acids can be stored together, except perchloric acid (see below):

Nitric acid shall be stored separate from other acids.

Segregate acids from bases and active metals such as potassium and magnesium.

Segregate acids from chemicals that could generate toxic gases upon contact, such as sodium cyanide.

Segregate acids from solvents such as toluene and xylene.

Organic acids (e.g., glacial acetic acid) are combustible and should be stored separately or with flammables rather than with inorganic acids. Several inorganic acids are oxidizers and are therefore incompatible with organics.

Corrosives: Bases

Bases are corrosive and react violently with acids.

Examples: ammonium hydroxide, sodium hydroxide, calcium hydroxide, organic amines.

Segregate bases from acids. Bases are also corrosive to skin and tissue. Pay meticulous attention to PPE when using bases.

Reactive: Oxidizers

Oxidizers react vigorously with reducing materials. The reaction can lead to fires or explosions. Oxidizers will increase the burning rate of combustible materials and contribute oxygen to a combustion reaction.

Examples: halogens, ammonium persulfate, hydrogen peroxide, sodium dichromate, potassium permanganate, perchloric acid; at elevated temperature, ammonium nitrate (and other nitrate salts).

Keep oxidizers away from flammables, combustibles (such as paper, wood) and other reducing agents.

Reactive: Reducers

Reducing materials react vigorously with oxidizers. The reaction can lead to fires or explosions.

Examples: ammonia, carbon, metals, metal hydrides, phosphorus, silicon, sulfur.

Store reducing materials away from oxidizers.

Water-reactive Chemicals

Water reactive materials react with water, water solutions, moisture, or humidity in the air to produce heat and/or flammable gases, which can ignite.

Examples: sodium (elemental), potassium (elemental), calcium carbide, phosphorous pentachloride.

Store water reactives away from any sources of water or moisture. Review manufacturer's recommendations for special storage conditions, such as under an inert atmosphere or, as in the case of elemental sodium, under mineral oil.

Peroxide Forming Chemicals

Potentially explosive peroxides are formed by a free-radical reaction of hydrocarbons with molecular oxygen. Distillation, evaporation or other concentration of the peroxide can cause an explosion in contaminated hydrocarbons.

Examples: diethyl ether, tetrahydrofuran, acetaldehyde, isopropyl ether.

Store peroxide-forming chemicals away from light and heat. Carefully label all containers with the date received and the date opened. Monitor container dates and avoid keeping peroxide-forming chemicals on hand for more than a year after receipt and 6 months after opening.

Highly Hazardous Chemicals

Highly hazardous chemicals are defined as chemical carcinogens, reproductive toxins, acutely toxic substances, and highly reactive materials (ex. Ethidium bromide used in molecular laboratories).

Designate a Restricted Work Area. Conduct all transfers and work with these substances in a "controlled area" (i.e., a restricted access hood, glove box, or portion of a lab designated for use of highly-toxic substances) for which all personnel with access are aware of the substances being used and the necessary precautions that must be taken. Only trained and authorized personnel should work in or have access to controlled areas.

Signs and labels. Assure that the controlled area is conspicuously marked with restricted access and warning signs, such as, "WARNING: Highly-Toxic Substance in Use: Authorized Personnel Only" or "WARNING: Cancer-Suspect Agent: Authorized Personnel Only." All containers of these substances must be appropriately labeled with identity and warning such as, "Warning: High Chronic Toxicity or Cancer Suspect Agent."

Storage. Store containers of these chemicals in a ventilated, limited access area in appropriately labeled, unbreakable, chemically resistant, secondary containers.

Establish Decontamination Procedures. The need for routine decontamination of designated work area, equipment, or personnel depends on the laboratory circumstances.

Medical surveillance. When using a highly toxic substance on a regular basis (e.g., 3 times per week), consult with your supervisor concerning medical surveillance or other health concerns you may have.

Cleanup and Waste Disposal. Use chemical decontamination whenever possible. Use a vacuum cleaner equipped with a High Efficiency Particulate Air (HEPA) filter, instead of dry sweeping when the toxic substance is a dry powder. A wet mop may also be used when the chemical is not water reactive or otherwise incompatible with water. Ensure that all vacuum filters, bag debris, mop heads or cleaning rags, as well as waste chemicals are transferred from the designated control according to a hazardous waste disposal container. Ensure that contingency plans, equipment, and materials are available to minimize exposures to personnel and property in the event of an accident. Do not ask/expect custodial staff to clean hazardous materials spills, unless they are already members of the facility's trained response team.

Hazardous Waste Disposal and Spill Control

Each container of hazardous waste is to be labeled with the following legends:

"HAZARDOUS WASTE"

Contents (be specific as to chemical):

Accumulation start date:

If a reagent container label has been removed or becomes illegible, and the identity of the contents is unknown, the container must be disposed of as soon as possible by arrangement with the facility hazardous waste coordinator.

Prior to the departure of staff, chemicals for which that person was responsible should be inventoried and discarded or returned to storage.

Pouring hazardous waste chemicals down the drain, adding them to regular trash, or evaporating them in a local exhaust hood could be illegal actions!

Section 8. Training in Basic Laboratory Procedures and Protocols

Training and education in laboratory safety need to be an ongoing process, not just an annual presentation. The most effective way to reinforce good work practices is to involve all personnel from principal researchers to volunteers in regular, periodic reviews and updates of this Basic Laboratory Safety Guide. Documentation of all forms of training is to be maintained in the laboratory as well as reported to the facility safety coordinator.

INITIAL BASIC LAB HAZARD AWARENESS TRAINING is **mandatory for all staff** and must be provided to all employees doing field and laboratory work prior to actual lab and field work, and prior to assignments involving new potential exposures. Information provided during trainings should include:

The location and availability of the Laboratory Safety Plan, chemical inventory, Material Safety Data Sheets (MSDSs), applicable regulatory exposure limits, and other reference material regarding the safe handling, storage, and disposal of hazardous chemicals (or hazardous collections) in the lab.

Signs and symptoms associated with exposures to hazardous chemicals and biological agents used in the laboratory, as well as the health hazards themselves.

Methods that may be used to detect the presence or release of a hazardous chemical. This could include industrial hygiene monitoring, the use of continuous monitoring devices, visual appearance, or odors of chemicals.

Methods employees can take to protect themselves from hazards, including work practices, personal protective equipment and emergency procedures listed in the LSP. This should include a discussion of the proper use and limitations of engineering controls and safety devices, including chemical and biological hoods.

Emergency response plans established by each facility's Emergency/Disaster Response Plan, any medical or first aid response specifically recommended, extinguishment of clothing fires (Stop, Drop, and Roll), and Chemical Spill Response Plans established by each facility.

Section 9. Basic Standards and Guide Checklists

- Coordinators should provide a "Useful Contacts" list with address and numbers of local medical and emergency response services.
- Personnel should know the locations of the emergency supplies (fire extinguishers, first aid kits, spill kits, safety showers and eye wash stations), phone numbers of supervisor and exits.
- Coordinators must verify that a Material Safety Data Sheet (MSDS) for each product to be used during PREDICT activities is readily available, complete and updated.
- Personnel should know where the MSDSs are located.
- Coordinators must ensure that personnel have read and understood the MSDS before using a chemical product
- Coordinators must have MSDS data available for emergency responders.
- Individuals that have been exposed to any hazardous chemical or biological agent should immediately report the exposure to medical authorities and supervisor.
- A complete list with the contents of the PPE kit should be available to the personnel.
- Personnel should wear appropriate PPE (lab-coat, protective glasses, gloves, closed toed shoes) for laboratory procedures.
- Inspect your PPE to ensure that it is in proper working condition before use (goggles, gloves, etc.).
- If you are working with PPE kits, ensure that the kit is stocked and material has not expired.
- Personnel must use a chemical, fume or laminar flow hood when indicated.
- All needles, scalpel blades and any other sharp instruments should be used and disposed of in a manner that prevents accidental human injury.
- All stored and in-process chemicals should be labeled clearly and completely.
- Segregate incompatible chemicals and store in appropriate cabinets or special cold-storage.
- Develop special controls for highly hazardous materials.
- Purchase chemicals only in the quantities needed and in containers of the smallest practical size.
- Inventory your chemical supplies at least annually and actively share or distribute excess stocks with other departments.
- Dispose of all unused and outdated chemicals through appropriate hazardous waste programs and NOT down the drain or by adding them to regular trash.
- Sinks and eye wash stations should be kept clear and in proper working condition.
- Staff should wash their hands and forearms after they have removed and disposed their PPE or after removing gloves.
- Food and beverages are NOT allowed in any of the labs.
- Report any lab failure (equipment, facilities, etc.) to the supervisor.
- Staff should keep BUTTONED lab coats at all times when working in the laboratory.
- All human and animal tissues, fluids and excrement should be handled in a Class II Biosafety Cabinet so that the potential for human exposure is minimized.
- Specific Biosafety levels 1 and 2 practices should be followed by personnel as warranted.
- Personnel must be familiar with hazard controls and safe operating procedures.

Section 10. List of Equipment and Supplies

- Lab-coat
- Nitrile gloves ideal, latex if not available
- Face-mask
- Goggles
- Face-shield
- Closed toed shoes
- Disposable (Tyvek) suit
- Sharp-container
- Medical waste box
- Respirator
- PPE Kits or Supplies
- Eyewash station
- Liquid nitrogen gloves

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Biosafety and Personal Protective Equipment (PPE) Use

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Objective: To provide principles and general guidelines for the use of Personal Protective Equipment (PPE) to prevent exposure to and transmission of infectious pathogens during PREDICT activities.

Section 1. Learning Objectives and Confirmation

After studying this guide, you will be able to:

- Implement basic biosafety precautions.
- Describe the factors to consider when assessing the biological risk of handling animals and collecting human and animal samples, and other field and laboratory activities that may have potential risk for zoonotic disease exposure.
- Understand factors to consider when choosing appropriate PPE based on identified risks.
- Identify and describe the functions of each component of PPE.
- Correctly put on and take off appropriate PPE for PREDICT sample collection and handling activities in a non-outbreak setting. For collecting samples from hospital and clinic patients and during disease outbreaks, specific PPE components and procedures to put on and take off PPE should be adapted based on the determined risk level.
- Describe the importance of respirator fit and fit testing.

Section 2. Biosafety Overview

Personal Safety Responsibilities

- Individuals have the primary responsibility for their own health and safety. Nothing substitutes for good training and vigilance.
- Follow safety procedures outlined in PREDICT protocols regarding each activity that involves potential exposure to infectious pathogens.
- Use appropriate safety equipment.
- Report unsafe or hazardous situations, injuries, and accidents immediately to your supervisor or instructor.
- Report any illness to your PREDICT supervisor.
- Participate in required safety training.

Follow PREDICT waste disposal procedures (see *Basic Laboratory Safety and Safe Disposal of Carcasses and Infectious Waste Guide* consistent with the *PREDICT Environmental Mitigation and Monitoring Plan*).

Responsibilities of the Country Coordinator and Field Supervisors

- Provide and document training for all personnel who will participate in PREDICT project activities.
- Ensure compliance with relevant PREDICT or organizational task protocols.
- Ensure compliance with the PREDICT Environmental Mitigation and Monitoring Plan.
- Ensure compliance with local permit requirements and regulations.
- Report injuries/accidents and ensure compliance with associated mitigation.
- Ensure that all field personnel are trained on the safe use of field equipment.

General Zoonoses Biosafety Precautions

There is a risk of exposure to pathogens, including zoonotic pathogens, when handling animals, and human and animal samples in the field. Therefore, it is important to implement measures to minimize the risk of pathogen transmission.

The following list of general precautions applies to most situations:

- Inform all who enter potential zoonotic pathogen risk areas of their potential for exposure and the associated risks.
- Review information regarding the zoonotic agents likely to be found in the samples or animals to which you or others may be exposed.
- Wear the appropriate PPE based on protocols for the activity and species and as directed by the Country Coordinator or Field Supervisor.
- Use disposable supplies whenever possible.
- Wash hands and wrists after removing your gloves.
- Don't wear field or lab clothing or shoes outside of work areas where there may be zoonotic pathogen exposure. Change clothing and shoes before getting into your vehicle.

- Launder contaminated protective clothing at work. Don't take your protective clothing home with you.
- Never eat or drink in areas where human sampling, animals, their wastes, or their products (e.g., blood) are present.
- Wash your hands frequently and practice good hygiene. Avoid touching your face while working with animals, human and animal samples, or other sources of pathogens. Although a normal, healthy adult person may have only mild symptoms of a zoonotic disease, that person may unknowingly spread the disease to others. Unfortunately, animal handlers have “carried home” zoonotic pathogens to their infants with fatal consequences. Therefore, good hygiene is not only to protect the person working directly with human and animal samples; but it is also for all persons and animals with whom they have contact.
- When seeking medical advice for any illness, inform your physician of your work with humans and animals.
- Make sure a first aid kit is immediately available during all field and laboratory activities.
- Refer to established procedures for how to respond to a bite, cut, scratch, puncture or other injury that results in possible zoonosis exposure.
- Refer to established procedures for disinfecting all equipment, samples, cages, and traps according to guidance provided below.

Hand Washing - Teach and Practice Good Hand Washing Technique

The importance of hand washing in preventing infection and the spread of infectious pathogens cannot be over emphasized.

Always wash your hands before:

- Putting on PPE for handling animals or collecting or handling human and animal samples
- Contact with a sick or injured person or animal
- Treating wounds or administering medications
- Preparing food
- Eating
- Inserting or removing contact lenses

Always wash your hands after:

- Taking off PPE
- Touching an animal, human and animal samples, waste, products or animal equipment
- Collecting and handling diagnostic samples
- Visiting field sampling sites or clinics/hospitals
- Preparing foods, especially raw meat or poultry
- Using a toilet
- Changing a diaper
- Blowing your nose, coughing or sneezing into your hands
- Treating wounds

- Touching a sick or injured person
- Touching garbage or other potentially contaminated materials
- Finishing work in the laboratory

Plan for hand washing:

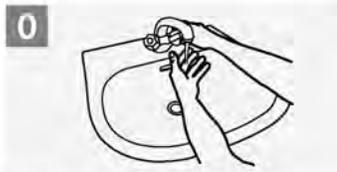
- Plan for hand washing in the field by identifying any locations with running water near the site and bringing supplies (i.e., water, soap, bucket, paper towels, hand sanitizing gels and germicidal wipes that contain at least 60% alcohol)
- Plan when you will need to wash to ensure supplies are ready and available

See the WHO guidelines below for proper hand washing technique. If soap and water are not available, use an alcohol-based hand sanitizing gel that contains at least 60% alcohol. These products significantly reduce the number of microbes on the skin and are fast acting. However, they are not effective if hands are visibly dirty. Organic matter and natural oils on hands create a barrier that blocks the effectiveness of the sanitizer. See <http://www.cdc.gov/handwashing/show-me-the-science-hand-sanitizer.html> for more information.

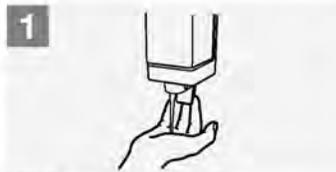
How to Handwash?

WASH HANDS WHEN VISIBLY SOILED! OTHERWISE, USE HANDRUB

 Duration of the entire procedure: 40-60 seconds



0 Wet hands with water;



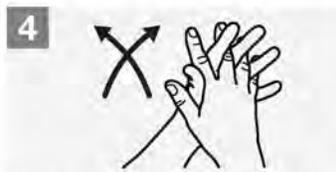
1 Apply enough soap to cover all hand surfaces;



2 Rub hands palm to palm;



3 Right palm over left dorsum with interlaced fingers and vice versa;



4 Palm to palm with fingers interlaced;



5 Backs of fingers to opposing palms with fingers interlocked;



6 Rotational rubbing of left thumb clasped in right palm and vice versa;



7 Rotational rubbing, backwards and forwards with clasped fingers of right hand in left palm and vice versa;



8 Rinse hands with water;



9 Dry hands thoroughly with a single use towel;



10 Use towel to turn off faucet;



11 Your hands are now safe.



World Health Organization

Patient Safety

A World Alliance for Safer Health Care

SAVE LIVES

Clean Your Hands

Disinfection of Surfaces and Materials

Dirt and organic matter can protect microbes from decontaminants (antiseptics, chemical germicides and disinfectants). Therefore, precleaning contaminated surfaces as well as reusable supplies, equipment and PPE is important to achieve proper disinfection. Precleaning should be carried out cautiously to avoid exposure to pathogens.

Contact times for disinfectants are specific to the type of solution and the manufacturer. Therefore, it is important to follow the manufacturers' specifications. Further, solutions used for precleaning and disinfection should be the same or chemically compatible.

There are several types of disinfectants on the market and formulations should be selected for specific needs. High temperatures can degrade chemical disinfectants, so shelf-life may be decreased in areas with high ambient temperatures.

Chlorine bleach or Virkon disinfectant solution are commonly used as general-purpose disinfectants. See the WHO Laboratory Biosafety Manual (<http://www.who.int/csr/resources/publications/biosafety/en/Biosafety7.pdf>) for frequently used classes of disinfectants, with general information on their applications and safety profiles, as well as recommended dilutions for chlorine-releasing compounds, such as chlorine bleach.

Section 3. Assessing Biosafety Risk of Zoonotic Pathogens and Selecting PPE

Key to the practice of biosafety is assessing the risk of infection associated with a specific procedure under specific environmental conditions. There are many considerations in the assessment of risk and it is the job of the supervisor to weigh these considerations to determine the appropriate measures to protect humans and animals from infection.

Factors to Consider when Assessing Biological Risk of Procedures to Determine Necessary PPE

1. Species to be handled and sampled.
2. Pathogens likely to be present in these species/samples.
3. Pathogenicity of these pathogens (see WHO classification of infective microorganisms by risk group below).
4. Potential exposure opportunities and routes of infection for the pathogens given the planned activity.
5. Potential result of exposure to the pathogens.
6. Estimated infectious dose and stability of the pathogens in the environment.
7. Information available in the literature, including animal studies and clinical reports that would help inform on risk.
8. Measures to reduce the risk of exposure, such as sanitary measures (e.g., food and water hygiene) and control of animal reservoirs or arthropod vectors, the movement of people or animals, and the importation of infected animals or animal products.
9. Local availability of effective prophylaxis and treatment. Prophylaxis may include vaccination or antisera. Treatment options may include passive immunization and post-

exposure vaccination, antibiotics, and chemotherapeutic agents, taking into consideration the possibility of the emergence of resistant strains.

Based on the risk assessment considering the factors listed above, the following should be determined by the PREDICT activity supervisor (often Country Coordinators):

1. Hazards and risk of exposure.
2. Appropriate PPE required to implement the activity safely and to prevent transmission of infectious pathogens. (Components of PPE to consider are discussed later in this document).
3. Special procedures, such as disinfection procedures between handling individual animals and people or between site visits, that may be required to reduce risk of transmission and provide adequate protection for humans and animals.
4. Vaccinations or prophylaxis required for PREDICT personnel before the activity.

World Health Organization (WHO) Classification of Infective Microorganisms by Risk Group (2004)

WHO provides the guidelines below for classifying biological risk categories, based on pathogenicity of the organism and modes of transmission and host range of the organism. These primary factors are affected by existing levels of immunity, density and movement of host population (human or animal), presence of appropriate vectors and environmental conditions, and availability of effective preventive measures and treatment. Countries usually adopt a similar set of risk categories. The WHO risk group classification was developed for laboratory work. See <http://www.absa.org/riskgroups/> for more information and a link to the Risk Group Database where information on risk can be obtained for specific microbes and/or microbe families.

The WHO risk categories are:

WHO Risk Group 1 (no or low individual and community risk) -- A microorganism that is unlikely to cause human disease or animal disease.

WHO Risk Group 2 (moderate individual risk, low community risk) -- A pathogen that can cause human or animal disease but is unlikely to be a serious hazard to laboratory workers, the community, livestock or the environment. Laboratory exposures may cause serious infection, but effective treatment and preventative measures are available and the risk of spread of infection is limited.

WHO Risk Group 3 (high individual risk, low community risk) -- A pathogen that usually causes serious human or animal disease but does not ordinarily spread from one infected individual to another. Effective treatment and preventive measures are available.

WHO Risk Group 4 (high individual and community risk) -- A pathogen that usually causes serious human or animal disease and that can be readily transmitted from one individual to another, directly or indirectly. Effective treatment and preventive measures are not usually available.

Appropriate PPE for PREDICT Activities

While PREDICT field staff will be working in very different environments with varying levels of biological risk, there are some tasks for which **minimum PPE requirements** have been established and detailed in Table 1.

Table 1. Minimum PPE to wear for some PREDICT Tasks:

Taxa/Task	Respirator (N95 or respirator with comparable filtering rating)	Goggles, Face shield or protective glasses	Gloves*	PPE Coveralls or Dedicated Clothing with washable shoes
Handling human and animal specimens	Yes	Yes	Yes	Yes (either PPE or coveralls or dedicated clothing)
Handling primates (live or carcass)	Yes	Yes	Yes	Yes (either PPE or coveralls or dedicated clothing)
Handling rodents or bats (live or carcass)	Yes	Yes	Yes	Yes (either PPE or coveralls or dedicated clothing)
Sampling in bat caves	Yes	Yes	Yes	PPE coveralls
Sampling or necropsy of sick/dead animals	Yes	Yes	Yes	Yes (either PPE or coveralls or dedicated clothing) with apron
Sampling bushmeat	Yes	Yes	Yes	Yes (either PPE coveralls or dedicated clothing) with apron
Handling poultry or waterfowl	Yes	Yes	Yes	Yes (either PPE or coveralls or dedicated clothing)
Handling livestock	Depends**	Depends**	Yes	Yes (either PPE or coveralls or dedicated clothing)
Sampling apparently healthy humans	Depends***	Depends***	Yes	Depends***
Collection of animal feces or urine from the environment	Depends****	Depends****	Yes	Depends****
Sampling an animal once it has been anesthetized	Recommended if in close contact with the animal during sampling activity	Recommended for those in close contact with the animal during sampling activity	Yes	Yes (either PPE or coveralls or dedicated clothing)

Table Definitions

* When handling live animals that pose a bite or scratch risk, it is recommended that leather gloves be worn above nitrile gloves for added protection. Nitrile gloves are more puncture resistant than latex and may reduce the risk of exposure from a bite or scratch. In many cases chemical restraint (anesthesia) is recommended to prevent injury to either the handler or the animal during sample collection.

** It is recommended to use a respirator, full protective clothing and eye protection when in contact with livestock suspected of harboring a biohazardous agent and pregnant livestock or livestock recently giving birth, and upon entering and/or working in abattoir settings or other settings where livestock are being slaughtered and/or butchered.

*** For routine sample collection from apparently healthy people, gloves are recommended. For collecting samples from hospital and clinic patients and during outbreaks, PPE should be adapted based on the determined risk level.

**** In some cases, such as during the collection of urine underneath a colony of fruit bats roosting in trees where there is a high risk of aerosolizing of excreta and microbial agents, then it is recommended to use a respirator (N95 respirator is recommended as the minimum level of protection), full protective clothing and eye protection.

Higher Risk Taxa

Below is a summary of special biosafety considerations for some of the key groups of species (bats, rodents, and non-human primates) to be handled as part of PREDICT activities.

Rodents, bats, non-human primates and other wild species may harbor pathogens that are transmittable to, and highly pathogenic in, humans. When handling these rodents, bats or non-human primates, careful consideration needs to be given to conscientious use of PPE, good personal hygiene (i.e., hand washing), safety training, and application of good animal handling and sampling techniques to minimize exposure to infection or injury.

In the event of an injury while handling animals that pose risk of zoonotic pathogen exposure, appropriate first aid must be applied. The risk of infection can be significantly reduced with immediate and thorough scrubbing of the wound with soap or antiseptic.

Vaccination to prevent rabies infection: Personnel who are handling animals that are known reservoirs for rabies (i.e., bats and dogs) should be immunized against rabies virus according to World Health Organization and CDC recommendations.

Investigators should familiarize themselves with known biohazards specific to species under study and with the procedures for the isolation and control of zoonotic pathogens.

Specific considerations with regard to working with rodents, bats and non-human primates are discussed below:

Rodents

Wild rodents have the potential to carry a variety of zoonotic bacteria and viruses that can be passed on to those handling them. Because of the serious consequences of becoming infected, personnel must always follow good personal hygiene and animal handling procedures and use the provided PPE to protect against exposure.

Special Precautions:

- Wear the minimum PPE for handling rodents including an N95 mask, eye-protection, gloves and coveralls, or clean dedicated clothing.
- Personnel who are handling animals should be immunized against rabies virus according to the World Health Organization and CDC recommendations.

Bats

Exposure to wild bat roosts (in caves or trees), handling of bats in the field or handling bat excreta (urine or feces) presents a potential for exposure to zoonotic pathogens. Rabies, Nipah virus, Ebola virus, and the fungal disease histoplasmosis are examples of zoonotic pathogens carried by some bat species. Bat bites, scratches and wound and mucous membrane exposure to bat saliva are the ways in which rabies can be transmitted. Spores of histoplasmosis can be present in soil and debris enriched with bird and bat droppings. When this dry soil is disturbed, spores can become airborne and cause infection by inhalation.

Special Precautions:

- When working around bats in enclosed spaces, such as in a cave, wear at a minimum an N95 respirator, goggles, gloves and Tyvek coveralls (or dedicated long-sleeved clothing).
- Personnel who are handling animals such as bats should be immunized against rabies virus and be aware of appropriate post exposure prophylaxis in the case of bites according to World Health Organization and CDC recommendations.

Non-Human Primates

Non-human primates may be infected with a number of potentially serious zoonoses. For example, all macaque monkeys and their fluids should be considered to be infected with **Herpes Simian B virus**. Marmosets, although they do not carry the herpes B virus, can carry other disease agents that affect humans such as lymphocytic choriomeningitis virus and *Trypanosoma cruzii*, the cause of Chagas' disease. It is critical that work with non-human primates be done while wearing the appropriate personal protective equipment and with the well-established safe protocols and procedures.

Special Precautions:

- Personnel must follow strict hygiene procedures. Frequent and thorough hand washing, although too often overlooked by the staff, is critical to physically remove bacterial contamination and prevent ingestion exposure.
- PREDICT personnel must wear the minimum PPE for handling non-human primates including an N95 mask, eye-protection, gloves and coveralls or clean dedicated clothing.

Section 4. Use and Disposal of PPE

Considerations When Using PPE

Personnel wearing PPE may experience heat stress and general discomfort in hot or humid environments. It is important to remain hydrated by drinking adequate water before and after wearing PPE. Length of time wearing full PPE should be limited, based on environmental conditions, to avoid the risk of heat exhaustion or heat stroke. Personnel should inform their supervisor(s) if they experience severe discomfort during animal capture or sampling activities, so that they may take a break.

When workers are heat-stressed, uncomfortable, or unable to see out of their fogged goggles, they are more likely to remove their goggles or mask in risky environments, exposing themselves to potential pathogens.

Most PPE items to be worn during PREDICT activities are disposable and designed to be used only once, and should be properly disposed of as medical waste after each use. Plastic goggles and rubber boots may be re-used, but must be disinfected between each use.

Designate a clean area for putting on PPE. It should ideally be a clean area away from any potentially contaminated animal equipment, such as cages, crates, or farm tools. All personnel should use this area to put on their PPE. Also, designate a decontamination and PPE removal site.

Always wear the respirator properly when you are working. Ensure that there is a tight seal formed around the mask and never hang it around your neck.

When wearing coveralls, ensure there is no exposed skin between your sleeves and gloves. If any piece of PPE is torn, it should be changed at the PPE decontamination site as soon as possible following the steps outlined in the section on how to take off PPE.

It is beneficial to have a colleague confirm that PPE is properly worn. Working in teams when putting on and removing PPE can help avoid mistakes and react immediately if accidents occur.

Planning and Preparations for PPE Use

1. Prior to going to the field, the level of risk for the field tasks and the appropriate PPE needed to safely perform the field tasks should be determined.
2. PPE kits should be assembled for each person who will be involved in the field tasks. Multiple kits per person may be required, based on the number of animals to be handled, the number of breaks that personnel may take, and to account for potential tears in gloves and coveralls, etc.
3. Prior to going to the field, PPE supplies should be organized. Along with required sets of PPE, supplies should include disinfectants, alcohol-based hand sanitizing gel and germicidal wipes, large color coded bags for infectious waste disposal according to national codification, and collection bags for equipment (such as plastic goggles, face shields and rubber boots) that will be disinfected for re-use.
4. Bottled water should be available for consumption before and after use of PPE. PPE can be very hot, and personnel are more likely to suffer heat stress if they do not consume adequate amounts of water.
5. Bring additional tape and extra collection and disposal bags. Tape can be used to secure shoe covers and protective clothing and seal bags.
6. Plan for disposing of PPE:
 - a. An area for removing PPE should be identified. This area should be away from the contaminated area and away from animals. All personnel should use this area to remove their PPE.
 - b. Remove all of your PPE carefully, following the recommended steps for PPE removal (below) and discard them (or put reusable items in bags for disinfection) before taking a break. Put on a new set after the break.
 - c. Immediately after removing PPE, place it directly into the color coded infectious waste bag (or marked biohazard waste bag).
 - d. Color coded infectious waste bags should be sealed and properly disposed. Follow the instructions of the local officials or person supervising the work on where to dispose infectious waste bags when they are full.
 - e. Disposal methods (such as burning or burial) may differ by situation or location. Local officials and/or those supervising the work will likely decide on how best to dispose of used PPE and other disposable items that are potentially contaminated. For guidelines, see PREDICT Safety Guide: Laboratory Operations, Environmental Guidelines for Small-Scale Activities in Africa (EGSSAA) Ch. 8: Healthcare Waste: Generation, Handling, Treatment and Disposal (<http://www.encapafrika.org/egssaa/medwaste.pdf>); and WHO Safe Management of Wastes from Health-Care Activities (http://www.who.int/water_sanitation_health/medicalwaste/wastemanag/en/).

Components of PPE Kits

1. Coveralls, dedicated clothing and shoes, and aprons – for high-risk tasks, full coverage may be warranted. In that case, Tyvek or Tychem coveralls, shoe covers or boots, and an apron may be used. For lower-risk tasks, just an apron and/or dedicated clothing and shoes may be appropriate. An apron should be a disposable type that is properly disposed of together with

gloves and masks after each use. Dedicated clothing (e.g., cotton coveralls) at the work site should be removed and laundered after each use.

Regarding the use of Tyvek or Tychem coveralls:

- Wear these coveralls to protect your skin and/or clothing against contamination when in contact with human samples, animal droppings, dust, animal urine or droppings, or animal fluids such as blood, saliva, and mucous.
- The synthetic material Tyvek is water resistant and Tychem is water proof, so even if the coveralls get dirty or wet, they will offer protection. Tychem offers more protection from liquids and should be considered in situations with high risk of exposure to blood-borne pathogens (e.g., hemorrhagic disease, EVD outbreak investigations).
- You can wear your dedicated shoes and clothing under the coveralls.

2. Shoe Covers or Washable Rubber Boots

- Because pathogens in human and animal samples including feces, secretions, or blood can easily contaminate your footwear, it is important to have disposable shoe covers or rubber boots that can be disinfected.
- The shoe covers provided in some PPE kits fit over your coverall feet, or over your shoes.
- Rubber boots may be worn with dedicated pants pulled over the top of them. If using PPE coveralls with rubber boots, purchase the coveralls without feet (or cut the feet off) and pull the pant legs of the coveralls over the top of the boots.
 - A footbath should be prepared with either chlorine bleach or Virkon disinfectant. This can be used to disinfect boots and other footwear upon leaving the field site. A boot brush should be available for scrubbing surfaces of footwear prior to using the footbath. It is critical to remove all organic material from footwear prior to disinfection to ensure effectiveness of disinfectants.

3. N95 Respirator

- N95 respirators (masks) protect you from inhaling droplet or aerosolized pathogens into your nose and lungs. Surgical masks are not respirators. They do not protect against aerosol and small droplets. They filter out large-size particles in the air and offer protection from large droplets and direct contact.
- There are several different models, styles, and sizes of N95 and comparable respirators that fit a variety of face shapes and sizes. Each person requiring a respirator for PREDICT activities should be individually fit tested to identify a respirator that appropriately and comfortably fits her or his face.
- Respirators with exhalation valves are generally more comfortable as the exhalation valve prevents resistance to exhalation when the filters load with dust.
- See [Section 5](#) on respirator use to learn more about respirators and fit testing.

4. Goggles and Face Shields

- Goggles protect your eyes from splashes and liquids.
- They are adjustable to ensure the best fit. Adjust the head strap before putting on all of the PPE. The goggles should fit snugly over and around your eyes.
- Personal glasses are not a substitute for goggles or safety glasses; if you wear eyeglasses, the goggles or safety glasses should be placed over them.
- If ordering goggles, be sure to order fog-free goggles. If they are not fog-free, they are likely to fog up in a few minutes, rendering them useless. If all you have are non-fog-free (regular) goggles, you may rub a little soapy water on the inside of the lens prior to use to reduce fogging.
- Goggles (and rubber boots) are one of the few components that may be re-used if disinfected properly after each use.



5. Gloves

- Nitrile gloves are best for use for infectious agent exposure protection. **Gloves are a component of minimum PPE required for sample collection and handling tasks conducted under PREDICT.**
- Two pairs of nitrile gloves are recommended when using sharps.
- Heavy rubber gloves or leather gloves may be required when handling animals and can be worn over the nitrile gloves. PREDICT teams have good success with Hexarmor Hercules 400R6E gloves.



6. Disinfecting Wipes and Alcohol-based Hand Sanitizing Gel (at least 60% alcohol) -- for disinfecting gloves and hands.

- Disinfecting wipes that contain at least 60% alcohol should be used to clean your gloves and other PPE before removing them.
- Alcohol-based wipes or hand sanitizing gel can be used to clean areas of skin that may have been contaminated. It is critical to remove organic material before using sanitizers to ensure effectiveness of disinfectant.
- It is recommended that you ALWAYS disinfect and wash your hands after removing gloves, regardless of contamination.

7. Infectious Waste Bag—for the safe disposal of PPE and other medical waste.

- A color coded infectious waste bag (or otherwise labeled biohazard bag) should be available at the field site for containing and disposing of used PPE items.
- As soon as you remove a contaminated item, place it in the infectious waste bag.
- Do not over fill bags and ensure they can be closed and tied.
- Tie the bag at the top and spray the outside of the bag with disinfectant once it is closed and tied. Wet waste should be double-bagged to prevent leakage.
- Leave it at the designated collection site or place it in in a secure container for transport to a proper disposal site.
- Containers should be constructed to contain all contents and prevent leakage of fluids during handling, storage, and transport.
- It is strongly recommended that field teams do not burn or bury medical waste at the field site. Incomplete burning may leave infectious or dangerous materials, and animals or children may dig up buried waste. All bio-hazardous waste should be contained and returned to a medical center for autoclaving or incineration. **See *Safe Disposal of Carcasses and Infectious Waste Guide* for information regarding guidelines for waste disposal.**

Procedure for Putting on PPE

All of the components of PPE discussed below are not necessary or appropriate for all PREDICT tasks. For instance, Tyvek or Tychem coveralls and aprons are not necessary for many PREDICT tasks. However, when investigating disease outbreaks or other potentially high-risk situations, the PPE and donning and doffing procedures may be substantially enhanced to reduce risk of exposure. See <http://www.cdc.gov/vhf/ebola/hcp/ppe-training/index.html> for CDC Guidelines for Personal Protective Equipment (PPE) Donning and Doffing Procedures during management of Ebola virus disease cases.

1. Wash your hands and/or disinfect them with alcohol-based hand sanitizing gel prior to putting on PPE.

2. Coveralls or dedicated clothing go on FIRST. Always start with the coveralls (which should be big and loose to fit over clothing and not restrict movement) or dedicated clothing. Be certain to zip up coveralls or button up clothing.



3. Shoe covers or boots go on SECOND. Shoe covers fit over the coverall feet. Pant legs of dedicated clothing and coveralls should fit over the boots.



4. Respirator or surgical mask goes on THIRD. Of the equipment to be worn around the head and face, the mask or respirator is always first on and last off. On a mask with a metal nose clip, be sure to form the clip around the nose for a nice fit. If wearing a respirator, perform a seal check by inhaling sharply. If there is air leakage around the edges of the mask, readjust to ensure a proper seal.



5. Goggles go on after the respirator. Goggles should fit snugly over and around your eyes. Goggle straps should be adjusted to fit your head.

Once the respirator and goggles are in place, pull the hood on your coveralls over your head (or put on the separate head cover if the coveralls do not have a hood).



6. Tie on the apron over the coveralls or your dedicated clothing. Place the apron over your head and then tie it in the back.



7. Put on two pairs of gloves. The inner glove should go under the sleeve of the coverall to prevent exposed skin between the coverall and the glove. Coveralls with finger loops that secure the sleeve over the first pair of gloves are ideal to avoid exposure of the wrist area (or you can make a small cut in the coverall sleeve and introduce your thumb). Otherwise, tape the coverall sleeve to the inner glove. Put the second pair of gloves on over the first pair and extend the gloves over the coverall cuffs.



Procedure for Removing PPE

After completing your work, assume the exterior of the PPE is contaminated. The goal of correct removal of PPE is to minimize contact between your clothes and skin and the contaminated outer surfaces of the PPE.

- 1. Wipe off any visible contamination of the PPE** using germicidal or alcohol-based wipes and dispose of the used wipe in the infectious waste bag.
- 2. Remove and dispose of the apron** in the infectious waste bag.
- 3. Wipe off outer gloves with a germicidal wipe and dispose of the used wipe** in the infectious waste bag.



- 4. Remove boots or remove shoe covers** by holding the top and rolling them off of your feet. Place the shoe covers in the infectious waste bag. Place the boots in the equipment collection bag for disinfection and re-use.



5. Remove the outer gloves and place them in the infectious waste bag. Using one gloved hand, grasp the outside of the opposite glove near the wrist. Pull and peel the glove inside-out and away from the hand. Hold the removed glove in the opposite gloved hand. Then, slide one or two fingers of the ungloved hand under the wrist of the remaining glove. Peel glove off from the inside, creating a bag for both gloves. Dispose of the gloves in the infectious waste bag.



6. Disinfect your inner gloves with alcohol-based hand sanitizing gel.

7. Unzip and roll down the coveralls until they are inside out and place them in the infectious waste bag.



8. Disinfect gloves with alcohol-based hand sanitizing gel.

9. Remove the goggles by the strap and place them in the infectious waste bag or equipment collection bag for disinfection and re-use if re-usable. Re-usable goggles can be disinfected using a chlorine bleach solution.



10. Disinfect gloves with alcohol-based hand sanitizing gel.

11. Close the biohazard bag by tying the corners of the top of the bag together.



12. Remove the respirator by grabbing the top and then the bottom elastic bands, and pulling the bands up over your head or by grabbing the nose and pulling forward and then off. Place the respirator in a second clean red infectious waste bag.



13. Disinfect gloves with alcohol-based hand sanitizing gel.

14. Remove the inside gloves using the procedures listed in #5 above and place them in the second infectious waste bag. Dispose of infectious waste bags according to guidelines in Section 4, #6 e above.



15. Disinfect your hands with alcohol-based hand sanitizing gel.

16. Wash your hands and wrists using soap and running water (from a tap or poured) following the guidelines presented in Section 2.



If PPE is compromised, falls off, rips or is removed while you are handling or are exposed to biological hazardous materials, stop your current activity, remove PPE in the designated area, and wash or disinfect the exposed skin/surfaces. In addition, immediately inform your supervisor to determine if prophylaxis is indicated.

Section 5. Respirator Use

- Using respirators alone will not fully protect you from acquiring an infection – the respirator must be used in combination with all of the other PPE components.
- Each person using respirators must be fit tested to identify a respirator that he or she can comfortably and securely wear. Fit testing is a process that takes approximately 15-20 minutes to complete and should be performed for each member of the field team before he or she uses any respirators in the field. Qualitative fit test kits are available for purchase through 3M. A video on fit testing is available online at



- <https://www.youtube.com/watch?v=7IAsoU6h-8g>. After passing a fit test with a respirator, you should always use the same make, model, style, and size of respirator that was found during the fit test process to create an effective seal around your face. If you have facial hair, it is unlikely that you can properly fit a disposable particulate respirator. Workers who cannot ensure a proper fit because of facial hair or other fit limitations should consider a loose-fitting (i.e., helmeted or hooded) powered air purifying respirator equipped with high-efficiency filters. More information on respirators and respiratory protection can be found at: <https://www.osha.gov/SLTC/etools/respiratory/index.html>.
- Do not use or provide others with respirators without instruction on the health risks associated with them. For example, workers with respiratory problems may not be able to wear these respirators. Anytime someone indicates they are having trouble breathing while wearing a respirator, they should go to the PPE removal site and remove their respirator.
- When disposable particulate respirators become wet from saliva, sweat, or respiratory secretions, they lose their protective properties and must be changed.

- If a respirator is splashed and becomes wet, it should be changed using gloves and the gloves disinfected or washed following hand washing procedures.
- Respirators should be discarded and replaced after 4-6 hours of use.
- Respirators should not be hung around your neck when working. Always wear them when working.

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Emergency Preparedness

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and the PREDICT One Health Consortium
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Objective: To provide guidance for PREDICT personnel to prepare for and respond to field emergencies.

Section 1. Overview and Resources

This material is intended to supplement other PREDICT guides and protocols that detail safety and protection measures for field situations. Namely it is imperative that all personnel are thoroughly familiar with the PREDICT guides relevant to their job tasks (e.g., PREDICT guides for ***Safe Animal Capture and Handling, Human Biological Sampling, Biosafety and PPE Use, Basic Laboratory Safety Guides*** as well as the relevant sampling guides for specific taxa. This document is intended to provide guidance and a collection of materials and resources for personnel use.

In performing fieldwork in their role for PREDICT, personnel may encounter a wide variety of hazards that they should be prepared for ahead of time. These hazards and the risks associated with them will vary and depend on many factors. This guide is intended to help personnel identify and prepare for the hazards, emergencies, and accidents they are most likely to encounter and that are not otherwise well-covered in PREDICT materials. It must be understood that the risk of accidents and emergencies can never be eliminated, but that careful planning and good preparation can minimize many of the most serious risks and resulting negative outcomes.

Emergency and accident preparedness encompasses a large body of information and materials beyond the scope of this guide. Personnel seeking further information on topics relating to emergency preparedness for disasters, general building operations, laboratory procedures, and related activities are advised to seek information on what are generally referred to as 'emergency action plans' (EAP) or 'accident preparedness plans' (APP). Additional information on those topics can be found at the following links:

Emergency Action Plans

- <http://www.nuc.berkeley.edu/sites/default/files/resources/safety-information/Building%20Emergency%2007%20FINAL.pdf>
- <http://www.lni.wa.gov/Safety/TrainingPrevention/Programs/?F=SHPN>
- www.osha.gov/SLTC/etools/evacuation

General Disaster Preparedness:

- http://multimedia.peacecorps.gov/multimedia/pdf/library/T0123_dpm_pst.pdf
- <http://www.redcross.org/prepare/disaster>

Section 2. Plan for Field Emergencies

Accidents and emergencies are inherently unplanned events, but many of them can be anticipated and prepared for. Being prepared for emergencies requires planning. Good planning is particularly important when working with field teams and in remote locations.

A basic process for emergency planning should include the following steps (adapted from the Global Safe Haven Network, which is targeted to individual student travel planning but has useful resources; www.globalsafehaven.org):

1. **Understand the hazards** and issues you may face. Consider the following categories of hazards: health, security, travel requirements, weather environment, transportation, legal, financial, communications, culture, language. (See following section for more information.)
2. **Evaluate the risks.**
3. **Communicate** with all field team members and supervisors to make sure everyone understands, is comfortable with, and is prepared for identified risks.
4. **Address and mitigate each issue** to your team's comfort level. Most risk mitigation strategies have inherent financial costs. Regardless of whatever else is addressed, develop an emergency communication plan.
5. **Monitor the local situation** in the event something changes.
6. **Respond to any change** or incident as necessary by preplanning.

More details can be found at: http://www.globalsafehaven.org/downloads/step_broch.pdf

Identify Hazards

The types of hazards and emergencies that any team may encounter will depend on many variables. Some will be consistent with all field activities while others may depend on site or time specific field activities. Therefore, hazards should be identified and evaluated before each field activity, and plans should be developed appropriately.

The following list (with worksheet in [Appendix I](#)) is provided in order to assist field teams to compile appropriate lists for their specific activities and sites.

Some Potential Field Hazards and Issues

1. Health
 - a. Exposure to infectious diseases not associated with the project (malaria, dengue fever, cholera, etc.)
 - b. Pharmacy availability
 - c. Access to emergency medical care
 - d. Handled animal bite/scratch/goring
 - e. Non-target animal bite/scratch/goring (including snakebite)
 - f. Staff anesthetic exposure
 - g. Other toxic exposure

- h. PPE breach/infectious disease exposure (needlestick, scalpel cut)
 - i. Burn, chemical injury
 - j. Fall/trauma
 - k. Spontaneous (heart attack, appendicitis, heatstroke, hypoglycemic crisis)
 - l. Accidental gunshot wound
2. Security
 - a. Robbery, car jacking
 - b. Coup, riot, political uprising
 - c. Passport lost or stolen
 3. Travel requirements
 - a. Insufficient visa/entry paperwork for any/all staff
 - b. Improper vehicle paperwork
 4. Weather and environment
 - a. Extreme temperature or conditions
 - b. Flood
 - c. Severe storm
 - d. Earthquake
 5. Transportation
 - a. Auto accident
 - b. Vehicle breakdown
 - c. Inability to refuel
 6. Legal
 - a. Police/military detainment (warranted or unwarranted)
 - b. Insufficient permits for samples, supplies (including dart guns), chemicals
 7. Financial
 - a. Unexpected expenses (including bribes)
 - b. Access to cash (ATMs, banks, etc.)
 - c. Emergency evacuation costs
 8. Communications
 - a. Lack of mobile phone coverage
 - b. Loss of primary communications (dead phone battery, robbery)
 9. Culture
 - a. Lack of local permission to perform activities
 - b. Lack of cooperation (suspicion, lack of communication)
 10. Language
 - a. Inability to communicate with local population in event of emergency

Once hazards are identified, addressed, and discussed, field teams should reach a consensus on appropriate measures to take and plan accordingly. In addition to those measures, field teams should always prepare at least the two types of documents described below for each field site.

Prepare “Emergency Communications Plan” (template provided in [Appendix II](#)).

The purpose of an Emergency Communications Plan is to make sure that field teams can access necessary resources in the event of an emergency. Critical to this planning is having a well-informed understanding of what communications will be available at the field site. In many regions mobile phone coverage may not exist and/or be limited to only certain carriers. Field teams should always have a basic or back-up plan for how to communicate if an emergency arises whether directly from a field site or by reaching the nearest resource. In many cases the team may have only one vehicle, which poses a risk if the vehicle breaks down and there is no local communication. It is recommended that each field team have a satellite phone to secure communication capacity for the field team.

Prepare “Field Personnel Emergency Information Records” (template provided in [Appendix III](#)).

The purpose of Personnel Emergency Information Records is to make sure that critical information about each team member is known and readily available in case of emergency. Emergency planning should consider worst-case scenarios and in this context a team member may be unconscious or otherwise unable to communicate. The information gathered for this type of documentation may be imperative for emergency responders and other medical authorities. It should be noted that ‘emergency responders’ may not always (or even usually) be available and that those responsibilities would then fall upon other team members until medical services can be engaged.

Emergency Planning Checklist:

A checklist for emergency planning is provided as Appendix IV and should be supplemented and edited as needed.

Section 3. First Aid

A comprehensive presentation of First Aid is beyond the scope of this document and personnel are referred to any recently published First Aid manuals, booklets, or guides. Those seeking further information may find the subcategory of First Aid referred to as “Wilderness First Aid” particularly useful because it deals with emergencies in remote settings. The Wilderness Medical Society has a number of resources, including guides and bibliographies, at their website: www.WMS.org.

Field teams should all have at least two members who are properly trained in basic First Aid techniques including cardiopulmonary resuscitation (CPR)¹ and wound management.

Personnel should also always operate under the basic tenets of First Aid: preserve life, prevent further harm, and promote recovery. Field teams must also always have a First Aid Kit available (see below). It is the responsibility of the Country Coordinator to seek training for personnel and ensure field teams follow this basic premise.

If no other resources are available, the following basic online First Aid resources can be consulted: <http://www.redcross.org> or <http://www.firstaidweb.com>.

¹ Note: most CPR training certificates must be renewed every 12 months.

While PREDICT field teams will typically be equipped with extensive medical supplies for field anesthesia of wildlife, sampling and diagnostics, they should also carry basic First Aid kits (best kept in waterproof containers) with dedicated materials for personnel emergencies.

Below is a basic First Aid kit list to which you can add on as the length and remoteness of your trip dictates:

- 10 pairs of nitrile gloves (medium and large)
- 1 CPR mask (with one-way valve)
- 4 absorbent compress dressings (5 x 9 inches)
- 25 adhesive bandages (assorted sizes)
- 1 adhesive cloth tape (10 yards x 1 inch)
- 5 antibiotic ointment packets (approximately 1 gram)
- 5 antiseptic wipe packets
- 2 packets of aspirin (81 mg each) (within the expiration date)
- 2 packets of ibuprofen (within the expiration date)
- 4 packets of anti-diarrheal tablets such as loperamide
- 1 blanket (space blanket)
- 1 instant cold compress
- 2 hydrocortisone ointment packets (approximately 1 gram each)
- 2 antibiotic ointment packets (approximately 1 gram each)
- Scissors
- 1 roller bandage or vet wrap (3 inches wide)
- 1 roller bandage or vet wrap (4 inches wide)
- 5 sterile gauze pads (3 x 3 inches)
- 5 sterile gauze pads (4 x 4 inches)
- Oral thermometer (non-mercury/non-glass)
- 2 triangular bandages
- Compression wrap for supporting ankles or knees
- Tweezers
- First aid instruction booklet
- Headlamp or other light source
- +/- EpiPen for life-threatening allergic reactions to be administered by trained personnel. A training video can be found here: <http://www.epipen.ca/en/about-epipen/how-to-use-epipen> Print out instruction form found here and include in kit: http://www.epipen.ca/sites/default/files/pdf/en/Instruction_Sheet.pdf. EpiPen users must observe the expiration date of the individual pens and replace accordingly. Expired EpiPens are considered hazardous waste and must be returned to the pharmacy where they were purchased for proper disposal.

Section 4. Employee Health

Personnel safety is covered in the PREDICT guides for *Safe Animal Capture and Handling*, *Human Biological Sampling* and for *Biosafety and PPE Use*. This section supplements that information and refers specifically to practices relating to institutional occupational health and safety programs.

In the United States, the Occupational Safety and Health Administration mandates that employers “assure safe and healthful working conditions” for employees, and that medical testing is available to employees exposed to potential hazards to determine whether the health of such employees is adversely affected by such exposure” (Occupational Safety and Health Act 1970). All PREDICT partner institutions are assumed to be appropriately managing general occupational health programs for their staff both domestically and abroad.

With an understanding that institutional practices may vary, the following recommendations apply to all PREDICT field personnel:

General Practices

1. Individuals with known allergies associated with animals, with immune deficiency diseases, or who are on immunosuppressant therapy, should not engage in studies involving the handling of animals and sick people.
2. Pre-exposure screening for tuberculosis is required for personnel who will be handling non-human primates. Tuberculosis screening and interpretation of results should only be conducted by a human health professional.
3. If within institutional capacity and guidelines, it is advised that periodic (annual) blood/serum samples be collected from all staff and banked.
4. All accidents, injuries and medical emergencies should be recorded and reported to direct supervisors immediately (see following section and report templates in Appendices VII a, b, and c).

Immunizations

1. The Country Coordinator or field supervisor should ensure that personnel have consulted with a human health professional with regard to the immunizations required prior to travel or participating in fieldwork that involves handling animals, human and animal samples. Required vaccines and immunizations will vary depending on the geographical area, animal species to be handled, whether staff member will be conducting human sampling, and personal medical history. Only a human health professional can recommend and provide vaccination and immunizations to personnel.
2. Due to the significant risks of rabies exposure when working with wild mammals (bats, carnivores, etc.), pre-exposure rabies vaccination is required for all personnel handling these species.
3. Tetanus immunization is also required for all personnel.

Health Records

All personnel health records must be guarded with the strictest confidentiality as directed by institutional requirements. Templates for employee medical history and vaccinations are provided in Appendix V and VI.

Section 5. Incident or Accident Reporting

It is important that any on the job accident or injury requiring even basic medical attention, including self-treatment, is documented and reported. PREDICT field personnel are presumed to be operating in environments often characterized by unhygienic conditions and with many known and unknown hazards (infectious agents, animals, human samples, scalpels, needles, darts, chemicals, etc.). Not all consequences of even the most minor injuries can always be foreseen and even minor cuts or abrasions can lead to life-threatening infection with pathogenic, treatment-resistant agents, especially in remote settings. Basic information collected at the time of injury can help to identify health hazards for future preventative actions and may also be critical for future treatment, clinical interventions, or even legal proceedings.

Accident and incident reporting may be mandated by each PREDICT partner institution. In the absence of other guidelines, very basic template accident reporting forms, provided in Appendix VII, can be used as-is or edited as needed. These templates include formats for both personal injury as well as motor vehicle accidents.

Section 6a. Appendix I. Hazard Identification Worksheet

Field Activity: _____
Date: _____
Location: _____
Team Leader: _____

A. Health *(e.g., animal injuries, human sampling, traumas, toxins)*

- a. _____
- b. _____
- c. _____
- d. _____
- e. _____
- f. _____

B. Security *(e.g., robbery, unrest)*

- a. _____
- b. _____
- c. _____

C. Travel Requirements *(e.g., visas, permits)*

- a. _____
- b. _____
- c. _____

D. Weather and Environment *(e.g., storms, natural disasters)*

- a. _____
- b. _____
- c. _____

E. Transportation *(e.g., auto accident, breakdown, fuel)*

- a. _____
- b. _____
- c. _____

F. Legal *(e.g., detainment, permits)*

- a. _____
- b. _____
- c. _____

G. Financial *(e.g., extra expenses, evacuations)*

- a. _____
- b. _____
- c. _____

H. Communications (*e.g., loss of primary form of communication*)

- a. _____
- b. _____
- c. _____

I. Culture (*e.g., lack of local cooperation*)

- a. _____
- b. _____
- c. _____

J. Language (*e.g., inability to communicate with locals*)

- a. _____
- b. _____
- c. _____

Section 6b. Appendix II. Emergency Communications Plan Template

Planned Activity Date(s): _____

Team Leader: Name: _____ **Phone:** _____

Team Members

Name: _____ Phone: _____

Name: _____ Phone: _____

Name: _____ Phone: _____

Name: _____ Phone: _____

Satellite phone number: _____

Local or Regional Supervisor or Contact (not with team):

Name: _____ **Phone:** _____, _____, _____

International Emergency Supervisor or Contact

Name: _____ **Phone:+** _____, _____, _____

Field Site: _____

Country: _____ **Region, Province, State:** _____

City/Village/Local: _____

GPS Coordinates: _____, _____ **Reference:** _____

EXPECTED MOBILE PHONE SERVICE: _____

Local Point(s) of Contact: Name: _____

Phone: _____ **Address:** _____

Local Emergency Number, if any (e.g., 911 service) _____

Nearest Hospital and Contact Info: _____

Nearest Clinic, Dispensary and Contact: _____

Nearest Airport: _____

Nearest Phone Line: _____

Local Police: _____ **National Police:** _____

Other Emergency Contacts (fire, ambulance): _____

Local Authority (mayor, district supervisor, district authority):

Legal Contact or Lawyer: _____

Embassy, Consulate Mission Contacts: _____

Section 6c. Appendix III. Field Team Emergency Information Template

Name	Date & place of birth	Passport info (Country, #)	Personal/family emergency contact information	Health insurance (provider, policy, primary physician)	Med-evac insurance (provider, policy)	Blood type	Medical conditions	Known allergies

Section 6d. Appendix IV. Emergency Checklist for PREDICT Field Activities

- Copy of emergency contact list/communications plan to accompany team (originals should be stored in office files).
- Copy of field team personnel info data to accompany team
- Copies of above documents accessible in office and/or with emergency contacts
- First aid kit
- Primary communications equipment (cell phone, sat phone, two-way radio)
- Back-up communications equipment
- Vehicle emergency equipment (spare tires, triangles, fire extinguisher, extra food and water, etc)
- Printed current maps of field location and surrounding areas
- GPS unit
- Emergency funds
 - Local cash
 - 'Hard' currency (dollars, Euros, pounds sterling)
 - Internationally accepted credit cards
- Original and/or photocopies of passports, permits, and insurance cards
- Spare batteries, car/DC charger adapter
- Flashlights
- Emergency kits for expected procedures (e.g., Ebola or B virus exposure kits)

Section 6e. Appendix V. Adult Vaccine Record

CDC Format

(Page 1 of 2)

Vaccine Administration Record for Adults

Patient name: _____

Birthdate: _____ Chart number: _____

Clinic name and address

Before administering any vaccines, give the patient copies of all pertinent Vaccine Information Statements (VISs) and make sure he/she understands the risks and benefits of the vaccine(s). Always provide or update the patient's personal record card.

Vaccine	Type of Vaccine ¹	Date given (m/d/yy)	Funding source (F,S,P) ²	Route ³ & Site ³	Vaccine		Vaccine Information Statement (VIS)		Vaccinator ⁵ (signature or initials & title)
					Lot #	Mfr.	Date on VIS ⁴	Date given ⁴	
Tetanus, Diphtheria, Pertussis (e.g., Td, Tdap) Give IM. ³									
Hepatitis A⁶ (e.g., HepA, HepA-HepB) Give IM. ³									
Hepatitis B⁶ (e.g., HepB, HepA-HepB) Give IM. ³									
Human papillomavirus (HPV2, HPV4) Give IM. ³									
Measles, Mumps, Rubella (MMR) Give SC. ³									
Varicella (VAR) Give SC. ³									
Pneumococcal (e.g., PCV13, conjugate; PPSV23, polysaccharide) Give PCV13 IM. ³ Give PPSV23 IM or SC. ³									
Meningococcal (e.g., MenACWY, conjugate; MPSV4, polysaccharide) Give MenACWY IM. ³ Give MPSV4 SC. ³									

See page 2 to record influenza, Hib, zoster, and other vaccines (e.g., travel vaccines).

How to Complete This Record

- Record the generic abbreviation (e.g., Tdap) or the trade name for each vaccine (see table at right).
- Record the funding source of the vaccine given as either F (federal), S (state), or P (private).
- Record the route by which the vaccine was given as either intramuscular (IM), subcutaneous (SC), intradermal (ID), intranasal (IN), or oral (PO) and also the site where it was administered as either RA (right arm), LA (left arm), RT (right thigh), or LT (left thigh).
- Record the publication date of each VIS as well as the date the VIS is given to the patient.
- To meet the space constraints of this form and federal requirements for documentation, a healthcare setting may want to keep a reference list of vaccinators that includes their initials and titles.
- For combination vaccines, fill in a row for each antigen in the combination.

Abbreviation	Trade Name and Manufacturer
Tdap	Adacel (Sanofi Pasteur), Boostrix (GlaxoSmithKline [GSK])
Td	Decavac (Sanofi Pasteur), generic Td (DA Biological Labs)
Hib	HiVax (GSK), Wida (Merck)
HibB	Ergen-B (GSK), Recombinant HB (Merck)
Hib-HepB	Tandem (GSK)
HPV2	Cervarix (GSK)
HPV4	Gardasil (Merck)
MMR	MMR II (Merck)
VAR	Varivax (Merck)
PCV13/PPSV23	Prevnar 13 (Pfizer), Pneumovax 23 (Merck)
MenACWY	Menactra (Sanofi Pasteur), Menveo (Novartis)
MPSV4	Meningovax (Sanofi Pasteur)

This form is printed on recycled paper with 50% recycled content.

For additional copies visit www.imzinnovize.org/utg_d/p2023.pdf • Item # P2023 (4/13)

This form was created by the Immunization Action Coalition • www.imzinnovize.org • www.vaccineinformation.org

Section 6f. Appendix VI. USAID Medical History and Examination Form



Bureau for Economic Growth, Agriculture And Trade Office of Education			
MEDICAL HISTORY AND EXAMINATION FOR FOREIGN APPLICANTS (Medical History To Be Completed By Applicant)			
1. LAST NAME – FIRST NAME – MIDDLE NAME		2. DATE OF BIRTH (MO/DAY/YR)	
3. NATIONALITY	4. SEX <input type="checkbox"/> Male <input type="checkbox"/> Female	5. Contact information for monitoring contractor or implementing partner who can be contacted related to medical claims in your absence	
6. TRAINING LOCATION (City, State for U.S. training) (Country for third country training)		7. LENGTH OF TRAINING (Weeks, Months, Years)	8. ESTIMATED DATE TO BEGIN TRAINING (Month/Year)
IMPORTANT NOTICE			
<p>Before You Complete The Medical History Questionnaire, You Are Hereby Notified That:</p> <ul style="list-style-type: none"> - USAID does not provide medical insurance for dependents that accompany or join the applicant. - A Medical condition resulting from an undisclosed pre-existing condition will not be covered by the USAID HAC insurance and may result in termination of your training program. Likewise, a medical condition resulting from a previously undiagnosed condition may not be covered by the USAID HAC insurance and may become the responsibility of the applicant. Your training program may be terminated if it is determined that your condition will significantly impact on your program, or if you cannot cover the cost of the medical care. Public funds may not be used to cover the cost of medical care. - I understand that by accepting USAID sponsorship I hereby waive any privacy rights that I have to such medical claims and agree to permit my insurance provider or its authorized representatives to release all information related to such claims to USAID. Such notification will include the date of the claim, the nature of the claim and copies of all documentation related to the claim. USAID shall use such claims information for reviewing its entire insurance program. I understand that I have the right to revoke this authorization by providing written notice to USAID. Such revocation will result in automatic termination of USAID's sponsorship of the program, unless USAID otherwise agrees in writing. 			
9. I Understand And Accept The Terms Of This Notice. <input type="checkbox"/> Yes <input type="checkbox"/> No			
10. CHECK EACH ITEM "YES" OR "NO," EVERY ITEM CHECKED "YES" MUST BE FULLY EXPLAINED IN BLANK SPACE ON RIGHT			
YES	NO		
<input type="checkbox"/>	<input type="checkbox"/>	a. Have you ever had any significant or serious illness or injury? (if hospitalized, give place & dates)	
<input type="checkbox"/>	<input type="checkbox"/>	b. Have you had any surgery or been advised by a physician to have surgery? (Give place & dates)	
<input type="checkbox"/>	<input type="checkbox"/>	c. Do you currently use any drugs for treatment of a medical condition? (Give name of & dose)	
<input type="checkbox"/>	<input type="checkbox"/>	d. Have you ever been a patient in a mental hospital or sanitarium or treated by a Psychiatrist? (Give place & dates)	
11. DO YOU NOW HAVE, OR HAVE YOU EVER HAD THE CONDITIONS LISTED BELOW? (Indicate "Yes" or "No" To Each Item)			
YES	NO	(Check Each Item)	(Check Each Item)
<input type="checkbox"/>	<input type="checkbox"/>	a. Epilepsy, convulsions, "fits"	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	b. Eye disease, vision defect in both or either eye	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	c. Tooth or gum disease (periodontal disease)	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	d. Asthma, emphysema, or other lung conditions	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	e. Tuberculosis or live with anyone who has tuberculosis	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	f. High blood pressure, heart disease	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	g. Stomach, liver (hepatitis), gallbladder disease	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	h. Hernia (rupture)	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	i. Kidney or bladder disease, stone or blood in urine	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	j. Diabetes (sugar in the urine)	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	k. Joint disease or injury, swollen or painful joints	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	l. Back pain, wear a back brace or support	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	m. Tropical disease (malaria, bilharzias, amoebas, leprosy, filariasis, yaws, etc.)	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	n. Depression, excess worry, attempted suicide, or other psychological symptoms	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	o. Drug or narcotic habit such as marijuana, cocaine, heroin, LSD, or any derivatives	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	p. Bleeding disorder, blood disease (sickle cell anemia)	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	q. Acquired Immune Deficiency Syndrome (AIDS)	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	r. Tumor, abnormal growth, cyst, or cancer	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	s. Skin disorder, growths, psoriasis	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	t. Female disorder, growths, psoriasis	<input type="checkbox"/> <input type="checkbox"/>
<input type="checkbox"/>	<input type="checkbox"/>	u. Pregnancy	<input type="checkbox"/> <input type="checkbox"/>
I CERTIFY THAT I HAVE READ THE ABOVE INSTRUCTIONS AND ANSWERED ALL QUESTIONS TRUTHFULLY AND TO THE BEST OF MY KNOWLEDGE.			
12. PRINTED NAME OF APPLICANT		13. DATE	14. SIGNATURE OF APPLICANT
NOTE For the Examining Physician: Please review this Medical History and make appropriate remarks on the Physician's Examination Form for any boxes checked yes. Any additional tests must be indicated on the Examination Form. Any test results that indicate a pre-existing condition(s) must be noted and explained.			

ADMINISTRATIVE REVIEW OF MEDICAL EXAMINATION
(For Use By Post Training Office)

1. NAME OF CANDIDATE: *(Last, First, Middle)*

MEDICAL CLEARANCE ACTION

ACTION BY SPONSORING UNIT OR DESIGNEE

- Recommend Approval of Applicant's Entry Into Training Program
- Recommend Disapproval of Applicant's Entry Into Training Program
- Recommend waiver of Applicant's medical ineligibility for the following reasons. Health cost liability for pre-existing medical conditions will be assumed by the Mission or Bureau. (USAID signature located at bottom of this page)
- Health cost liability for pre-existing medical conditions will be assumed by the responsible party noted below:

REASON FOR REJECTION / WAIVER OF INELIGIBILITY

SIGNATURE	PRINTED NAME	DATE
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REVIEWED BY:

SIGNATURE	PRINTED NAME
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MISSION/BUREAU MEDICAL WAIVER ACTION

Applicants rejected for training because of medical problems may be re-evaluated for training with a waiver of HAC coverage for specified pre-existing condition.

The USAID Mission/Bureau may determine to grant a waiver when:

1. It is felt that the period of training will be of short duration and medical condition is unlikely to be activated or aggravated during that period; or
2. The training is considered essential to the program objective.

By granting this waiver request, the USAID Mission/Bureau accepts full responsibility to ensure payment of all claims arising from waived conditions. This determination by the USAID Director or U.S. officer designee must be obtained prior to further processing of the applicant.

Waived Condition(s):

SIGNATURE	DATE
PRINTED NAME	POSITION TITLE

Section 6g. Appendix VII. OSHA Form for Injury and Illness Report

Version A

OSHA Form 301- Injury and Illness Incident Report

Case # _____
 Recordable Non-recordable
To be completed by EH&S

Information about the Injured person

1) Full name: _____

2) Street _____
 City _____ State _____ Zip _____

3) Injured persons "A" # _____

4) Date of birth _____ Date hired _____

5) Male Female

6) Employee
 Job title _____
 Hrs/day _____ Days/Wk _____
 Student
 Visitor

7) Program area _____ Phone # _____

8) Injured persons Signature _____

9) Supervisor _____ Phone # _____
 Signature _____ Date _____

Information about the Medical Treatment

10) Extent of treatment: None First Aid Medical Treatment

11) If treatment was given away from the worksite, where was it given?
 Dr. Name _____
 Facility _____
 Street _____
 City _____ State WA Zip _____

12) Was the Injured person treated in an emergency room?
 Yes No

13) Was the Injured person hospitalized overnight as an in-patient?
 Yes No

Information about the case

14) Date of injury or illness _____

15) Time of event: _____ AM PM Unknown

16) Time Injured person began work _____ AM PM

17) Dates lost from work: _____ to _____

18) Dates on restricted duty: _____ to _____

Completed by: _____

Title: _____

Phone: _____

Date: _____

Attention: This form contains information relating to Injured persons health and must be used in a manner that protects the confidentiality of the information while being used for occupational safety and health purposes to the extent possible.

Complete this form for all injuries and illnesses. When complete, print form, get necessary signatures, & make two photocopies. Forward the original to the EH&S Coordinator in 1254 LAB11 and forward a photocopy to Business Services L 1125. The affected person keeps the remaining photocopy. This form should be completed within 24 hours of the incident.
www.evergreen.edu/facilities/docs/accidentreport.pdf

19) Did injured person file a Labor & Industries report? Claim # _____
 Yes No

20) If the injured person died, Date of death: _____

21) Location: _____

22) Witness: _____

23) What was the injured person doing just before the incident occurred? Describe the activity, as well as the tools, equipment, or material the injured person was using. Be specific. Examples: "climbing a ladder while carrying roofing materials"; "spraying chlorine from hand sprayer"; "daily computer key-entry."

24) What happened? Tell us how the injury occurred. Examples: "When the ladder slipped on wet floor, worker fell 20 feet"; "Worker was sprayed with chlorine when gasket broke during replacement"; "Worker developed soreness of wrist over time."

25) What was the injury or illness? Tell us the part of the body that was affected and how it was affected; be more specific than "hurt," "pain," or "sore." Examples: "strained back"; "chemical burn, hand"; "carpal tunnel syndrome."

26) What object or substance directly harmed the injured person? Examples: "concrete floor"; "chlorine"; "radial arm saw". If this question does not apply to the incident, leave it blank.

Mark part of body injured on diagram above

Section 6h. Appendix VIII. United States General Services Administration Motor Vehicle Accident Report Form

MOTOR VEHICLE ACCIDENT REPORT	Please read the Privacy Act Statement on Page 3	INSTRUCTIONS: Sections I through IX are filled out by the vehicle operator. Section X, items 72 thru 82c are filled on by the operator's supervisor. Section XI thru XIII are filled out by an accident investigator for bodily injury, fatality, and/or damage exceeding \$500.			
SECTION I - FEDERAL VEHICLE DATA					
1. DRIVER'S NAME (Last, first, middle)		2. DRIVER'S LICENSE NO./STATE/LIMITATIONS		DATE OF ACCIDENT	
4a. DEPARTMENT/FEDERAL AGENCY PERMANENT OFFICE ADDRESS			4b. WORK TELEPHONE NUMBER		
5. TAG OR IDENTIFICATION NUMBER	6. EST. REPAIR COST \$	7. YEAR OF VEHICLE	8. MAKE	9. MODEL	
				10. SEAT BELTS USED <input type="checkbox"/> YES <input type="checkbox"/> NO	
11. DESCRIBE VEHICLE DAMAGE					
SECTION II - OTHER VEHICLE DATA (Use Section VIII if additional space is needed)					
12. DRIVER'S NAME (Last, first, middle)		13. SOCIAL SECURITY NO./ TAX IDENTIFICATION NO.	14. DRIVER'S LICENSE NO./STATE/LIMITATIONS		
15. a. DRIVER'S WORK ADDRESS			15b. WORK TELEPHONE NUMBER		
16a. DRIVER'S HOME ADDRESS			16b. HOME TELEPHONE NUMBER		
17. DESCRIPTION OF VEHICLE DAMAGE			18. ESTIMATED REPAIR COST \$		
19. YEAR OF VEHICLE	20. MAKE OF VEHICLE	21. MODEL OF VEHICLE		22. TAG NUMBER AND STATE	
23a. DRIVER'S INSURANCE COMPANY NAME AND ADDRESS			23b. POLICY NUMBER		
			23c. TELEPHONE NUMBER		
24. VEHICLE IS <input type="checkbox"/> CO-OWNED <input type="checkbox"/> RENTAL <input type="checkbox"/> LEASED <input type="checkbox"/> PRIVATELY OWNED		25a. OWNER'S NAME(S) (Last, first, middle)		25b. TELEPHONE NUMBER	
26. OWNER'S ADDRESS(ES)					
SECTION III - KILLED OR INJURED (Use Section VIII if additional space is needed)					
27. NAME (last, first, middle)		28. SEX	29. DATE OF BIRTH		
30. ADDRESS					
A	31. MARK "X" IN TWO APPROPRIATE BOXES <input type="checkbox"/> KILLED <input type="checkbox"/> DRIVER <input type="checkbox"/> PASSENGER <input type="checkbox"/> FED <input type="checkbox"/> INJURED <input type="checkbox"/> HELPER <input type="checkbox"/> PEDESTRIAN <input type="checkbox"/> OTHER (2)		32. IN WHICH VEHICLE	33. LOCATION IN VEHICLE	34. FIRST AID GIVEN BY
	35. TRANSPORTED BY		36. TRANSPORTED TO		
37. NAME (last, first, middle)		38. SEX	39. DATE OF BIRTH		
40. ADDRESS					
B	41. MARK "X" IN TWO APPROPRIATE BOXES <input type="checkbox"/> KILLED <input type="checkbox"/> DRIVER <input type="checkbox"/> PASSENGER <input type="checkbox"/> FED <input type="checkbox"/> INJURED <input type="checkbox"/> HELPER <input type="checkbox"/> PEDESTRIAN <input type="checkbox"/> OTHER (2)		42. IN WHICH VEHICLE	43. LOCATION IN VEHICLE	44. FIRST AID GIVEN BY
	45. TRANSPORTED BY		46. TRANSPORTED TO		
47. Pedestrian	a. NAME OF STREET OR HIGHWAY		b. DIRECTION OF PEDESTRIAN (SW corner to NW corner, etc.)		
			FROM	TO	
	c. DESCRIBE WHAT PEDESTRIAN WAS DOING AT TIME OF ACCIDENT (crossing intersection with signal, against signal, diagonally; in roadway playing, walking, hitchhiking, etc.)				
NSN 7540-00-634-4041 Previous edition not usable			STANDARD FORM 91 (2/2004) Prescribed by GSA-FMR 102-34.295		

SECTION IV - ACCIDENT TIME AND LOCATION (Use section VII if additional space is needed.)

48. DATE OF ACCIDENT 49. PLACE OF ACCIDENT (Street address, city, state, ZIP Code; Nearest landmark; Distance nearest intersection; Kind of locality (industrial, business, residential, open country, etc.); Road description).

50. TIME OF ACCIDENT
 AM
 PM

51. INDICATE ON THIS DIAGRAM HOW THE ACCIDENT HAPPENED

Use one of these outlines to sketch the scene. Write in street or highway names or numbers.

a. Number Federal vehicle as 1, other vehicle as 2, additional vehicle as 3 and show direction of travel with arrow.

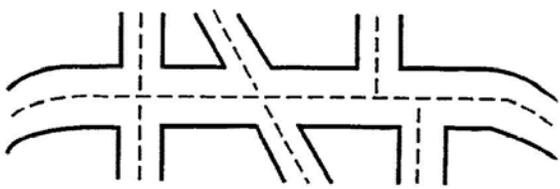
Example: 

b. Use solid line to show path before accident and broken line after the accident.

c. Show pedestrian by 

d. Show railroad by 

e. Place arrow in this circle to indicate NORTH 



52. POINT OF IMPACT (Check one for each vehicle)

FED	2	AREA
		a. Front
		b. R. Front
		c. L. Front
		d. Rear
		e. R. Rear
		f. L. Rear
		g. R. Side
		h. L. Side

53. DESCRIBE WHAT HAPPENED (Refer to vehicles as "Fed", "2", "3", etc. Please include information on posted speed limit, approximate speed of vehicles, road conditions, weather conditions, driver visibility, condition of accident vehicles, traffic controls (warning light, stop signal, etc.), condition of light (daylight, dusk, night, dawn, artificial light, etc.), and driver actions (making a U-turn, passing, stopped in traffic, etc.)

SECTION V - WITNESS/PASSENGER (Witness must fill out SF 94, Statement of Witness) (Continue in Section VIII.)

54. NAME (Last, first, middle)	55. WORK TELEPHONE NUMBER	56. HOME TELEPHONE NUMBER
A 57. WORK ADDRESS		58. HOME ADDRESS
59. NAME (Last, first, middle)	60. WORK TELEPHONE NUMBER	61. HOME TELEPHONE NUMBER
B 62. WORK ADDRESS		63. HOME ADDRESS

SECTION VI - PROPERTY DAMAGE (Use Section VIII if additional space is needed.)

64a. NAME OF OWNER (Last, first, middle)	64b. WORK TELEPHONE NUMBER	64c. HOME TELEPHONE NUMBER
64d. WORK ADDRESS		64e. HOME ADDRESS
65a. NAME OF INSURANCE COMPANY	65b. TELEPHONE NUMBER	65c. POLICY NUMBER
66. ITEM DAMAGED	67. LOCATION OF DAMAGED ITEM	68. ESTIMATED COST

SECTION VII - POLICE INFORMATION

69a. NAME OF POLICE OFFICER	69b. BADGE NUMBER	69c. TELEPHONE NUMBER
70. PRECINCT OR HEADQUARTERS	71a. PERSON CHARGED WITH ACCIDENT	71b. VIOLATION(S)

SECTION VIII - EXTRA DETAILS

SPACE FOR DETAILED ANSWERS. INDICATE SECTION AND ITEM NUMBER FOR EACH ANSWER. IF MORE SPACE IS NEEDED, CONTINUE ITEMS ON PLAIN BOND PAPER.

PRIVACY ACT STATEMENT

The information on this form is subject to the Privacy Act of 1974 (5 U.S.C. section 552a). Authority to collect the information is Title 40 U.S.C. Section 491 and the title 31 U.S.C. Section 7701. The formation is required by federal Government agencies to administer motor vehicle programs, including maintaining records on accidents involving privately owned and Federal fleet vehicles, and collecting accident claims resulting from accidents. Federal employees, and employees under contract, will use the information only in the performance of their official duties. Routine uses of the collected information may include disclosures to: appropriate Federal, State, or local agencies or contractors when relevant to civil, criminal, or regulatory investigations or prosecutions; the Office of personnel Management and the General Accounting Office for program evaluation purposes; a Member of Congress or staff in response to a request for assistance by the individual of record; another Federal agency, including the Department of Treasury and Justice, or a court under judicial proceedings; agency Inspectors General in conducting audits; private insurance and the collection agencies (including agencies under contract to Treasury to collect debt), and to other agency finance offices for federal management and debt collection. Furnishing the requested information is mandatory, including the Social security Number or Taxpayer's Identification Number(TIN) for use as a unique identifier to ensure accurate identification for individuals or firms in the system.

SECTION IX - FEDERAL DRIVER CERTIFICATION

I certify that the information on this form (Sections I thru VII) is correct to the best of my knowledge and belief.

72a. NAME AND TITLE OF DRIVER	72b. DRIVER'S SIGNATURE AND DATE
-------------------------------	----------------------------------

SECTION X - DETAILS OF TRIP DURING WHICH ACCIDENT OCCURRED

73. ORIGIN	74. DESTINATION
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75. EXACT PURPOSE OF TRIP

76. TRIP BEGAN	DATE	TIME (Include AM or PM)	77. ACCIDENT OCCURRED	DATE	TIME (Include AM or PM)
----------------	------	-------------------------	-----------------------	------	-------------------------

78. AUTHORITY FOR THE TRIP WAS GIVEN TO THE OPERATOR <input type="checkbox"/> ORALLY <input type="checkbox"/> IN WRITING (Explain)	79. WAS THERE ANY DEVIATION FROM DIRECT ROUTE? <input type="checkbox"/> NO <input type="checkbox"/> YES (Explain)
---	--

80. WAS THE TRIP MADE WITHIN ESTABLISHED WORKING HOURS? <input type="checkbox"/> YES <input type="checkbox"/> NO (Explain)	81. DID THE OPERATOR, WHILE ENROUTE, ENGAGE IN ANY ACTIVITY OTHER THAN THAT FOR WHICH THE TRIP WAS AUTHORIZED? <input type="checkbox"/> NO <input type="checkbox"/> YES (Explain)
---	--

82. COMPLETED BY DRIVER'S SUPERVISOR	<input type="checkbox"/> YES <input type="checkbox"/> NO	a. DID THIS ACCIDENT OCCUR WITHIN THE EMPLOYEE'S SCOPE OF DUTY b. COMMENTS
--------------------------------------	---	---

83a. NAME AND TITLE OF SUPERVISOR	83b. SUPERVISOR'S SIGNATURE AND DATE	83c. TELEPHONE NUMBER
-----------------------------------	--------------------------------------	-----------------------

SECTION XI - ACCIDENT INVESTIGATION DATA

84. DID THE INVESTIGATION DISCLOSE CONFLICTING INFORMATION. NO YES (If checked, explain below.)

85. PERSONS INTERVIEWED

NAME	DATE	NAME	DATE
a.		c.	
b.		d.	

86. ADDITIONAL COMMENTS (Indicate section and item number of each comment).

SECTION XII - ATTACHMENTS

87. LIST ALL ATTACHMENTS TO THIS REPORT

SECTION XIII - COMMENTS/APPROVALS

88. REVIEWING OFFICIAL'S COMMENTS

89. ACCIDENT INVESTIGATOR

a. SIGNATURE		b. DATE
c. NAME (First, middle, last)		
d. TITLE		
e. OFFICE		
f. OFFICE TELEPHONE NUMBER		
AREA CODE	NUMBER	EXTENSION

90. ACCIDENT REVIEWING OFFICIAL

a. SIGNATURE		b. DATE
c. NAME (First, middle, last)		
d. TITLE		
e. OFFICE		
f. OFFICE TELEPHONE NUMBER		
AREA CODE	NUMBER	EXTENSION

Implementing Cold Chain for Safe Sample Transport and Storage

Prepared by
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and the PREDICT One Health Consortium
Last updated: 29 November 2016

Objective: To provide principles and general considerations for cold chain maintenance, the safe transport and storage of samples collected during PREDICT surveillance activities, and the safety of personnel.

Section 1. Introduction to Cold Chain

This guide focuses on implementing an efficient cold chain and sample transport/storage plan appropriate for PREDICT disease surveillance activities. The guidance provided is to ensure that all PREDICT materials arrive at their end laboratories in suitable condition for PREDICT diagnostics and pathogen testing. When you are familiar with the information in this Guide, take the PREDICT quiz on ***Implementing a Cold Chain for Safe Sample Transport***.

A cold chain is a monitored temperature-controlled supply chain. The goal of the cold chain is to keep a sample or material within a certain temperature range during all stages of delivery, processing and storage (Figure 1). Cold chains are widely used to ensure the viability of products in the pharmaceutical and agricultural sectors, and are critical components of vaccination programs and bio-medical surveillance activities.

Many biological samples deteriorate when exposed to heat, sunlight, or fluorescent light. When transporting and storing such biological substances, it is imperative that field and laboratory teams control environmental conditions, ensuring that exposure to potentially damaging environmental factors is minimized.

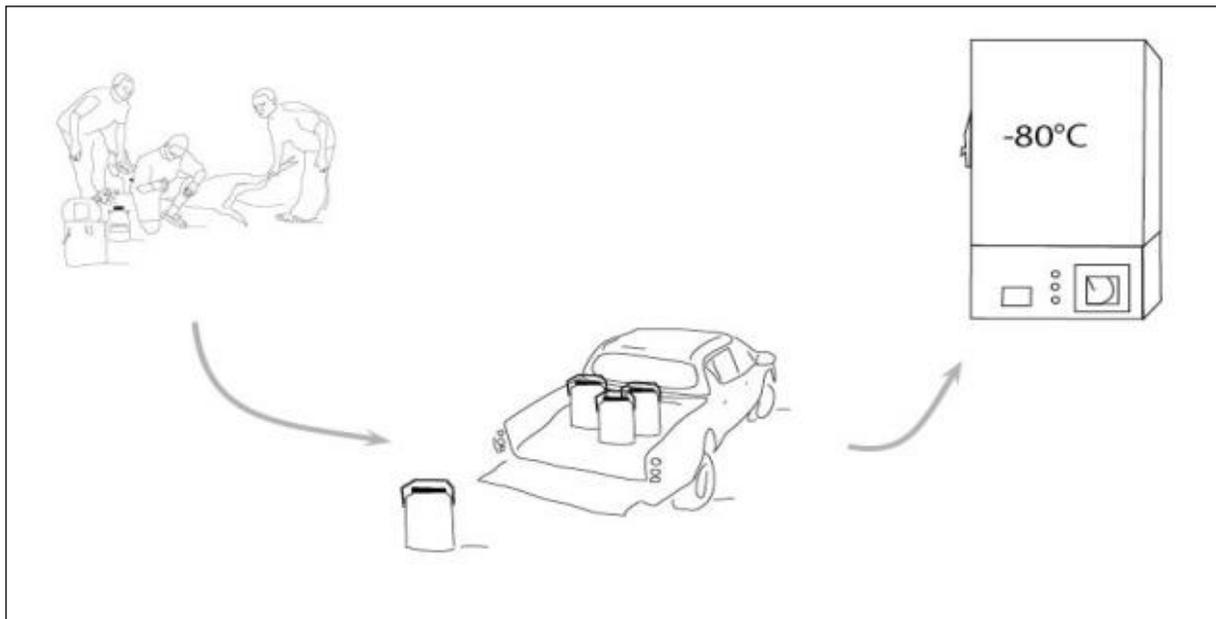


Figure 1: Illustration of a typical cold chain from field to lab storage for PREDICT biological samples. Field teams sample an animal and place specimens in liquid nitrogen dewar for storage. The dewar with specimens is transported in the back of a project vehicle to long-term storage at a PREDICT laboratory or field station, inventoried, and archived until testing in an ultra-low temperature freezer ($<-80^{\circ}\text{C}$).

Freezing is the simplest way to ensure that the biological samples remain viable for laboratory analysis. The cold chain for PREDICT samples can be maintained through the use of ice packs, coolers and dry ice (for a very brief period immediately following collection), liquid nitrogen (LN2) containers and freezers, and the use of ultra-low temperature (-80°C and colder) freezers. It is recommended that PREDICT samples be placed in LN2 or ultra-low temperature freezers as soon as possible to optimize sample viability for diagnostics and pathogen testing.

Repeated exposure to heat leads to a cumulative and irreversible loss of sample viability and may render a sample useless for laboratory analysis.

PREDICT Sample Cold Chain Requirements: All biological samples from PREDICT surveillance activities should be stored and transported at temperatures colder than -80°C suitable for the preservation of targeted PREDICT pathogens and viral detection.

Section 2. Implementing the Cold Chain

This section introduces recommended steps for cold chain planning and implementation.

Section 2a. Planning

The first step in implementing the cold chain is planning. Your team must identify the cold storage needs for your sampling activities, then identify and procure all necessary materials and resources. In addition, it is critical to train your team to understand the logistics of the cold chain, how to monitor cold chain temperature, and how to maintain system records.

Considerations for Cold Chain Planning:

1. What is your surveillance plan and what type of cold chain is appropriate for that plan? What types of samples are you collecting? What are the temperature requirements for safely storing these samples?
2. Assess local context and conditions. Do you have access to long-term sample storage facilities? Are your sampling activities located in remote rural locations several days or weeks from the project infrastructure or laboratory?
3. Determine where the cold chain ends. If your field team delivers samples to a laboratory with an ultra-low temperature freezer, then initiating your cold chain may require simply extending it from laboratory to sampling site through the use of LN2 dry-shippers or dewars. If you are developing a cold chain without any pre-existing infrastructure, mapping out an appropriate cold chain from sample collection to endpoint is essential (Figure 2).
4. Determine the maximum amount of time samples will be located outside of long-term cold storage. If your field activities are 5 days away from long-term storage, then you will need a minimum of 5 days mobile cold storage in LN2. If you plan to export samples, how long will it take to ship from origin to destination?

5. Determine the minimum amount of time samples will stay in long-term storage. Planning for long-term storage requires assessing the space necessities of your cold chain. Are you maintaining a sample bank or archive? If so, you will need to plan for sufficient storage space for the life of the project to preserve sample viability.
6. Establish procedures for monitoring the cold chain and tracking the samples moving through the cold chain. Confirm all team members have been trained in cold chain maintenance and record keeping. Prepare forms for data logging and recording. Prepare a schedule for re-filling LN2 containers and contingency plans for equipment failure.

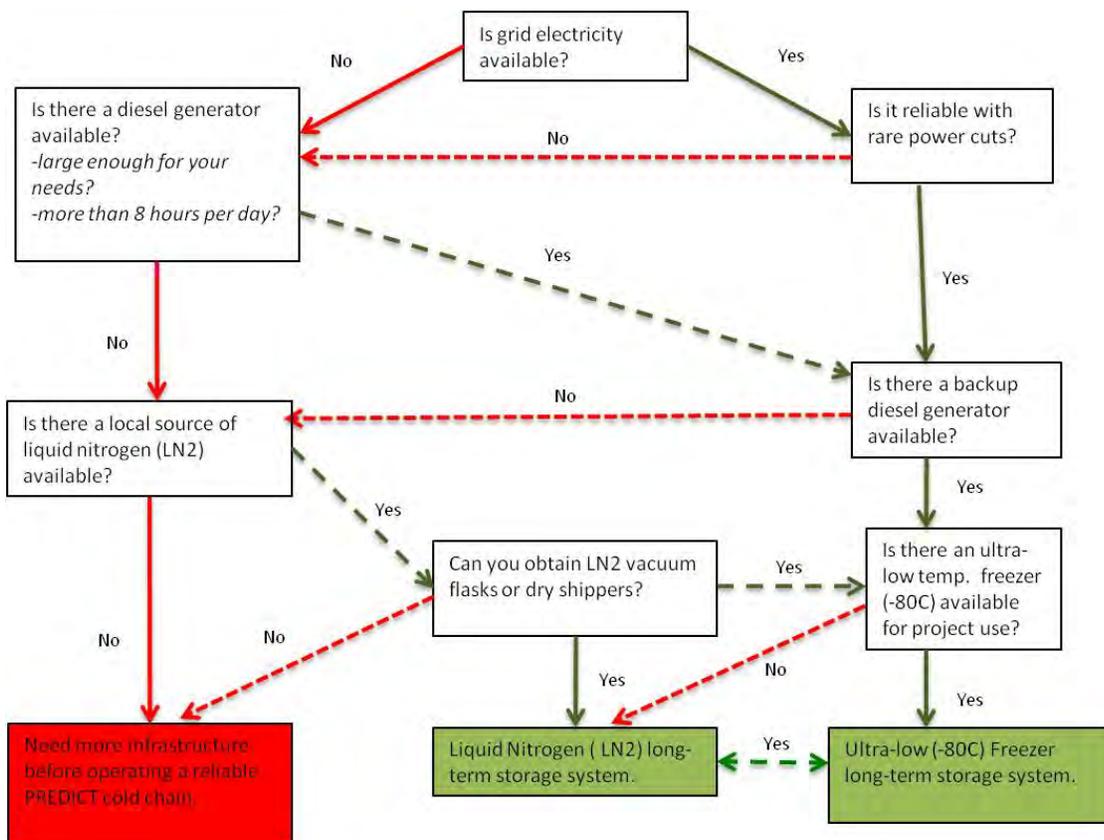


Figure 2: A decision tree for cold chain planning. Based on United Nations World Food Program Logistics Cluster “Logistics Operational Guide”.

Developing a Cold Chain System

To develop and maintain a cold chain, a series of simple and routine processes must be established. These processes should be designed to function efficiently in each team’s environmental and local conditions, and should be easy to maintain with available materials and resources.

1. Assess the opportunities and constraints to developing a cold chain in your area. These may include:
 - a. Access to a pre-existing cold chain

- b. Access to LN2, and LN2 transport and storage supplies
 - c. Access to an ultra-low temperature (sub -80°C) freezer available for use
 - i. If freezer available, does it have a backup generator and alarm system?
2. Identify the appropriate materials and resources needed to implement and maintain the cold chain. Required materials and resources may include:
 - a. Personal Protective Equipment (PPE) for working with LN2 and -80°C freezers
 - b. Coolers
 - c. Ice/gel freezer packs
 - d. Liquid Nitrogen (LN2) dewars and/or LN2 vapor-phase dry shippers (see distinctions below in Table 2)
 - e. Source of LN2
 - f. Large capacity LN2 storage dewars or ultra-low temperature (<-80°C) freezers for longer-term sample storage
 - g. Temperature gauges, thermometers, data loggers (as needed), alarm systems, and an alert network for staff when facilities are unoccupied
 - h. Appropriate sample storage containers and racks for sample organization
 3. Identify local suppliers or other sources for procurement of materials and resources. (Note: Carefully assess the reliability/sustainability, and costs of any suppliers to assure procurement of reliable supplies and ability to service equipment.)
 4. Establish a written protocol for monitoring the cold chain and stored samples. The protocol should cover:
 - a. Temperature regulation and record
 - b. Sample storage and tracking system
 - c. Equipment maintenance schedules
 - d. Response procedures in event of container/freezer failure or power outage
 - e. Training programs to ensure continued and safe operation of cold chain system
 - f. Annual review of cold chain operation and sample storage procedures

Cold Chain Materials and Resources

A cold chain can consist of any combination of materials and resources that serve to maintain samples at a desired temperature. **For all PREDICT samples, that temperature is -80°C or lower.** This temperature range requires the use of specialized cooling technologies and specially designed freezers. Gas-based coolants (LN2) do not require electricity, and can be deployed to remote and rural areas. In contrast, ultra-low temperature (< -80°C) commercial freezers are dependent upon an electrical grid and emergency generators in the event of blackouts or grid failure.

Safety Considerations for Coolants

Working with cold chain coolants can be dangerous if appropriate precautions are not taken. The recommended PREDICT cold chain requires samples to be stored in temperatures well below freezing. Exposure to these temperatures can cause severe burns and damage to living tissue. There are three coolants commonly used in implementing a cold chain: 1) ice/gel packs, 2) dry ice, and 3) liquid nitrogen (LN2). Dry ice and LN2 give off gases that can cause asphyxiation

and should only be handled by trained personnel in ventilated areas. In addition, dry ice and LN2 containers must be able to vent evaporated gas to avoid the risk of explosion. Characteristics and safety considerations for working with cold chain coolants are listed in Table 1. For more information on human safety when working with PREDICT field and laboratory activities, please review the *PREDICT guide Biosafety and PPE Use*.

Table 1: Characteristics and safety considerations for PREDICT cold chain coolants.

Coolant	Characteristics	Use and Maintenance	Safety Considerations
Ice Packs	Ice packs are water filled packs that obtain the temperature of a standard freezer (approx. -18°C). Ice packs DO NOT achieve temperatures sufficient for the preservation of PREDICT biological samples.	Ice packs must be kept in a freezer for 12-24 hours to achieve maximum coldness. Keep at a temperature colder than the freezing point of the ice pack, to ensure longer cold life.	None (water-based product). Do not chill ice packs used for samples in refrigerators or freezers used for food and beverages.
Gel Packs	Gel packs consist of a liquid blend of chemicals that depress the melting point of a cold pack allowing the gel pack to remain colder than 0°C for longer time intervals than an ice pack. Gel packs DO NOT achieve temperatures sufficient for the preservation of PREDICT biological samples.	Before purchase, request documentation from the manufacturer to validate manufacturer claims on the product's cold life, and to obtain instructions on appropriate use of the product, including packaging a cooler with biological samples and the gel packs. Gel packs take at least 24 hours to reach their lowest temperature and can take even longer if chilled in a domestic refrigerator.	Though most gel packs are non-toxic, be careful to not ingest gel from ruptured gel packs. Consult manufacturer guidelines for product use on safety.
Dry Ice	Dry ice is the solid form of carbon dioxide (CO ₂), and is approximately -78.5°C. In ambient conditions, dry ice is unstable and evaporates quickly. Therefore, samples packed in dry ice should be transferred to a <-80° container within 24 hours. Dry ice is recommended as a SHORT-TERM COOLANT ONLY , to be used for transporting samples from the field to more reliable temperature controlled storage containers.	Dry ice is easily manufactured, often as a byproduct of other processes, and is widely used in the food industry for preservation. Dry ice can frequently be sourced from breweries, importers of frozen products like ice cream, and meat processing facilities. Any specimens transported on dry ice must be placed in specially insulated containers capable of venting gaseous CO ₂ . Note: sealing seams of containers like Styrofoam cold boxes prevents ventilation of the gas and can lead to unsafe pressure build-up.	Wear insulated gloves. Always work in well-ventilated areas. Always transport dry ice in containers approved for transport, ensuring that the CO ₂ can diffuse minimizing pressure build-up.

Liquid Nitrogen (LN2)	LN2 is a readily transportable and highly effective compound used for the cryopreservation of blood, reproductive cells, and other biological samples and materials. LN2 is produced through the distillation of liquid air, and is stored and transported in vacuum flasks insulated from ambient heat.	LN2 can often be locally obtained through international airports (urban areas), and services that work with artificial insemination (beef/dairy industry located primarily in rural areas). LN2 boils at -196°C, and can cause rapid freezing on contact with living tissue, and severe damage to materials if split.	Wear insulated gloves, a thermal apron and a face shield. Always work in well-ventilated areas. LN2 tanks feature pressure relief devices, which if not routinely checked and properly maintained can fail resulting in tank explosion and considerable damage. Consult the manufacturer's recommendations for tank maintenance to ensure compliance. Transporting LN2 tanks or dewars inside project vehicles can be dangerous: there is a risk of rupture or tank failure, and the tanks can potentially explode. When possible, transport LN2 in dry shippers or vacuum flasks approved for transport. If using LN2 tanks or dewars, be sure to secure these containers on the exterior of the vehicle to maximize safety in transport. LN2 tanks should only be placed in an upright position.
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Containers for Cold Chain Transport and Shipping

There are two main types of LN2 containers: dry shippers (vapor shippers) and vacuum flasks (dewar flasks). The insulating capacity of LN2 containers varies considerably from a few hours to weeks, requiring constant vigilance for signs of leakage, and routine assessment of container temperature.

Dry shippers (vapor shippers)

Dry shippers are large vacuum containers that contain an absorbent material to hold LN2. A properly prepared dry shipper does not contain any free LN2, and can safely store samples at the optimal temperature range for a period of 24 hours to several weeks depending on the type. Dry shippers are highly recommended for sample storage when samples need to be transported or shipped (bicycle, car, airplane, etc.). Because of their transport utility, dry shippers are often smaller and more compact, and well suited to more short-term storage applications.

Vacuum (dewar) flasks

Vacuum flasks are non-pressurized LN2 containers lacking absorbent material, in which biological samples or specimens are suspended in LN2 within the container. Vacuum flasks should not be used to transport or ship biological specimens. Rather, vacuum flasks are suited for longer-term storage application (storage time dependent on size of the flask – consult the manufacturers guidelines) in laboratories, field offices, or other locations where samples are expected to reside for longer period of time. Vacuum flasks come in a range of sizes from small to very large capacity containers.

Recommended steps for using dry shippers/vacuum flasks:

- Always consult and follow the manufacturer's instructions for filling, as procedures for each type of container can vary.
- Always wear a face shield and insulated gloves made for handling liquid nitrogen.
- Always work in well-ventilated areas, as a significant amount of nitrogen gas will be generated as the cold liquid contacts the warm surfaces inside the shipper.

Refrigerators and Freezers

Domestic (e.g., household/home) refrigerators and freezers are designed and built for food and drink storage; they do not meet the requirements for sample storage, and do not reach the temperature levels needed for preservation of PREDICT biological material (e.g., specimens for viral screening). **DO NOT STORE SAMPLES IN REFRIGERATORS OR FREEZERS THAT CONTAIN FOOD OR BEVERAGES FOR CONSUMPTION.** In addition, temperature in domestic refrigerators varies significantly with door opening, defrosting, and variable ambient temperatures; they should not be used in a cold-chain for storage of PREDICT samples. Additionally, freezers designated as "frost free" should not be used for sample storage; because the temperature cycling mechanisms they utilize to avoid ice accumulation can damage samples.

Only specially designed ultra-low temperature (< -80°C) commercial freezers are recommended for use with samples when viral isolation is an objective.

Ultra-low temperature (< -80°C) commercial freezers

Commercial freezers come in a variety of temperature settings (-20, -40, -50, -85, and cryogenic freezers at -150°C), and in a variety of configurations (upright, chest, and bench top freezers). It is important to be sure any commercial freezers utilized for biological sample and specimen storage are able to consistently maintain a sub 80°C environment.

Operating a commercial freezer requires a constant source of electricity to maintain temperatures colder than -80°C temperatures and ensure the viability of the cold chain. In many places where PREDICT projects are being conducted, electricity is intermittent and blackouts are common. **It is imperative that the electrical source for a commercial freezer be supported by a back-up generator to ensure continued power for the freezer and viability of the samples.** It is equally imperative that each team has a contingency plan for power outages, to ensure that the back-up generator is functioning and that the freezer remains operational. Teams should clearly mark the power source to the freezer to prevent accidental disconnection, which can cause heat damage if unnoticed over long periods of time. The power source can also be protected by placing a sticker above the power plug or switch, or by installing a lockable switch. Additional steps on maintaining the cold chain during blackouts are included in Section 3 below.

The location of the freezer in the laboratory or field office impacts performance. Avoid placing a freezer in direct sunlight or near heat sources (hot water or a warm external wall), because that

makes the freezer work harder to maintain cool temperatures. In addition, -80°C freezers often require a certain amount of airspace in their immediate surroundings for ventilation and to function efficiently; -80°C freezers should not be located in close proximity to other freezers, equipment, counters, etc. When possible, leave at least 1 meter of space between the -80°C freezer and other freezers or equipment.

Temperature Gauging Equipment

Continual temperature monitoring of the cold chain assures that all samples remain in an optimal environment for preservation. There are a number of methods to monitor cold chain temperatures, from simple thermometers to more complex temperature gauges, cold chain monitors, and data loggers. When combined with an appropriate record keeping system, temperature monitoring provides an ideal method to evaluate the viability of the cold chain and to respond accordingly to any interruptions.

Table 2: Temperature gauging equipment used in the cold chain.

Type	Description	Guidelines for use
Thermometers	Minimum/maximum thermometers are essential equipment for temperature monitoring, and come in two main types: dial and digital.	All thermometers used for temperature monitoring should be set to Celsius, must be reset on a daily basis, and require annual checks to ensure accuracy, as battery failure or damages temperature probes can impact readings. In addition, a temperature-monitoring chart should be maintained to provide a record of variation in temperature that may indicate problems with the freezer or thermometer.
Temperature Chart Recording Systems	Temperature Chart Recording Systems are automated systems that record temperature and provide visual or audio alarms at signs of malfunction.	These systems are fully automated and provide digital output of temperature variations over time. These are typically after-market modifications to freezers, and if installed, should be verified to function with the freezer manufacturer as they may void product warranty.
Data loggers	Data loggers are used to record temperature patterns over time by recording temperature data electronically, and providing an electronic and downloadable record.	Data loggers are not a replacement for manual monitoring, and daily minimum and maximum temperatures should still be recorded to ensure the maintenance of the cold chain. When used for routine temperature monitoring, a data logger must be equipped with a visual min/max temperature display to allow for daily real-time recordings.

Cold chain monitors	Cold chain monitors generally consist of dual-time temperature indicators (WarmMark™ and MonitorMark™) and function by displaying changes in temperature through color change on an indicator strip. Other types of cold chain monitors include freeze indicators (Freeze Watch™, ColdMark™) consisting of color bulbs that release a dye at a threshold temperature. There are also combined indicators featuring dual time-temperature indicators and freeze indicators.	Cold chain monitor color change allows for an estimation of the amount of time a temperature exceeds a pre-determined threshold. No color change means the cold chain was not interrupted and temperature remained safe for sample transport. Note: these monitors are often for temperatures warmer than -80°C (e.g. cold boxes/coolers, refrigerators, -20°C freezers) and are often not designed for samples kept at or colder than -80°C.
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Management of Cold Chain Equipment.

Procuring the needed equipment is only one aspect of keeping a functional cold chain.

Equipment management and maintenance is equally important, and requires:

- Maintaining an equipment inventory
- Planning and budgeting for equipment operation (e.g., electricity), maintenance, and repair
- Planning and budgeting for equipment replacement
- Emergency response or contingency planning in the event of cold chain breach or equipment failure

Equipment Inventory

An inventory should be developed to track all equipment, tools, and parts that are used as part of the cold chain. A good inventory will allow team members to track the location of all materials used in the cold chain, schedule maintenance and repair, arrange for replacement and evaluate the project supplies. Table 3 includes some information recommended for a sample storage equipment inventory.

Table 3: Sample storage equipment inventory database example.

Item	Specifications (brand, model, SN, date of acquisition)	Current Location	Current Condition (Working, Under repair, Out of commission)	Date of purchase – warranty number	Estimated Replacement Date	Notes
Ultra-low freezer	TS Revco ElitePlus, S/N 007054568, Oct. 2010	Morogoro	Working	September 2010	October 2013 (MFR warranty expiration)	Recently purchased and installed
Dryshipper	MVE Cryomoover, S/N 9989900745, Oct. 2010	Serengeti, PREDICT Mobile team	Working	September 2010	October 2013 (MFR warranty expiration)	Field sampling with TAWIRI team

LN2 Generator	StirLITE, S/N 356777456, Oct. 2010	Iringa, PREDICT office	Out of Commission	September 2010	October 2013 (MFR warranty expiration)	Installation issues: working with support services to resolve; sourcing LN2 from supplier in Dar es Salaam currently.
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Equipment Operation, Maintenance, and Repair

All equipment requires maintenance to protect against failure and degradation. Maintenance planning involves identifying procedures and plans to keep equipment functioning properly, as well as planning for emergency repair in the event of equipment failure. Some equipment requires routine maintenance (daily, weekly, or monthly), while others may require maintenance following use (dry shippers, vacuum flasks, cold boxes, etc.). Maintenance instructions are usually included with the equipment, and can often be obtained from the manufacturer. It is important that team members receive training in routine maintenance and repair within reason, while skilled technicians should be identified for complex maintenance and repair procedures.

In addition, it is important to estimate the costs of installing, operating, and maintaining the equipment. Ultra-low temperature freezers utilize significant quantities of electricity, though newer models are designed to minimize power consumption. It is possible that the installation of new equipment will drastically increase power consumption requiring a re-budget of operational costs.

You may use the following equation to estimate the cost of your electrical equipment using the manufactures specifications to obtain the value for kilowatt hours (kWh).

$$[\text{kWh} / 24 \text{ h}] \times [\text{kWh costs in your location}] \times [365 \text{ days}] = \text{Operational Cost} / \text{Year}$$

Maintenance of equipment over time will also require a budget, and should be included in operational cost planning.

Equipment Replacement

Equipment will eventually wear out, and if plans are not in place to address equipment failure, a significant cold chain breach may occur (See [Section 3.](#)). It is important that teams understand the lifecycle of all cold chain materials and equipment, and that plans are in place to address equipment failure when it occurs. Most manufacturers provide estimates of equipment life expectancy. When developing the equipment inventory, estimated replacement dates should be included in documentation to assist in replacement planning. As equipment can often take months for order and delivery, temporary cold chain storage plans should be considered to ensure no breach or interruption.

Emergency Planning

Cold chains are fragile, material dependent, and subject to interruption through breakdowns of background infrastructure (electricity failure) and equipment failure (leakages of cold storage containers or freezer malfunction). Team members must set up emergency planning for identifying equipment failure early, along with arrangements for maintaining the cold chain during repairs or replacement. Equipment outages caused by shortages of spare parts or materials should not occur.

Power surges and “brown-outs” are often frequent occurrences in areas where PREDICT teams are active. A brown-out is a drop in voltage in an electrical power supply, most commonly observed by the dimming of lights. Black outs are covered below in the [Section 3](#). To prevent adverse impacts to cold chain equipment during power surges, it is imperative to have stand-by generators, back-up power sources, and other mechanisms in place (surge protectors, CO2 backup systems, etc.). Often electrical equipment is sensitive to undercurrent (for example a 220V system running at 205V temporarily), and equipment failure and destruction is possible.

Section 2b. Recommended Temperature Requirements for Sample Transport and Storage

An essential component of cold chain planning is knowing the optimal temperature requirements for different diagnostic methods, sample types and storage media.

For PREDICT purposes all samples (stored in VTM and Trizol) must be frozen in liquid nitrogen immediately in the field and transferred to a -80°C freezer once back in the lab. If the location of the field site allows, you may use short term (maximum 48 hrs.) refrigeration (i.e., ice/gel packs) prior to transfer to -80°C freezer or LN2 dewar.

ONLY if there is no **short term** access (i.e., within 24 hours) to cold chain such as in an emergency situation samples can be collected in 200 µL of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows: 1 day at 37°C (i.e., room temperature), 1 week in the refrigerator, and transfer to -80°C for long term storage as soon as possible and within 1 week until analysis.

Do not collect samples onto dried blot spot cards.

Section 2c. Cold Chain Initiation at the Sampling Sites

Following collection in the field, samples must be immediately introduced to the optimum temperature range. When possible, collected samples should be initially stored in cryotubes allowing for immediate introduction to the cold chain and minimizing any freeze/thaw issues involved in sample transfer at a later time.

Table 4 provides an overview of temperature ranges used in PREDICT activities, along with procedures for optimizing these ranges for short-term storage. This table is followed by recommendations on the use of referenced equipment.

Table 4: Maintenance of transit temperature by optimum temperature range.

4°C	-70°C	-80°C or colder
Commercial Refrigerator or “on-ice”	Dry ice	LN2
Time interval: 1-2 days (chilled). Limit to a minimum	Time interval: 1-2 days (frozen).	Time Interval: Indefinite (as long as LN2 quantities are maintained)
*Procedure: The sample transport container (cold box or cooler) should be fitted with as many ice/gel packs as possible. Temperature should not exceed 4°C. If available, a cold chain monitor should also be inserted.	*Procedure: Place a minimum 1 kg of dry ice per 1 kg of samples (but double or triple dry ice amount if possible) for every 24 hours in transit. Place in a sturdy Styrofoam container, allowing for release of carbon dioxide gas to prevent explosion. Use solid dry ice cubes when possible as their duration greatly exceeds that of chips or snow.	*Procedure: Place samples into special cryotubes with screw-down lids (no snap-tops). Cryotubes are then inserted into a LN2 “charged” dry shipper or vacuum flask.

**Maintain at least 4 frozen gel packs and an additional transport container as a contingency plan in case of package or container failure with dry ice or LN2.*

Using temporary cold boxes or coolers

Insulated cold boxes or coolers may be used for sample transport of less than 48 hours duration for all samples requiring storage at -80° C or if no LN2/dry ice supplies are available, or during equipment failure or emergency maintenance periods.



Figure 3: The PREDICT Tanzania team packs blood specimens on ice in a cooler after sampling rodents. Other specimens from field collection were stored in LN2 consistent with sample storage guidelines (Table 6). Photo by Liz Vanwormer.

Recommended steps when using cold boxes or coolers:

- Samples must be protected from heat, sunlight and fluorescent light at all times.
- Check the temperature in the cold box using a mercury or digital thermometer every 3 hours. Note: repeated opening and closing of the cold box will cause temperatures inside the box to elevate more rapidly. Teams must use good judgment when deciding to monitor the cold box temperatures.
- Rotate ice/gel packs to maintain maximum coldness within the container. If possible have extra ice gels to replace thawing or thawed ones.
- Do not transport samples in the trunks of vehicles (or the floors of some vehicles) due to the risk of exposure to temperature extremes. Be familiar with the coolest part of the vehicles.
- Do not remove samples from cold box or cooler until ready to transfer to recommended vacuum flask, dry shipper, or commercial freezer.
- When transferring samples, do not leave them out on the counter or the floor subjected to room temperature and light.
- Keep records of amount of time samples were stored at temperatures warmer than -80°C, and record the date and time when samples were introduced to the -80°C cold chain.

Using containers with dry ice

Dry ice (-78.5°C) is colder than ice and gel packs and allows for maintenance of samples frozen in transit. Any specimens transported in dry ice must be placed in specially insulated containers capable of venting gaseous CO₂.

Recommended steps when using dry ice:

- Pack samples in a good insulated container. Thick polystyrene/styrofoam boxes work well with dry ice as they allow for the necessary off gassing of CO₂ (release of CO₂ gas) and are durable enough to last through transport.
- Sufficient dry ice is needed for maintaining samples consistently frozen. If dry ice quantities are insufficient samples will thaw and rendered useless.
- Use a minimum 1 kg of dry ice for each 1 kg of samples for every 24-hour transit period. Keep in mind however that depending on the quality of your shipping container and environmental conditions you will need to adjust these quantities to ensure constant temperatures. In hot conditions and whenever possible use double or triple the recommended dry ice quantity (i.e., 2 or 3 kg dry ice per kg of samples). For longer than 24 storage/transit times, double the amount of dry ice.
- When packaging items, place dry ice and sample containers as close together as possible and cover with additional dry ice. Fill any empty space with newspaper (ideal) or cloth, bubble packs, or Styrofoam peanuts. Empty space allows the dry ice to sublime (change from liquid to gas) more quickly.
- Dry ice blocks take longer to evaporate and are better at maintaining samples frozen for longer storage/transit periods. However, samples must be close to dry ice (or surrounded by it) for adequate preservation. Solid blocks of 2-3 kg are ideal, yet not always available. Avoid using “snow” or chip dry ice whenever possible as they evaporate very quickly.

Using dry shipper or vacuum flask storage (LN2)

Dry shippers and vacuum flasks when properly charged provide ideal low temperatures for preservation of PREDICT samples both in the short-term following sample collection, in transport, and in the long-term as samples await analysis and/or shipping for diagnostics.

Recommended steps for filling dry shippers/vacuum flasks for sample transport:

1. Use appropriate PPE!
2. Add the LN2 slowly into the container.
3. Stop filling the container when the liquid reaches the neck of the dry shipper. (**DO NOT OVERFILL**)
4. Then, attach the cap and set the container aside to saturate the absorbent for the period specified by the manufacturer. This is called “charging” the container.
5. Repeat the steps above until the liquid level no longer drops on standing (e.g. the container is “charged”). Some manufacturers provide empty and full weights for their containers. If the dry shipper will not reach the expected full weight specified by the manufacturer, there may be a problem with the absorbent’s ability to hold the LN2, and could indicate the container is compromised, and that samples transported or stored in the container may be at risk of degradation. In this case, contact the manufacturer or supplier of the equipment to assess whether the container is fit for use with biological samples.
6. Remove all free liquid nitrogen from the container prior to transport.
7. Empty the container by pouring the excess liquid nitrogen back into a large LN2 vacuum flask.
8. If the LN2 cannot be poured back into the flask, pour the LN2 into an appropriate area.
9. Do not pour LN2 onto the floor or onto hard surfaces. LN2 can crack and destroy concrete and other hard surfaces, and the liquid could splash onto your shoes or legs and cause severe burns.
10. Ensure that any area where LN2 is poured away is well ventilated. Remember that handling or spilling LN2 in a small, confined space has been known to cause fatalities via asphyxiation /displacement of oxygen. Appropriate safety precautions outlined in the Protocol above must be considered.
11. After pouring out excess LN2, hold the dry shipper or vacuum flask upside down to be sure that all liquid has stopped flowing.
12. Stand the dry shipper upright for the period specified by the manufacturer.
13. Repeat the LN2 removal steps as many times as necessary to make sure there is no excess LN2 in the container.
14. Put the samples into the dry shipper/container and replace the cap.
15. Record the date, time, and ID of the samples for when they were placed into the container to initiate the cold chain data log.
16. Ready the dry shipper/container for transport by securing the container in the vehicle. If using a protective bin for the container, then secure the container in the bin first, before securing the bin in the vehicle.

Recommended steps for using dry shippers or vacuum flasks for sample storage:

- Make sure containers are fully charged prior to deployment in the field or removal from dry ice/LN2 source (See steps on filling shippers/flasks above).
- Make sure containers are not leaking.
- Make sure to have sufficient quantities of LN2 on hand for sample storage and emergencies.
- Develop a plan for obtaining additional dry ice/LN2 supplies in the event of emergency or container failure.
- When in the field, always keep additional cold boxes with conditioned (e.g., properly prepared) ice/gel packs as back up in event of container failure.
- Following sample collection, organize samples in the containers according to animal or sample ID consistent with PREDICT sample tracking recommendations for rapid retrieval.
- Remove samples from containers only when ready to prepare for analysis or shipping.
- Record the length-of-time samples were kept in containers and document the number of times and duration containers were opened.



Figure 1: The PREDICT Tanzania team packs up equipment after collecting specimens from rodents. The mushroom shaped container in the background is a specially designed transport container for LN2 dryshippers, ensuring the dryshipper container is well protected during overland or air travel, and that all stored specimens are well within the temperature range required for viral isolation. Photo by Liz Vanwormer.

Section 2d. Sample Transport

Following sample collection, it is imperative that the field teams coordinate with the receiving laboratories or PREDICT Country Coordinators on all details involving sample transport and storage planning. In many cases, samples will be delivered from the field/collection site to a temporary storage facility prior to shipment to end-use processing laboratories, and may involve multiple phases of the cold chain. In the event of international transport of samples to a processing laboratory, all PREDICT personnel must follow the guidelines specified in *guide Packing and Shipping Biological Samples*.

All sample transport containers must be secured (e.g., tied down) in the transport vehicle. If possible, LN2 dryshippers should be secured in a separate compartment space from the passengers (e.g., rooftop bin or a covered canopy of a flatbed truck), and equipped with a spill kit containing absorbent materials to protect personnel from any accidents involving spillage. Non-LN2 containers with unprocessed samples may be secured in the project vehicle with proper secondary containment to minimize sample jostling during transport. There is a risk that containers may leak during transport, so it is imperative that teams understand the risk of asphyxiation in a closed vehicle and be prepared to address any spills and leakages with appropriate equipment. **PREDICT vehicles should be equipped with cold chain PPE (e.g., disinfectant, heavy reusable gloves, disposable gloves, mask, apron, goggles, and a sealable and leak proof disposal container) to respond to any incidents involving sample spillage.** To ensure maintenance of the cold chain, additional ice/gel packs, dry ice and appropriate containers, or an additional LN2 dry shipper should be available to prepare for travel delays or primary container failure.

Section 2e. Safe Storage of Samples

Upon delivery of samples from the field, it is the responsibility of the receiving party to ensure that cold chain is continued and samples are appropriately stored, documentation transferred (See [Section 3](#). Records below), and Country Coordinator or other supervisor notified. **For PREDICT purposes ALL SAMPLES must be stored frozen at -80°C or lower temperatures.**

Additional Sample Storage Guidelines

- Samples should be divided or aliquoted into the smallest useful units during initial processing in order to avoid excessive freeze-thaw cycles, and to avoid damage leading to a loss of infectivity.
- When samples are removed from cold storage and shipped to a laboratory facility for analysis, teams should follow the PREDICT training guidelines on *Packing and Shipping Biological Samples*.

Long-term Sample Storage

It is strongly recommended that all samples kept for long-term storage be maintained at temperatures at or below -80°C. This can be achieved either through the use of large capacity LN2 dewars or through ultra-low temperature freezers.

Using Liquid Nitrogen

There are generally two types of sample storage systems available for LN2 dewars: box/rack (or canister systems) and cane/straw systems. While cane/straw systems are acceptable for short-term storage, it is highly recommended that samples for long-term storage be kept in box/rack systems, which allow for quick retrieval and identification with minimal temperature reduction upon retrieval. Cane/straw systems have less storage capacity and often increase the amount of time required to locate samples for pathogen testing.

Recommended steps for using LN2 in long-term sample storage:

- Make sure containers are filled to capacity, functioning properly, and are not leaking.
- Develop a plan for obtaining additional LN2 supplies in the event of emergency or container failure.
- Maintain a supply of ice/gel packs to maintain temperature in the container in the event of container failure, or for use in emergency storage or transport.
- Organize samples in box/rack systems according to animal or specimen ID consistent with PREDICT sample tracking recommendations for rapid retrieval.
- Remove samples from containers only when ready for testing or shipping.
- Record the length-of-time samples were kept in containers and document the number of times and duration containers were opened.

Using Ultra-low Temperature Freezers

Like samples in LN2, samples stored in ultra-low temperature freezers (-70/80°C and colder) must also be easily identifiable and organized in a way to minimize the time required for sample location and access. Freezers must be well managed, and staff must be prepared for disruption of electricity, blackout, or other event where the freezer malfunctions.

Recommended steps for using ultra-low temperature freezers:

- Store material in the freezer leaving space between boxes/containers to allow for air to circulate.
- Organize samples according to animal or sample ID consistent with PREDICT sample tracking recommendations for rapid retrieval.
- Remove samples from freezer only when ready to prepare for testing or shipping.
- Minimize the number of times the freezer is opened, and make sure the freezer door is closed tightly.
- Secure the electrical outlet and freezer plug to prevent accidental disconnection and freezer failure.
- Post a highly visible sign or sticker by the electrical outlet to ensure the freezer is not unplugged, or cover the electrical outlet with a cage to prevent disconnection.
- Maintain a supply of ice/gel packs in the freezer to maintain temperature in the event of freezer failure, and for use in emergency storage or transport.
- Employ a temperature monitoring system.
- Train all staff members in monitoring and documenting temperatures.

Section 2f. Cold Chain Maintenance

Checking, Recording and Monitoring Cold Chain Temperature

Implementing a temperature-monitoring plan through consistent and regular thermometer readings is essential to maintaining a secure and reliable cold chain.

Recommended Steps for Cold Chain Temperature Monitoring:

- Check LN2 levels and container temperature (if using gauge), and ensure that the container is not leaking twice per day in the mornings and evenings.
- Check and record freezer temperature twice per day in the mornings and evenings (Figure X) as follows: (Note: these readings must be done more frequently if samples are temporarily stored in cold boxes or coolers).
 - Check and record the current freezer temperature.
 - Check and record the maximum freezer temperature.
 - Clear the maximum reading after it is documented.
 - Check and record the minimum freezer temperature.
 - Clear the minimum reading after it is documented.
 - Reset the thermometer.
- Do not open the freezer door to take the temperature readings; an external temperature gauge should be used for commercial freezers.
- Change the thermometer or temperature gauge battery every 6 months (i.e., seasonally with the time change) or as recommended by the manufacturer, as a low functioning battery may give false temperature readings.
- Keep a supply of spare batteries in case of device failure.

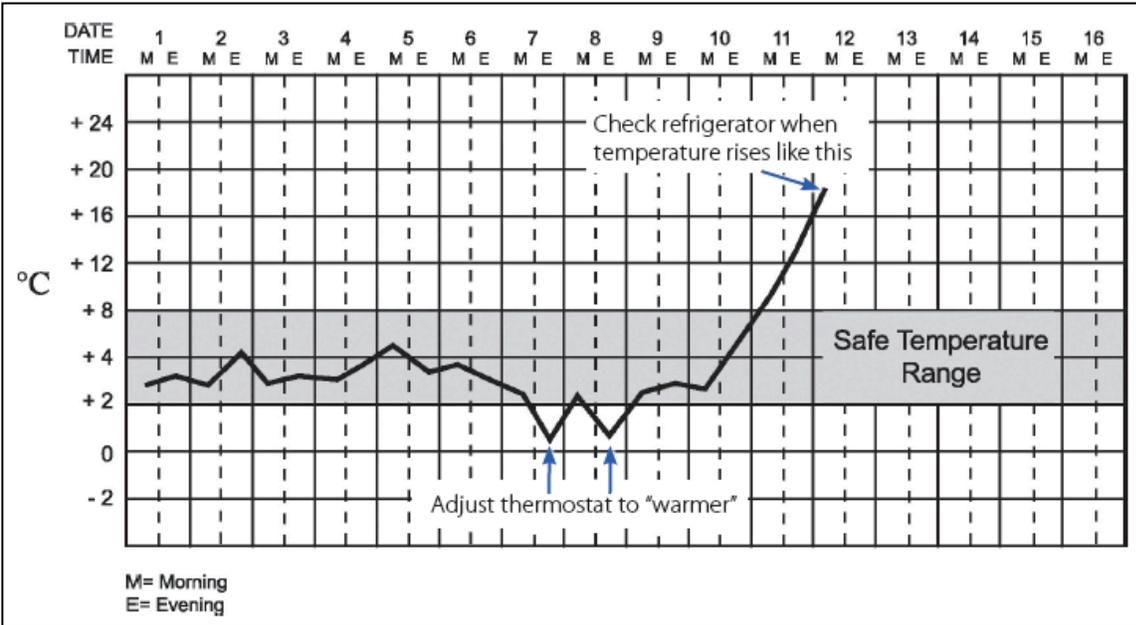


Figure 5. An example of a cold chain temperature monitoring chart. Source: WHO, 2004.
 Note: this chart is for a cold chain optimized for vaccines at 2-8°C. Use the chart included in the Appendix for the PREDICT cold chain at lower storage temperatures optimal for viral isolation.

Section 3. Contingency Planning and Responding to a Cold Chain Breach

Preserving and maintaining below freezing temperatures in tropical conditions requires attention to detail and intensive logistical planning, linking equipment, people, policies, and procedures into an integrated system. Country coordinators, laboratory technicians, and field personnel all have a role to play in ensuring that PREDICT samples are collected, transported, stored, and shipped (if necessary) without breaks in the cold chain. In addition, team members must be trained and prepared to address incidents in which there is a cold chain breach, to enact response measures for rapid cold chain rehabilitation.

Contingency Planning

It is imperative that all PREDICT teams have a pre-determined contingency plan for maintaining the cold chain in the event of freezer or container malfunction or electricity disruption. It is highly recommended that all facilities using commercial -80°C freezers be linked with a back-up generator for continued electrical operation (see box below). However, it is the team’s responsibility to make sure that the back-up generator is of sufficient capacity to operate the freezer, is functioning and has sufficient fuel to maintain electricity, or that alternative measures for maintaining the cold chain are necessary. Arrangements with other facilities for temporary sample storage (if necessary) should be made in advance, along with plans for rapid sample transfer with minimal cold chain disruption.

Essential Steps in Setting-up your Back-up Generator System

Generators should be connected to freezers before a power failure to determine:

- a) If the generator can effectively operate the freezer
- b) The temperature at which the freezer operates when connected to the generator, and whether an appropriate temperature is maintained for samples over an extended period of time
- c) How long the generator can be used in the event of a power outage

If these three conditions are met, then the generator is sufficient to act as a back-up system in the event of a breach. If these conditions are not met, please see "Recommended Steps for Contingency Planning" below.

Recommended Steps for Contingency Planning:

- Identify possible sources of cold chain interruption or breach (e.g., equipment failure, supply shortages, power outages, etc.).
- Identify preparations and solutions for possible chain interruptions
- Prepare back-up infrastructure for sample storage.
- Identify alternate storage facilities for samples and initiate communication to facilitate emergency use.
- Monitor and evaluate equipment regularly and maintain records to assist in understanding potential weaknesses in the cold chain.
- Ensure staff are trained on cold chain maintenance and monitoring for prevention of a breach.

Recommendations for a Power Failure Contingency Plan

Power Failure Contingency Plan (Example)

Start-up the Generator! If Generator is not working, or is insufficient to provide adequate backup (See Box above), then proceed with these steps below:

Samples stored in refrigerator

Monitor the temperature of refrigerator (temperature gauges should be battery powered).

During a power failure of 4 hours or less, the refrigerator door should be kept closed at all times.

If samples are at risk of warming, implement alternative storage arrangements. All samples must be transferred to cold boxes/coolers with prepared ice/gel packs. Monitor sample temperature through the use of a thermometer probe placed near the samples inside the cold box or cooler.

Samples stored in commercial freezer

Monitor the temperature of freezer (temperature gauges should be battery powered).

If samples are at risk of thawing, implement alternative storage arrangements (either in dry ice or LN₂, or in cold boxes and coolers with prepared ice/gel packs).

Responding to a Cold Chain Breach

A cold chain breach is an interruption in the cold chain exposing samples to temperatures above the required range for viral preservation (for prolonged periods – opening and closing a freezer door will often cause temperature fluctuation, but does not qualify as a “breach”). If not quickly rehabilitated, such an interruption can destroy sample viability and render samples useless for PREDICT pathogen testing activities. It is imperative that all teams have documented plans for addressing a breach in the cold chain, and that all team members have received training on appropriate response and cold chain rehabilitation.

Recommended steps in responding to a cold chain breach:

1. Contact your PREDICT Country Coordinator (or supervisor) as soon as possible for advice on emergency response measures, and consult your contingency plans.
2. Define the incident: check all temperature monitoring records, equipment, and discuss with staff possible explanations for the breach.
3. Confirm accuracy of equipment by referencing manufacturer specifications to ensure that the breach is not simply equipment malfunction (data loggers, cold chain monitors and temperature gauges may have operational failure. It is important that emergency measures are not implemented until staff is certain the failure is with the freezer or storage container).
4. Assess the condition of the freezer/storage container. Can the cause be identified (e.g., leaky dewar, freezer door no longer closing completely)?
5. Record:
 - a. When the cold chain was last guaranteed?
 - b. What monitoring has been recorded prior to breach?
 - c. What is the time interval of breach?
 - d. What is the temperature range of the breach period?
 - e. What samples were involved in incident? Enter record in sample database.
6. Continuously monitor temperatures of the containers/freezers and record the duration of time samples are exposed to temperatures warmer than -80°C.
7. If temperatures approach -30°C, begin planning for sample transfer to temporary cold boxes or coolers, or other laboratory facilities.
8. If temperatures climb to warmer than -20°C, transfer samples to temporary storage containers and continue monitoring temperature. If there is no -20°C capacity, actively pursue an alternative storage facility and prepare insulated boxes for sample transport.
9. DO NOT discard any samples until advice has been sought from PREDICT Country Coordinators and laboratory personnel.
10. Label all samples exposed to elevated temperatures in the PREDICT sample tracking information database.

Take active steps to correct and prevent the problem from recurring. In the event of a cold chain breach, it is important to keep records to guide in response implementation, to help prevent future breaches, and to inform PREDICT team members of any potentially affected samples. The following table includes an example data sheet for a cold chain breach. A blank data sheet is included in the Appendix.

Example data sheet for cold chain breach

Date and suspected time of the breach	Date: Aug. 13, 2010	Time: 5:14 PM
Do you store your samples in a commercial freezer or vacuum flask container?	Commercial Freezer	LN2 vacuum flask
Minimum and maximum temperature readings	Minimum: -88°C	Maximum: -57°C
When was the thermometer last reset	Date of reset: July 12, 2010	Time of reset: 11:12 AM

Section 4. References

Commonwealth of Australia, Department of Health. 2003. *Cold Chain and Immunization Operations Manual: Guidelines for handling heat sensitive vaccines and pharmaceuticals.*

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World Health Organization Department of Communicable Disease Surveillance and Response. 2000. *Guidelines for the collection of clinical specimens during field investigations and outbreaks.* WHO/CDS/CSR/EDC/2000.4.

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Data Sheet Template for Cold Chain Breach

Date and suspected time of the breach	Date:	Time:
Do you store your samples in a commercial freezer or vacuum flask container?	Yes	No
Minimum and maximum temperature readings?	Minimum	Maximum
Are Cold Chain Monitors (CCMs) stored with the samples? If 'yes', be ready to report the reading when breach was noticed.	Yes	No
When was the thermometer last reset?	Date of reset:	Time of reset:
When was the thermometer battery last changed?	Date of battery change:	Time of batter change:
When was the last check on the accuracy of the thermometer done?	Date:	Time:
How long do you think the temperature was above -80°C?	Minimum Estimate	Maximum Estimate:
How long do you think these problems have been occurring?	First breach	Recurring (state number):
Where is the temperature probe situated?	Location:	Notes:
What type and number of samples were exposed to the breach?	Type of samples:	Number of samples:
Are all samples labeled and accessible?	Yes	No
Are there ice/gel packs in the freezer to use if transfer is necessary?	Yes	No
What do you think was the cause of the cold chain breach?	Suspected cause:	Notes:
Has the cause of the cold chain breach been rectified?	Yes	No
Free fields for customization		

Temperature Monitoring Chart (-80°C and ultra-low temperature freezers).

Date	1		2		3		4		5		6		7		8		9		10	
Time	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
10																				
0																				
-10																				
-20																				
-30																				
-40																				
-42																				
-44																				
-46																				
-48																				
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-80																				
-82																				
-84																				
-86																				
-88																				
-90																				
-92																				
-94																				
-96																				
-98																				
-100																				
-102																				
<																				

M=Mornings; E=Evening **Red:** Critical zone above freezing temperatures; **Green:** Safe zone for PREDICT samples; **Yellow:** Temperature zone indicating thawing of samples and potential breach. **Note:** This Chart will produce a visible trend from dot plots of temperature like in Figure 6, showing your equipment’s temperature variation over time. You may customize the temperature column to use with other temperature ranges as needed. This form will need to be replaced every 10 days (with dates adjusted in the “Date Column”). If using grey-scale, feel free to remove the color shading and print a simple table format.

TEMPERATURE LOG

Site: _____

Refrigerator ID#: _____ Required Temp: _____

Freezer ID#: _____ Acceptable Range: _____

ENTER TEMPERATURE AND INITIALS DAILY!

	JANUARY	FEBRUARY	MARCH	APRIL	MAY	JUNE
1						
2						
3						
4						
5						
6						
7						
8						
9						
10						
11						
12						
13						
14						
15						
16						
17						
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31						

NOTE: CROSS OUT WEEKENDS AND HOLIDAYS – UPDATE FOR REMAINING MONTHS.

**This is a sample template for use with refrigerators and other equipment; it can be used together with the "Temperature Monitoring Chart" above.*

Packing and Shipping Biological Samples

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Last updated: 08 December 2016

Objective: To provide guidance to ensure safe, proper, and efficient packaging and shipping of biological samples.

THIS DOCUMENT IS NOT TO SERVE AS A REPLACEMENT FOR CERTIFIED TRAINING TO SHIP INFECTIOUS SUBSTANCES.

Section 1. Learning Objectives and Confirmation

If you understand the material in this Guide, you should be able to:

- Classify biological substances based on the specifications of US Code of Federal Regulations Title 49 (49 CFR) Parts 171-175.
- Select the proper shipping name and United Nations (UN) identification number for biological substances.
- Complete a shipper's declaration to accompany biological substance shipments.
- Package biological substances safely and in accordance with regulations.
- Mark and label shipments containing biological substances in accordance with regulations.
- Properly notify the receiver of a shipment containing biological substances.

Confirm you understand the material of this Guide:

When you are familiar with the information in this Guide, take the PREDICT quiz on [Packing and Shipping Samples \(Section 8.4.15.\)](#).

Section 2. Introduction

Correct packing and shipping of biological substances is necessary to prevent potential exposure to infectious diseases that affect people, animals or both. Samples collected from wildlife, domestic animals and people for the purpose of disease surveillance often fall into Categories A and B:

Infectious Substances, Category A -- Samples collected from sources that are known to be infected with a Category A pathogen (See Section 3 for definition of Category A).

Biological Substances, Category B -- Samples that *may be infected with a pathogen*, but are collected for diagnostic purposes,¹ or samples that are likely infected with a non-Category A pathogen. Most samples, such as blood serum and swabs collected from apparently healthy animals, fall into this category.

Non-regulated Biological Materials -- Samples that do NOT contain pathogens and/or samples that have been treated such that the pathogens have been inactivated (heat treated, formalin, etc.). This category also includes environmental samples such as food and water that are not considered to pose significant risk of infection. (However, it is important to note that certain sample preservatives, namely formalin, are considered "dangerous goods"; samples in formalin cannot be shipped without adhering to the appropriate restrictions, as discussed later in this guide.)

¹ Note that prior to 2006, *Category B* samples (whose status of infectiousness was unknown) were referred to as "Diagnostic Specimens".

Exempt Patient Specimens – Patient specimens not likely to contain a pathogen that are undergoing testing for non-infectious disease. In order to classify a patient specimen as Exempt, professional judgment is required. Factors such as the known medical history, symptoms and individual circumstances of the source, human or animal, and endemic local conditions must be considered. This label is inappropriate for specimens being tested for infectious disease.

Samples of Category A and Category B are legally considered to be “*Dangerous Goods*” and are regulated by the United States International Air Transport Association (IATA) and the United States Department of Transportation (DOT). Keep in mind that these organizations use the word “dangerous” as a technical term for substances that you may not normally consider being dangerous (including dry ice).

United States regulations state that anyone involved in preparing “dangerous goods” such as biological samples for shipment must be trained to perform these tasks. A record of current training must be maintained by the employer, and training must be provided for anyone involved in any aspect of packing and shipping samples, from the investigator and/or technician who handles the samples, to the administrative staff who may complete the DHL or Federal Express labels for the shipping containers.

If you are shipping anything into or out of the US, failure to comply with the correct packing and shipping regulations is punishable by significant fines and can jeopardize your and/or your home institution’s current and future ability to obtain permits to import and export samples.

Keeping good records and knowing the regulations will be helpful if you encounter problems when the carrier, the laboratory receiving the samples, or authorities, inspects a shipment.

Problems can and do arise even if you follow these instructions. Complications that may occur include differences in national and local laws that you must follow in each country regarding importing, exporting and shipping samples, in addition to the US and international regulations discussed here. In the event that the country’s local or national regulations are less stringent, use the regulations described here as your guide.² Sometimes problems arise because there are many authorities and individuals involved in the process, each of whom may interpret the regulations differently.

Be prepared by familiarizing yourself with the regulations and double check that you have completed each step using the checklist provided at the end of this section.

² These protocols follow rules for air transport of samples, which are somewhat stricter than those for ground transport; one cannot always control how samples are shipped once they are given to the carrier, and therefore it is safer to follow the stricter rules, in keeping with the precautionary principle.

The protocols discussed below will enable you and your staff to comply with the highest standard of safety for packing and shipping biological samples. The underlying principles are:

- 1) Minimize the risk of inadvertent exposure to an infectious agent through shipping, importing or exporting biological samples (including samples from domestic and wild animals and humans).
- 2) Prevent human injury, as well as damage to the samples, the environment and property, that can be caused by improper handling and packing of storage and shipping materials that are flammable and/or toxic (e.g., alcohol, formaldehyde) and/or volatile (e.g., dry ice).

Investigators and project supervisors must be trained to ensure that hazards to human and animal health are clearly identified and communicated to project personnel; that all personnel fully understand the techniques to be used for handling known biohazards specific to the species under study; and that written procedures and any necessary protective packing materials and equipment are made available.

Investigators or project supervisors are responsible for:

- Keeping a record of personnel who have been trained and the content and date of that training.
- Staying informed about changes in regulations at the regional or national level, so that appropriate updates in training can be provided to personnel.
- Providing a “Useful Contacts” list for staff, with numbers of local offices that handle import and export permits for domestic animals, wildlife, migratory birds, and CITES samples.
- Creating and posting first response guidelines in the event of exposure to a known or suspected infectious or toxic agent while handling, packing and/or shipping samples.

COORDINATE WITH US OFFICIALS WHEN IMPORTING SAMPLES:

When importing samples to the USA by air you must be met at the airport by an agent from 1, 2 or 3 US agencies (USFWS, USDA, CDC), depending on your samples. Work with them by phone and fax in advance to encourage efficient and positive interactions with agents (who have the power to help or hinder your imports and exports, and even to destroy samples.)

Example of samples being imported:

Agents to meet you at the airport:

Common duck serum and/or feathers	USFWS, USDA
Endangered duck serum and/or feathers	USFWS, USDA
Wild orangutan serum	USFWS, CDC
Serum from wild rats trapped in an urban market	USFWS
Ticks from rats at an urban market	USFWS, USDA
Serum from domestic cows	USDA

CHECKLIST FOR PLANNING TO SHIP SAMPLES

DO YOU HAVE...?

- Valid **export** permit from the country you are exporting from
- Valid **import** permit from the USA (or other country of import) to bring field samples into the USA (or other country) for analysis and/or storage
- Valid CITES I, II, III, and/or Migratory Bird permits, if applicable. (For CITES species search www.cites.org/eng/resources/species.html; for Migratory Birds: <http://www.fws.gov/birds/policies-and-regulations.php>)
- Permits that explicitly allow for the **fixative and containers** you will use. (Permits specify how biological samples are expected to be fixed and stored for shipping.)
- Arrangements with all the relevant authorities about your planned shipment, e.g.
 - United States Fish and Wildlife Service (USFWS), management authority for importation of ALL wildlife samples, whether from threatened, vulnerable, endangered, OR common species not listed by IUCN or CITES.
 - United States Department of Agriculture (USDA), regulator of importation of biological materials from wild and domestic birds, ungulates, and plants. Permits are required to import parasites and materials potentially containing bacteria, viruses, or fungi, which may pose a threat to U.S. livestock or agriculture.
 - United States Centers for Disease Control (CDC), regulator of importation of ALL non-human primate samples. (See www.fws.gov/le/ImpExp/Info_Importers_Exporters.htm)
- Flight arrangements that facilitate the safe arrival of your samples; specifically, if bringing the samples to the USA in person by air:
 - Fly into a *designated* U.S. port of entry (see list at: <http://www.fws.gov/le/designated-ports.html>).
 - Arrive during USFWS business hours (9:00 am-5:00 pm M-F) so that inspectors will be able to meet you with the least inconvenience to all of you. (It is your responsibility to know which inspectors must meet you and to alert the proper agencies; see text box 2 below.) Make arrangements with the agent IN ADVANCE if you cannot arrive at these times.
 - Have all your forms in order for imports:
 - USFWS Import Declaration Form 3-177
 - Sample Inventory
 - **Copies** of relevant **import** permits (CITES, USDA, CDC, Migratory Bird)
 - **Originals** of all relevant **export** permits (from country of origin) or letters of permission from the regional ministry, with required signatures and stamp.
 - Letter on official letterhead from your institution giving you permission to use EACH permit, if any of the permits are not in your name.
 - Make sure your inventory of all your samples contains NO errors and that it EXACTLY matches your physical samples.

Section 3. Summary of Key Terms

Biological Sample – A biological specimen including, for example, blood, tissue, hair, feathers, skin, urine, nail clippings etc. collected from a human, domestic or wild animal. Samples collected from any part of a plant are also biological samples.

Dangerous Goods (also known as Hazardous Materials) – The United Nations (UN) Economic and Social Council’s Recommendations on the Transport of Dangerous Goods defines these as: “substances which are capable of posing a risk to health, safety, property or the environment.” IATA and the DOT regulate the movement of dangerous goods within and between countries and global regions.

Infectious Substances – Substances which are known to contain, or can reasonably be expected to contain, pathogens including bacteria, viruses, parasites, fungi and other agents such as prions that can cause disease in humans or animals. Infectious substances include BOTH “Infectious Substances, Category A” and “Biological Substances, Category B.”

Infectious Substances, Category A – Infectious substances in a form(s) capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs. An exposure occurs when an infectious substance is released outside of its protective packaging, resulting in physical contact with humans or animals (49CFR 173.134).

Biological Substances, Category B – Potentially infectious substances not in a form generally capable of causing permanent disability of life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs. This includes Category B infectious substances transported for diagnostic or investigational purposes (49CFR 173.134). Most biological samples collected during disease surveillance among human and animal populations will fall into Category B.)

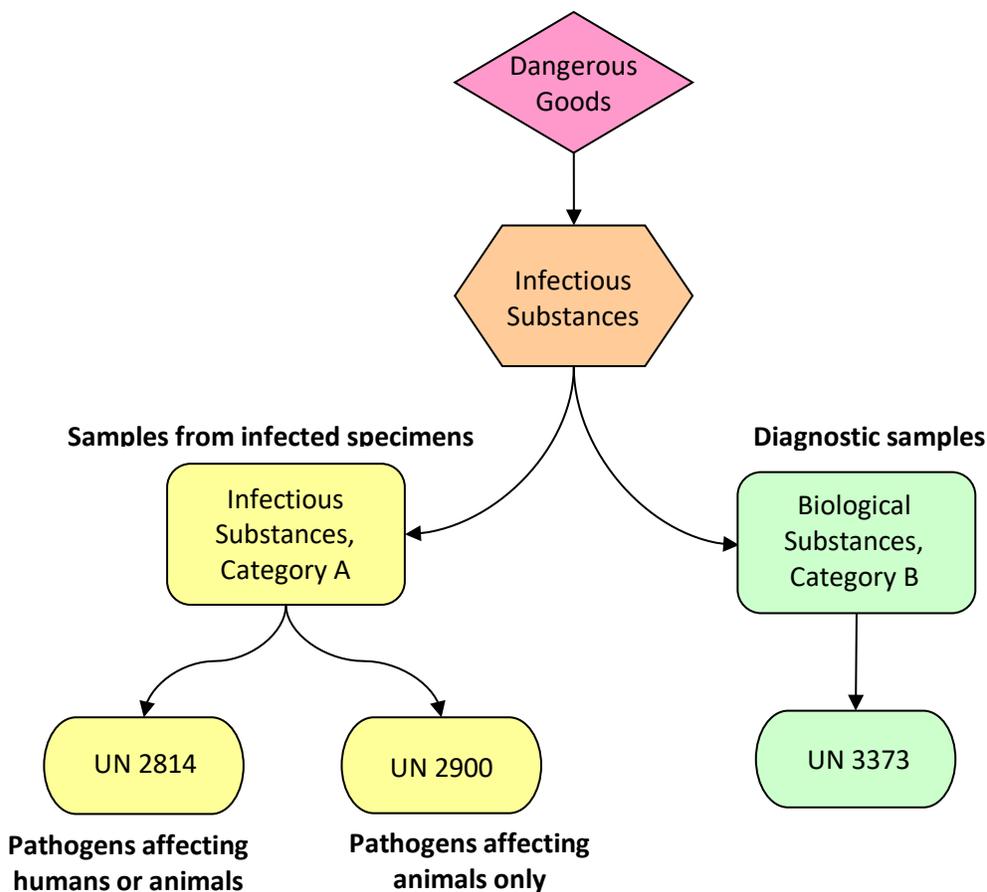
Exempt Patient Specimens – Patient specimens not likely to contain a pathogen that are undergoing testing for non-infectious disease. Examples include, but are not limited to, samples taken for routine testing not related to the diagnosis of an infectious disease (such as for drug/alcohol testing, cholesterol testing, blood glucose level testing). Factors such as the known medical history, symptoms and individual circumstances of the source, human or animal, and endemic local conditions must be considered in making this determination. If testing for infectious diseases is being performed, or if a medical history is not known, the sample must not be shipped as an exempt patient specimen. Samples are packaged the same as Category B substances, but do not require a UN number or PSN, and instead must have the term “Exempt Human Specimen” or “Exempt Animal Specimen” on the box.

Non-regulated Biological Substances – Substances that are not subject to the regulations unless they meet the criteria for inclusion in another Class or Division of dangerous goods. Examples include, but are not limited to, microorganisms that do not cause disease in humans or animals, DNA, RNA or other non-infectious genetic elements, environmental samples such as food and water, dried blood spots, or blood taken for the purpose of transfusion. For a full list of exemptions, please refer to 49 CFR 173.134, searchable at <http://www.ecfr.gov>.

Section 4. Classifying and Identifying Biological Samples

In order to determine the correct way to package and label your biological samples for shipment, you need to know how to classify and identify the sample.

STEP 1: Assign the sample to one of nine “hazard classes” as defined by the United Nations. The following diagram gives an overview of the process you will use to identify your biological samples, which will be explained in more detail in this Section . If the sample is known to be infected with a non-category A pathogen, it can be placed in Category B.



Hazard Classes

There are nine categories of “dangerous goods” specified by the UN Globally Harmonized System of Classification and Labeling for Chemicals (GHS). Some of the nine classes have further sub-categories. In the case of samples collected for disease surveillance in humans and animals, we will primarily be working with two hazard classes:³

Class 6.2 – Infectious Substances

Class 9 – Miscellaneous Dangerous Goods (dry ice and formalin)

Each hazard class is identified by a diamond symbol containing the class number, class name and a unique icon.

STEP 2: *Assign a UN Number based on the hazard classification and the composition of the sample.*

UN Identification Numbers

The United Nations Committee of Experts on the Transportation of Dangerous Goods has developed a system of 4-digit numbers to identify substances that fall into one of the nine hazard classes. This number is accompanied by a “proper shipping name” as well as a “technical name” for each substance. Proper shipping names are used in shipping documents, notifications and on package labels.

You should be familiar with the following four UN numbers:

UN 2814: assigned to Infectious Substances, Category A, which cause disease in humans or both in humans and animals. The proper shipping name for UN2814 is *“Infectious substances, affecting humans.”*

UN 2900: assigned to Infectious Substances, Category A that causes disease only in animals. The proper shipping name for UN 2900 is *“Infectious substances, affecting animals only.”*

UN 3373: assigned to *all* Category B (see above) infectious substances. The proper shipping name for UN 3373 is *“Biological Substance, Category B.”*

UN 1845: assigned to shipments that contain dry ice. The proper shipping name for UN 1845 is *“Carbon Dioxide, solid”* or *“Dry Ice.”*

³ The other classes include: Class 1-Explosives; Class 2-Gases; Class 3-Flammable Liquids; Class 4-Flammable Solids; Class 5-Oxidizing Substances and Organic Peroxides; Class 7-Radioactive Material; Class 8–Corrosives.

Class 6.2 – Infectious Substances and Class 9 – Miscellaneous Dangerous Goods:

Hazard class and UN numbers should be assigned to biological samples based on the known medical history and symptoms/signs of the source human or animal, endemic local conditions, and professional judgment concerning the individual circumstances of the source human or animal. Likely hazard classes for human and animal disease surveillance work includes the following:



Class 6.2 – Infectious Substances:

All samples collected from humans or animals which are known to contain, or are reasonably expected to contain, pathogens including bacteria, viruses, parasites, fungi and other agents such as prions which can cause disease in humans or animals should be assigned to hazard class 6.2 - Infectious Substances. Samples collected for the purpose of diagnosing an infectious disease fall within Class 6.2 – Infectious Substances.



Infectious Substances, Category A (UN 2814 and UN 2900):

If you think your sample contains a pathogen in a form that, when exposure to it occurs, is capable of causing permanent disability, life-threatening or fatal disease in otherwise healthy humans or animals, it should be assigned to Category A. These are assigned the following UN numbers and proper shipping names:

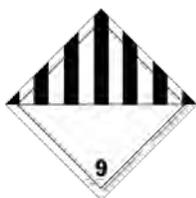
UN 2814 – Infectious Substance, affecting humans; or
UN 2900 – Infectious Substance, affecting animals only



Biological Substances, Category B (UN 3373):

Biological samples that do not meet the criteria for inclusion in Category A are assigned to Biological Substances, Category B. This includes samples collected for the purpose of diagnosing infectious diseases. They are assigned the following UN number and proper shipping name:
UN 3373 – Biological Substance, Category B

Most biological samples collected as part of human and animal disease surveillance activities will fall into Biological Substances, Category B.



Class 9 – Miscellaneous Dangerous Goods:

According to IATA and the DOT, dry ice is considered a “dangerous good”. Dry ice falls into Class 9 - Miscellaneous Dangerous Goods, which are hazardous substances that do not fall into the other categories (this class also includes asbestos, air-bag inflators, and self-inflating life rafts). The amount of dry ice that you are allowed to ship with your samples will vary with each carrier or airline, and you must determine this BEFORE shipping your samples. Dry ice is assigned the following UN number and proper shipping name:

UN 1845 – Carbon dioxide, solid, or
UN 1845 - Dry ice

Section 5. Packing Instructions

The classification and identification of your biological sample determine how you will pack and ship it. The following guidelines apply to shipments classified as 6.2 – Infectious Substances (both Category A and Category B).

Class 6.2 – Infectious Substances must be packed in triple packaging consisting of:

- 1) A primary receptacle that contains the infectious substance and must be watertight to prevent leakage. Primary receptacles include those made of glass, metal, or plastic and include screw-cap tubes or rubber-stopped glass vials fitted with metal seals. The primary receptacle should have a specimen ID label. The primary container must be capable of withstanding without leakage an internal pressure producing a pressure differential of not less than 95 kPa and temperatures in the range of -40°C to 55°C.
- 2) One or more primary receptacle placed in a water tight secondary packaging. The secondary packaging should also bear a label with the name, address, and telephone number of the shipper. If multiple fragile receptacles are in a single secondary package they must be individually wrapped to prevent contact. Absorbent material must be placed between the primary receptacle and secondary packaging. The absorbent material must be sufficient to absorb the entire contents of the primary containers. An itemized list of contents must be placed between the secondary packaging and outer packaging. Filling out the submission form of the laboratory receiving the samples can meet this requirement.
- 3) The secondary packaging should be placed within a styrofoam container inside of a shipping box. The outer shipping box must be of adequate strength for its capacity, mass, and intended use. You must be able to drop the complete package from a height of 1.2 meters without it suffering any damage. Outer package dimensions must be at least 4”L x 4”W x 4”D (10 cm x 10 cm x 10 cm).

It is best to obtain appropriate outer packing materials used by your carrier (both DHL and FedEx sell them). Packaging should be used exactly as written in the directions supplied with the packaging.

If dry ice is used as a refrigerant, tape only some of the seams between the dry ice and outer packaging. Seal the outside package such that the carbon dioxide gas that forms is able to escape, preventing the build-up of pressure that could rupture the package or put transporters at risk.

Examples of the CORRECT packaging of biological samples:

Example 1:



These three screw-cap tubes contain specimens being sent to a laboratory for diagnostic testing. The tubes have been sealed with parafilm, to prevent leakage around the cap. Parafilm should always be on hand when you package your samples for shipping.



Tubes should be packed with absorbent material into a leak-proof secondary container. Multiple primary receptacles are individually wrapped to prevent contact between them. The absorbent material must be sufficient to absorb the entire contents of the primary containers.



The container with the tubes is placed in the center of an insulated styrofoam carton inside a shipping box. Dry ice is added above the samples (and below if possible). The lid of the styrofoam carton

is added. The plastic liner is folded around the styrofoam carton.

REMEMBER: The lid of the styrofoam carton is NEVER taped shut when you are shipping with dry ice, to allow gas to escape cartons.

Example 2:

Absorbent material sufficient to absorb the entire contents of the primary receptacle

Leak-proof primary receptacles (screw-top plastic jar)



Leak-proof secondary packaging (Ziploc biohazard bag)

Examples of POORLY packaged biological samples:



Tubes with cork stoppers are NOT acceptable; evidence of leakage is visible on the labels and in the box.



Ensure that your packaging can withstand wear and tear.

Packaging must be constructed and closed so as to prevent loss of contents that might occur under normal conditions of transport, by vibration, or by changes in temperature, humidity or pressure.

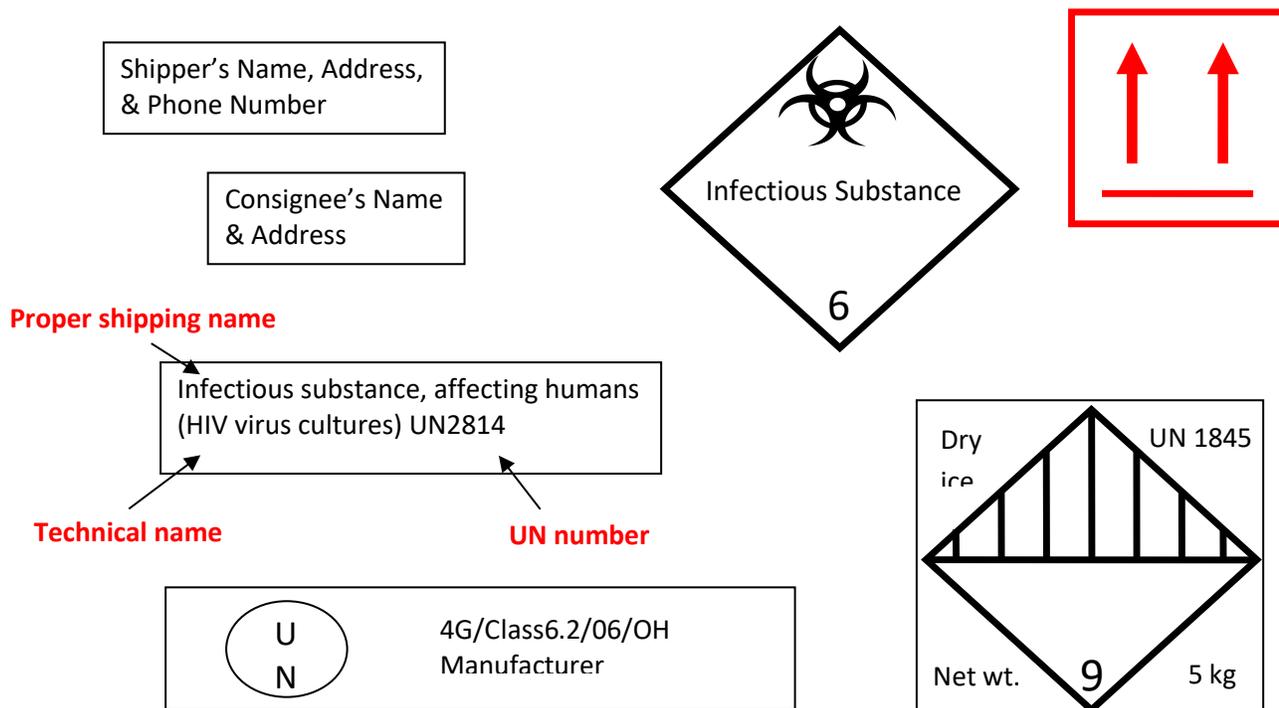
Section 6. Marking and Labeling

The labeling requirements differ depending on whether your biological samples are classified as Infectious Substances, Category A or Biological Substances, Category B. Make sure to follow the guidelines that are appropriate for the type of samples you have.

INFECTIOUS SUBSTANCES, CATEGORY A (UN 2814 & UN 2900), Marking and Labeling:

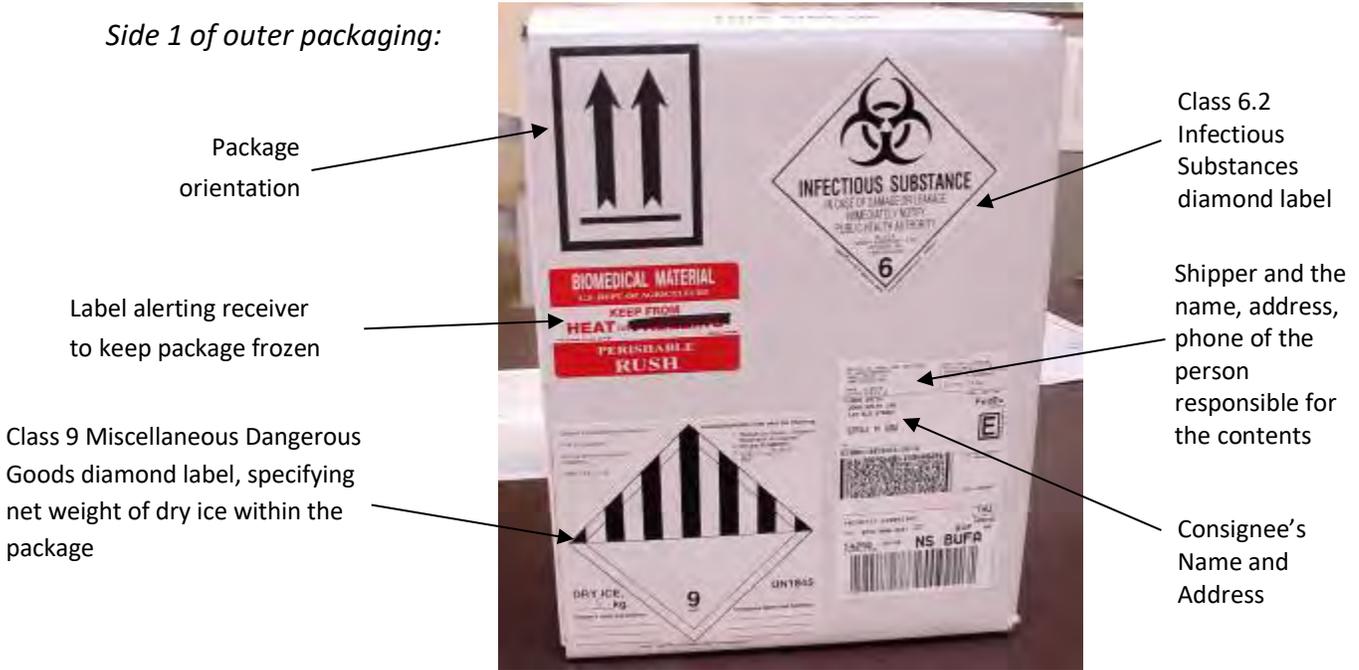
Packages containing Category A, Infectious Substances, must be marked on the outside of the shipping container with the following:

- Diamond shaped label for Class 6.2 – Infectious Substances.
- Diamond shaped label should be 50mm on all sides.
- Diamond shaped label should have a line width of 2mm.
- The proper shipping name of the dangerous goods and corresponding UN number in type that is at least 6mm. The technical name of the suspected infectious agent must be listed on the shipper's declaration per "Special Provision A140". It should NOT be marked on the box.
- Package orientation (This Way Up) labels affixed on opposite sides of the outer package.
- The full name and address of the shipper and the consignee.
- 24-Hour emergency response number and name of the person responsible for the contents (this information can be on the waybill for Category B).
- Successful drop and pressure test certification labels.
- For dry ice, Class 9 diamond label and the net weight of the dry ice contained.

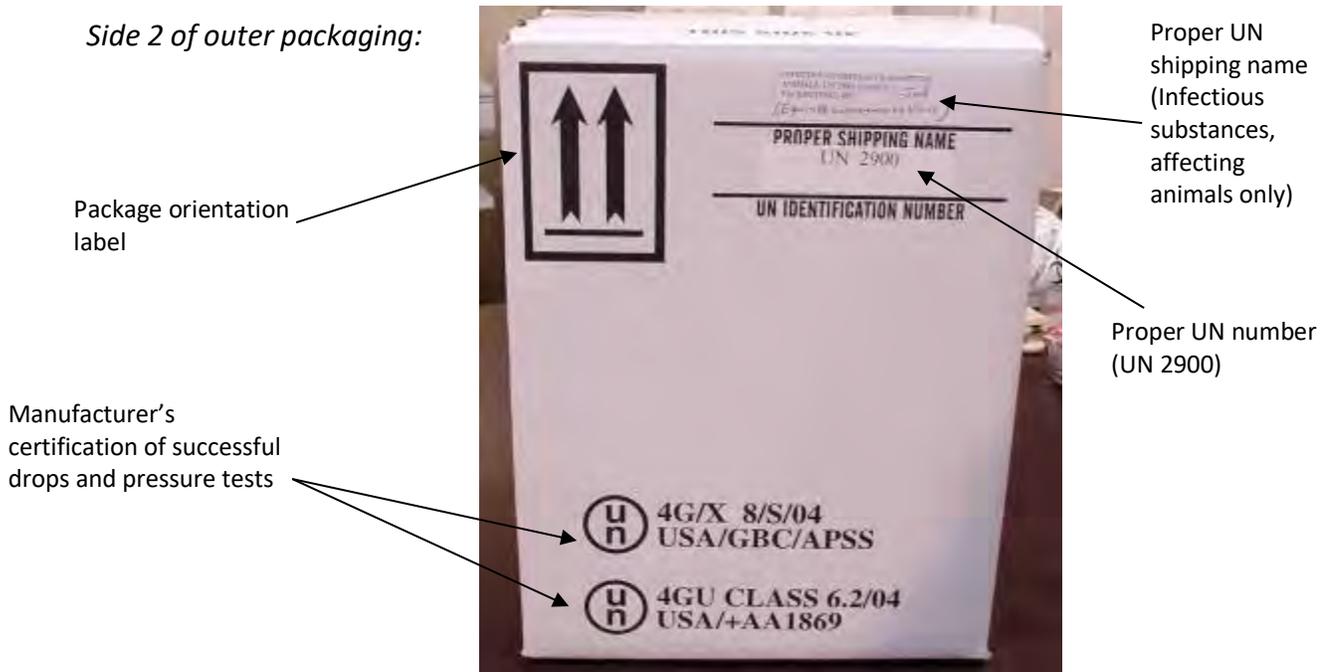


Example of PROPER Infectious Substances, Category A marking and labeling:

Side 1 of outer packaging:



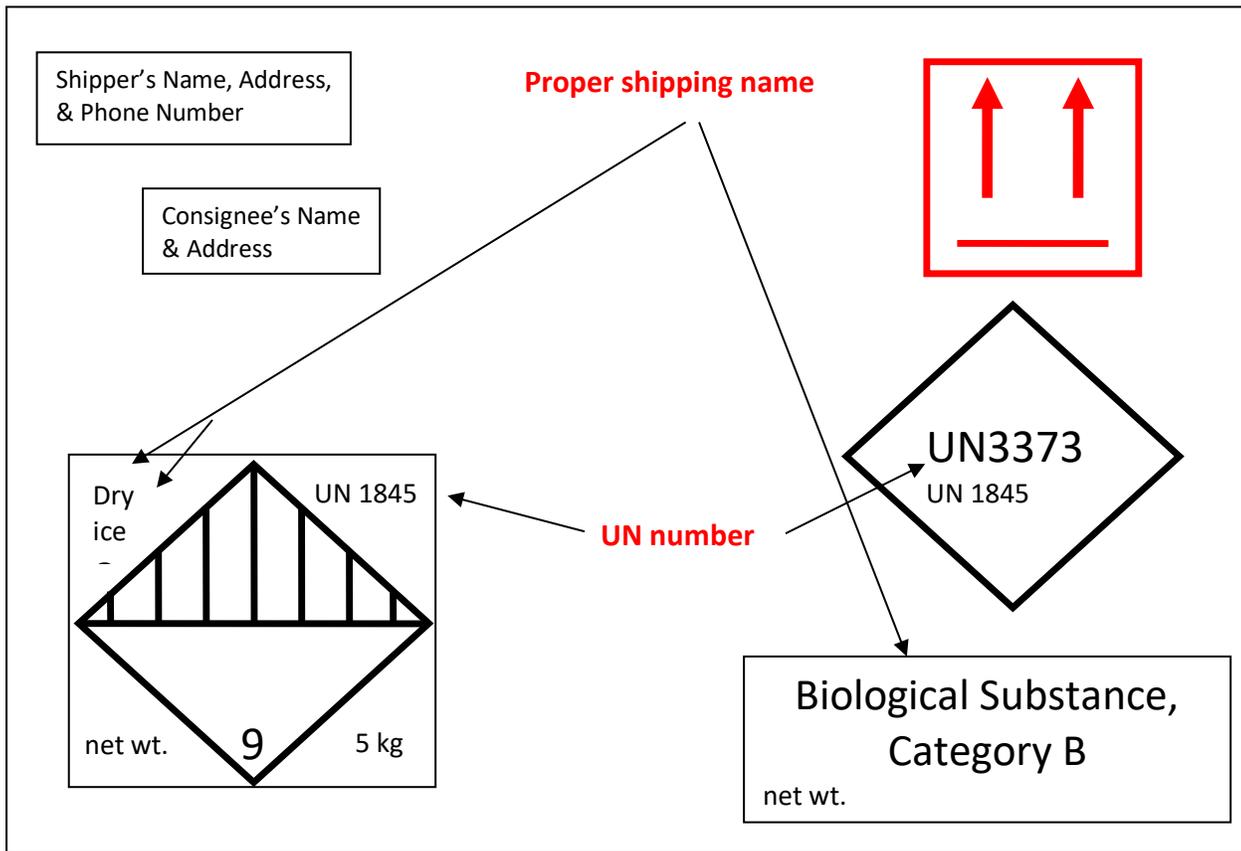
Side 2 of outer packaging:



Biological Substances, Category B (UN 3373) marking and labeling:

Packages containing Category B, Biological Substances must be marked on the outside of the shipping container with the following:

- The proper shipping name of “Biological Substances, Category B” and corresponding UN number 3373 in font at least 6mm tall.
- Package orientation (This Way Up) labels affixed on opposite sides of the outer package.
- The full name and address of the shipper and the consignee.
- For dry ice, Class 9 diamond label and the net weight of the dry ice contained.



Example of PROPER Biological Substances, Category B marking and labeling:

Side 1 of outer packaging:



Side 2 of outer packaging:



Section 7. Documentation

Shipper's Declaration of Dangerous Goods:

Packages containing Infectious **Substances, Category A** that are transported by air must contain a "Shipper's Declaration for Dangerous Goods" form. This is a legal document and must be fully and accurately completed by you, the shipper. Carriers will refuse incomplete, illegible or inaccurate documents.

Shipments of Infectious Substances, Category A require the shipper to make advance arrangements with the consignee and the operator to ensure that the shipment can be transported and delivered without unnecessary delay.

A Shipper's Declaration is NOT required for Biological Substances, Category B and it is not required for dry ice without other dangerous goods.

Enter the following information on the Shipper's Declaration:

Shipper – enter the full name and address of person responsible for sending the shipment

Consignee – enter the full name and address of person who will receive the shipment

Air Waybill Number – if known, enter the air waybill number provided by the courier, this information may also be entered or amended by the shipper, a brokering agent or by the airline or its handling agent

Page of pages – enter the page number and the total number of pages (for a single page Shipper's Declaration, enter "page 1 of 1 pages")

Transport Details – Indicate whether the shipment is packaged to comply with the limitations for passenger and cargo aircraft OR cargo aircraft only, mark X in the box that *does NOT apply*

Airport of Departure – enter the full name of the airport or city of departure, if known; this information may also be entered or amended by the shipper, a brokering agent, or by the airline or its handling agent

Airport of Destination – enter the full name of the airport or city of arrival, if known; this information may also be entered or amended by the shipper, a brokering agent or by the airline or its handling agent

Shipment Type – enter X's to block out "RADIOACTIVE" (for shipments which do not contain radioactive material) or to block out "NON-RADIOACTIVE" (for shipments which contain radioactive material)

Nature and Quantity of Dangerous Goods – Enter the required information strictly in accordance with IATA 8.1.6.9. Per IATA, the following information fields *must be* entered in sequence within the columns provided. If your information will not fit without going over the lines separating the columns, enter text on another line below the first line.

Emergency Contact Number – you must include a telephone number for a 24-hour emergency response agency (in the US this would be the Center for Disease Control, CDC, 1-800-232-0124).

Name and Title of Signatory – Enter the name and title of the person actually signing the Shipper’s Declaration. Only a *certified* shipper may fill out a declaration (this training guide does NOT count as a certification).

Place and Date – Enter the place and date to indicate where and when the form was actually signed.

Proper shipping name – Enter the appropriate UN proper shipping name either “Infectious substances, affecting humans” or “Infectious substances, affecting animals only” or “Carbon dioxide, solid”

Class or Division – Enter hazard class “6.2” for Infectious Substances or hazard class “9” for dry ice.

UN or ID Number – Enter the appropriate UN number to match the shipping name either *UN 2814* or *UN 2900* or *UN 1845*.

Packing Group – Leave blank (Class 6.2 does not have a packing group).

Subsidiary Risks – Leave blank.

Quantity and Type of Packing – List the amount of dangerous good included in shipment and type of packing used to contain it.

Packing Instruction – Enter *602* for Category A Infectious Substances; enter *904* for dry ice.

Dry ice -- If using dry ice in your shipment, be sure to include it in the declaration list.

Example of Shipper's Declaration for Dangerous Goods:

SHIPPER'S DECLARATION FOR DANGEROUS GOODS				(Provide at least three copies to the airline.)		
Shipper Dr. Jane Smith Ebola Research Program 123 Research Street New York, NY, 10000, United States				Air Waybill No. 12345678 Page 1 of 1 Pages Shipper's Reference Number		
Consignee Generic Laboratory 4567 Laboratory Avenue Chicago, IL, 60000, United States Telephone: 1-800-123-4567						
<i>Two completed and signed copies of this Declaration must be handed to the operator</i>				WARNING Failure to comply with all respects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties.		
TRANSPORT DETAILS						
This shipment is within the limitations prescribed for: <i>(delete non applicable)</i>		Airport of Departure <div style="text-align: center; font-size: 1.2em;">LaGuardia, NY</div>				
<input type="checkbox"/> PASSENGER AND CARGO AIRCRAFT	<input checked="" type="checkbox"/> CARGO AIRCRAFT ONLY					
Airport of Destination:		<div style="font-size: 1.2em;">O'Hare, IL</div>				
Shipment type: <i>(delete non-applicable)</i> <input type="checkbox"/> NON-RADIOACTIVE <input checked="" type="checkbox"/> RADIOACTIVE						
NATURE AND QUANTITY OF DANGEROUS GOODS						
Dangerous Goods Identification						
UN or ID No.	Proper Shipping Name	Class or Division (Subsidiary Risk)	Pack- ing Group	Quantity and type of packaging	Packing Inst.	Authorization
UN 2814	Infectious substance, affecting humans (Ebola virus)	6.2		5 ml in plastic screw-top vial	602	
UN 1845	Carbon dioxide, solid (Dry ice)	9		5 kg in unsealed styrofoam cooler	904	
Additional Handling Information						
Prior arrangements as required by the IATA Dangerous Goods Regulations 1.3.3.1 have been made						
I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name, and are classified, packaged, marked and labelled/placarded, and are in all respects in proper condition for transport according to applicable International and National Governmental Regulations. I declare that all of the applicable air transport requirements have been met.				Name/Title of Signatory John Doe Place and Date Ebola Research Program, New York, NY 1/1/2010 Signature [A typed signature may be used if the origin and destination are in the United States or its territories.]		
CDC, 1-800-232-0124			Emergency Telephone Number		John Doe	
FOR RADIOACTIVE MATERIAL SHIPMENT ACCEPTABLE FOR PASSENGER AIRCRAFT, THE SHIPMENT CONTAINS RADIOACTIVE MATERIAL INTENDED FOR USE IN OR INCIDENT TO RESEARCH, MEDICAL DIAGNOSIS, OR TREATMENT. ADR EUROPEAN TRANSPORT STATEMENT: CARRIAGE IN ACCORDANCE WITH 1.1.4.2.1						

Air Waybill:

A waybill is different from a Shipper's Declaration. All shipments require a waybill regardless of contents. Forms may vary depending on the shipping company (FedEx, DHL, Compass Forwarding, etc.). In the following example from FedEx you will need to specify in Box 6 that the package contains dangerous goods.

If you are shipping Biological Substances Category A mark “Yes (As per attached Shipper’s Declaration)”.

If you are shipping Biological Substance Category B, mark “Yes (Shipper’s Declaration not required).”

The proper shipping name and UN number need to be on the waybill. For FedEx, there is no designated area for this information and it should be written in by the shipper.

If your shipment includes dry ice, mark “Dry Ice (Dangerous Goods Shipper’s Declaration not required)” and include the number of blocks and their weight in kg.

When shipping infectious substances, your waybill is a legal document. It must be legible (typed), CANNOT contain spelling errors and must be in triplicate – one copy each for the shipper, the carrier and the recipient. Shippers must keep their copies for 375 days.

Section 8. Summary of Protocol for Packing Biological Samples for Shipment

Review the basic procedures:

Before you begin packing samples, refer to the checklist on page 7 regarding all relevant permits and regulating authorities that must be advised, and all travel-related details that can facilitate or hinder the success of your shipment.

Keep an updated log of all the sample shipments you send, and include relevant details including the contents, recipient, contact names and phone numbers of agencies, etc.

Assemble required materials: The following materials can be ordered from commercial suppliers:

1. *Outer packaging (box)* in good condition. If you re-use packing containers be mindful of when the container needs to be replaced. Outer package (box) must be:
 - a. At least 4 in. length x 4 in. width x 4 in. depth (10 cm x 10 cm x 10 cm)*
 - b. Sturdy
 - c. Able to be dropped from 1.2 meters without suffering damage*
2. *An insulating styrofoam container* that fits snugly into the cardboard outer box
3. *Primary containers* (containers in direct contact with the biological material) that are watertight (e.g. vacutainer tube, screw-top cryo-tube, etc.). Primary or secondary containers must withstand 95kPa internal pressure differential⁴. Parafilm works well to ensure that tubes do not leak in transit and is mandatory for Category A shipments.
4. *Absorbent material* (e.g. cotton or paper towels) to wrap each primary container individually, in an amount sufficient to absorb the entire contents of the primary container(s) in the event that they should leak or break.
5. *Watertight secondary container* (e.g. zip-lock bag) in which to place the individually wrapped primary containers
6. *Itemized list of contents* between the secondary and outer packaging.
7. *Diamond-shaped shipping labels* for Class 6.2 – Infectious Substances (UN 2814 or UN2900) and Class 9 – Miscellaneous Dangerous Goods (UN 1845, Dry ice).
8. *Shipping labels* for UN3373 – Biological Substances, Category B

⁴ When you purchase packing materials for shipping dangerous goods, they will have been tested to meet these specifications. However, you are only in compliance when using these packages if you follow the manufacturer's instructions on how to use them.

Shipping instructions for samples WITHOUT dry ice:

1. Prepare an itemized list of samples that you will ship and type the list in Excel or other easily read format. Keep a backup for your records.
2. Locate all the materials you will need from the above list.
3. Make sure all the samples are contained in watertight primary containers (e.g., vacutainer tube, screw-top cryo-tube, etc.)
4. Wrap each sample individually with absorbent material such as cotton or paper towels.
5. Place the individually wrapped samples in a watertight secondary container (such as a ziploc bag)
6. Place the secondary container in styrofoam container and secure it.
7. Place the Styrofoam container in the box.
8. Place the itemized list of contents between the styrofoam container and the box (outside the top of styrofoam container, but within cardboard box).
9. Seal the box with strong tape for shipping.
10. Label the box appropriately depending on the contents of the package:
 - a. Infectious Substances, Category A
 - Label with UN number and proper shipping name: **UN2814** - *Infectious substances, affecting humans* OR **UN2900** - *Infectious substances, affecting animals only*. The technical name for the pathogen of concern should be placed on the shipper's declaration.
 - Successful drop and pressure test certification labels
 - 24 hour emergency response contact and the name and telephone number of the person responsible for the shipment
 - b. Biological Substances, Category B
 - Label with UN number and proper shipping name: **UN3373** *Biological Substances, Category B*
11. Label the box with shipper's name & address and the receiver's (consignee) name & address. Include contact telephone information if applicable.
12. If shipping Category A, fill out a Shipper's Declaration and attach to the outside of the package (see instructions above).
13. Fill out the Air Waybill as per the courier's instructions.
14. Log your shipment for the record in a format agreed upon with the investigator or project supervisor.

Shipping instructions for samples WITH dry ice:

1. Prepare the itemized list of samples that you will ship and type the list in Excel or other easily read format.
2. Locate all the materials you will need from the above list.
3. Make sure all the samples are contained in watertight primary containers (e.g., vacutainer tube, screw-top cryo-tube, etc.)
4. Wrap each sample individually with absorbent material such as cotton or paper towels.
5. Place the individually wrapped samples in a watertight secondary container (such as a zip-lock bag)

6. Place the secondary container in styrofoam container and surround with dry ice. DO NOT SEAL the secondary container. You must allow for the carbon dioxide to escape as the dry ice sublimates.
7. Place the Styrofoam container in the box.
8. Place the itemized list of contents between the styrofoam container and the box (outside the top of styrofoam container, but within cardboard box).
9. Seal the outer box only for shipping, allowing gases to escape if necessary.
10. Label the box appropriately depending on the contents of the package:
 - a. Infectious Substances, Category A
 - Diamond-shaped label for Class 6.2 – Infectious Substances
 - Label with UN number, proper shipping name, and technical name for pathogen contained: **UN2814** - *Infectious substances, affecting humans* OR **UN2900** - *Infectious substances, affecting animals only* (Successful drop and pressure test certification labels)
 - 24 hour emergency response contact and the name and telephone number of the person responsible for the contents of the shipment
 - b. *Biological substances, Category B*
 - Label with UN number and proper *shipping name*: **UN3373** *Biological Substances, Category B*
11. Label with appropriate dry ice labels:

Diamond-shaped label for Class 9 – Miscellaneous Dry Goods

 - Label with UN number, proper shipping name, and technical name for dry ice: UN1845 *Carbon dioxide, solid (dry ice)*.
 - Include Net weight of dry ice contained within package.
12. Label the box with the shipper's name & address and the receiver's (consignee) name & address. Include contact telephone information if applicable.
13. If shipping Category A, fill out a Shipper's Declaration and attach to the outside of the package (see instructions above).
14. Fill out the Air Waybill as per the courier's instructions. Be sure to mark inside the box "contains dangerous goods" and specify the net weight of the dry ice contained within the package.
15. Log your shipment in your records in the format agreed upon with the investigator or project supervisor.

Section 9. References and Acknowledgments

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United Nations. (2009) Committee of Experts on the Transport of Dangerous Goods. Globally Harmonized System of Classification and Labeling of Chemicals. New York and Geneva.

Walsh, L. and C. Henry (2005) *Shipping Specimens to the National Veterinary Services Laboratories*. PowerPoint presentation. NVSL, USDA.

Walsh, P. and K. Ingerman (2008) *Shipping Training 2008 Transportation of Dangerous Goods*. PowerPoint presentation. Global Health Program, WCS.

A technical review incorporating recent modifications to USG regulations was conducted in June 2013, by Kenneth Conley, Wildlife Pathologist, Wildlife Conservation Society. The material was originally compiled and edited with the assistance of Sarah Pilzer

QGIS Users Guide

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Objective:

- To understand basic GIS terminology and theory.
- To learn the geographic user interface for QGIS.
- To learn how to import files and make layers in QGIS.
- To produce and export high quality maps for presentations and publications.

Section 1. Introduction to GIS

Geographic Information Systems (GIS) are the combination of computer hardware, software, data, and personnel, which makes it possible to describe and characterize the earth and other geographies for the purpose of visualizing and analyzing spatially referenced information. Broken down, the components of GIS are:

Geographic: a location at various levels of aggregation, e.g. a country, city, or a protected national forest.

Information: information about the location, e.g. population, number of sick or healthy people, species of animals sampled, etc.

Systems: helps capture, store, manipulate, analyze, manage, and present the above.

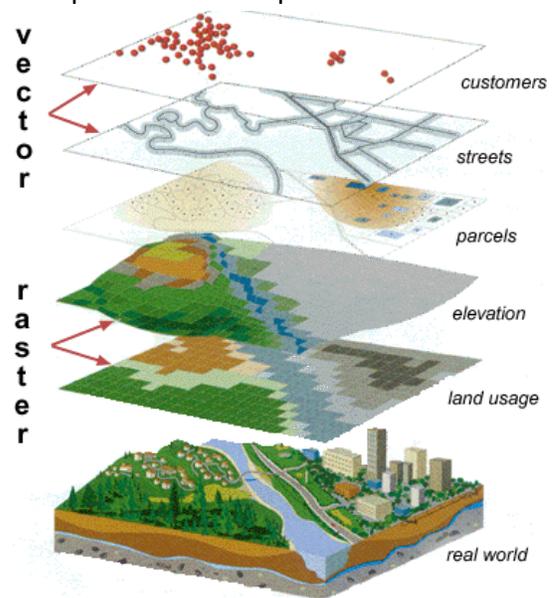
In short, GIS combines geographic data (latitude and longitude) and non-geographic information about the location (attributes like population or land use) with the help of software like ArcGIS, QGIS, GRASS, etc. This tutorial is designed to introduce you to QGIS, which is a free, open-source software. You will gain an understanding of some basic processes that can be executed using QGIS, like opening digital maps on your computer, creating new spatial information to add to a map, and creating printed maps customized to your needs.

Before you get started, it will be helpful to acquaint yourself with some basic GIS concepts.

A common function of GIS Applications is to display **map layers**, which are spatial data representing something in the real world — a roads layer for example will have data about the street network. Map layers mainly consist of two types of spatial data to represent their information:

1. **Vector data** represent information as points, lines, and areas and are most appropriate when used to represent discontinuous data, e.g. houses, rivers, national parks, etc.
2. **Raster data** represent space as a continuous field consisting of squares (called pixels) of a standard size, and are most appropriate when used to represent continuous data, e.g. land cover, soil maps, etc.

Fig1. Different GIS information layers, stacked together.Source: National Coastal Data Development Centre (NCDDC), National Oceanic and Atmospheric Administration (NOAA), USA 



Every GIS dataset has a **coordinate system**, which is a reference system used to represent the locations of geographic features, imagery, and observations such as GPS locations within a common geographic framework. Put simply, a coordinate system helps enable every location on the earth to be specified by a set of coordinates of known location (latitude and longitude) on a grid. Data is represented using either a geographic coordinate system or a projected coordinate system:

1. A **Geographic coordinate system (GCS)** uses a three-dimensional spherical surface to define locations on earth via latitude and longitude values.

Horizontal lines of latitude run parallel to the equator. Lines of latitude in the northern hemisphere are positive ranging from 0 degrees at the equator to 90 degrees at the North Pole. Lines of latitude in the southern hemisphere are negative ranging from 0 degrees at the equator to -90 degrees at the South Pole. Vertical lines of longitude are parallel to the prime meridian, ranging from 0 degrees (at the Prime Meridian) to a positive 180 degrees in the eastern hemisphere and 0 to -180 degrees in the western hemisphere.

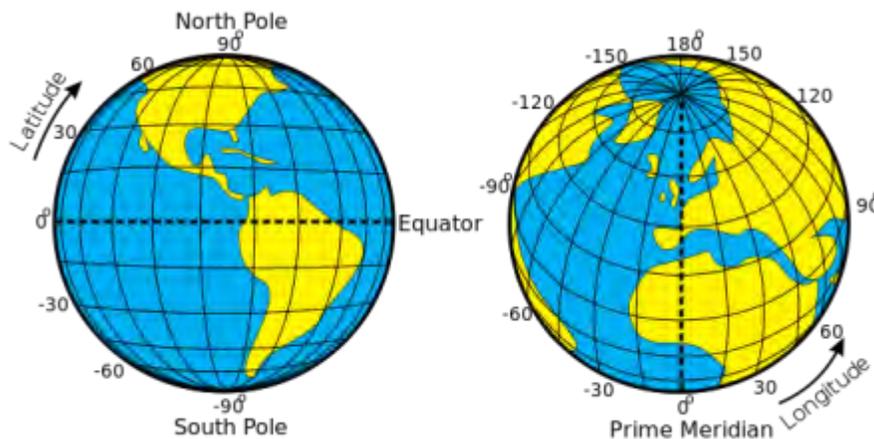


Fig 2. The geographic coordinate system.

(Source: <http://www.plux.co.uk/converting-radians-in-degrees-latitude-and-longitude/>)

2. A **projected coordinate system** is defined on a flat, two-dimensional surface. Unlike a geographic coordinate system, a projected coordinate system has constant lengths, angles, and areas across the two dimensions. This enables accurate measurements of distance, angles, and areas.

Since projected coordinate systems are based on a sphere that is projected onto a flat plane, the coordinate system defines, with the help of coordinates, how the two-dimensional, projected map in your GIS is related to real places on the earth.

Projected coordinate systems are often referred to as projections, and the choice of which coordinate system to use (there are many options, common ones being Universal Transverse Mercator (UTM), Lambert Conformal Conic, and Albers Equal Area) depends on the regional extent of the area your work is in and on the analysis you want to conduct. A discussion about the different types of projected coordinate systems is beyond the scope of this basic introductory section; for more information, refer to the glossary and helpful links sections of this tutorial. **It is important to understand, however, that a projected coordinate system is always based on a geographic coordinate system that is based on a sphere or spheroid (which represents earth).**

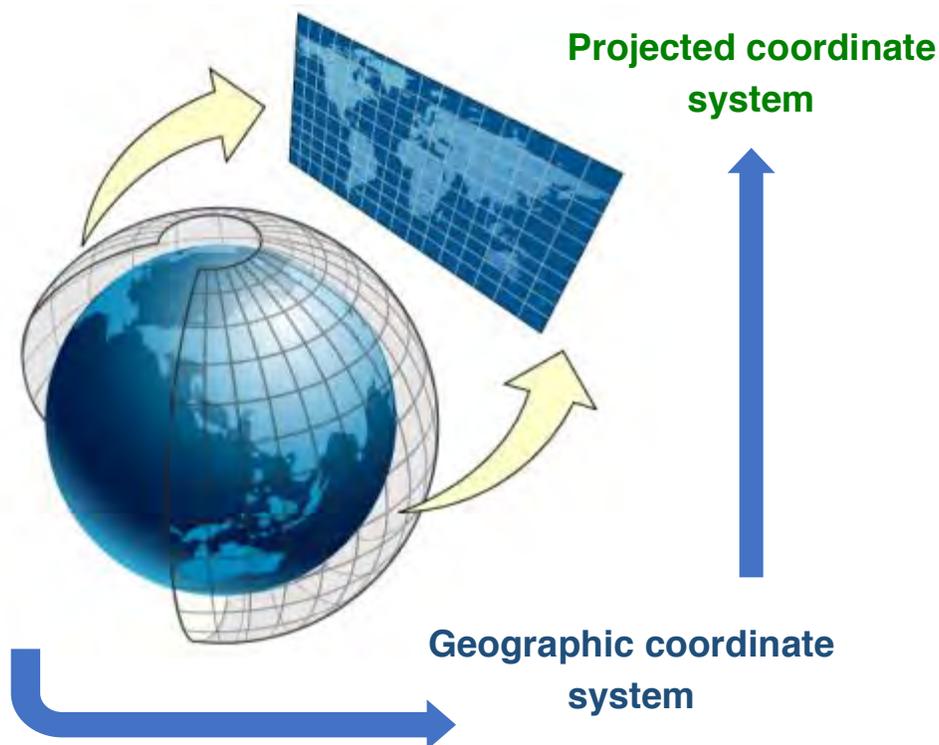


Fig 3. Mapping spherical data to a flat surface.
(Adapted from: <https://developer.apple.com>, "Displaying Maps")

Uses of GIS:

GIS has been widely employed in government, business and commerce, transportation, health, and natural resource sectors. It has been used for urban planning, resource allocation, surveying, emergency and disaster management, and tracking the occurrence and spread of disease.

Apart from displaying geographic data, one of its most notable functions is the ability to synthesize or combine geographic information to analyze and understand underlying patterns

that may not be obviously apparent. This process of finding patterns and trends to inform decision-making is called spatial analysis. Using GIS, we can:

- Summarize data associated with geographic features.
- Find locations that meet specified criteria.
- Identify, quantify and visualize spatial patterns.
- Combine geographic data for further analysis.

Section 2. QGIS Official Training Guides

The official training and user guides for QGIS are very detailed in all aspects of using QGIS. Listed below are links to User Guides and Training Manuals in different languages that may be helpful for you if you wish to further your knowledge about everything QGIS has to offer. *Note: these guides are for QGIS Version 2.6; however, will still be useful in version 2.8 as the user interface has not changed.*

Brazilian Portuguese: User Guide - http://docs.qgis.org/2.6/pdf/pt_BR/QGIS-2.6-UserGuide-pt_BR.pdf

Training manual - http://docs.qgis.org/2.6/pdf/pt_BR/QGIS-2.6-QGISTrainingManual-pt_BR.pdf

English: User Guide - <http://docs.qgis.org/2.6/pdf/en/QGIS-2.6-UserGuide-en.pdf>

Training Manual - <http://docs.qgis.org/2.6/pdf/en/QGIS-2.6-QGISTrainingManual-en.pdf>

French: User Guide - <http://docs.qgis.org/2.6/pdf/fr/QGIS-2.6-UserGuide-fr.pdf>

Training manual - <http://docs.qgis.org/2.6/pdf/fr/QGIS-2.6-QGISTrainingManual-fr.pdf>

Hindi: User Guide - <http://docs.qgis.org/2.6/pdf/hi/QGIS-2.6-UserGuide-hi.pdf>

Training manual - <http://docs.qgis.org/2.6/pdf/hi/QGIS-2.6-QGISTrainingManual-hi.pdf>

Indonesian: User Guide - <http://docs.qgis.org/2.6/pdf/id/QGIS-2.6-UserGuide-id.pdf>

Training manual - <http://docs.qgis.org/2.6/pdf/id/QGIS-2.6-QGISTrainingManual-id.pdf>

Spanish: User Guide - <http://docs.qgis.org/2.6/pdf/es/QGIS-2.6-UserGuide-es.pdf>

Training manual - <http://docs.qgis.org/2.6/pdf/es/QGIS-2.6-QGISTrainingManual-es.pdf>

Note: When you download and install QGIS, 6 different programs will be downloaded. They are:

QGIS Desktop: *This is the program you will be using for this guide. Here you can create, edit, visualize, analyze, and publish geospatial information. QGIS Desktop can be downloaded on Windows, Mac, Linux, BSD, and Android devices.*

QGIS Browser: *This program allows you to browse and preview your data and metadata (data that describes and summarizes basic information about other data).*

GrassGIS: *This is another free and open source GIS software program. We will not be using this; however, QGIS does have the ability through the tool box to use layers and tools GRASS has to offer.*

MSYS: This program allows the building of applications; we will not be using this program in this guide.

OSGeo4Shell: This is a package of open-source geospatial tools for Windows; we will not be using this program.

Saga GIS: This is another free and open source GIS software program; we will not be using this software in this guide.

Section 3. Download QGIS

1. Go to the QGIS website to download the software.

a) <http://www.qgis.org/en/site/>

i) Click the “**Download Now**” button

ii) Choose your platform (i.e. Windows or Mac)

(1) **For Windows**

(a) Choose the **QGIS Standalone Installer Version 2.8.1 (32 bit)** OR **QGIS Standalone Installer Version 2.8.1 (64 bit)** (or current version) depending on your operating system.

(b) Save the .exe file to your computer.

(2) Double click on the .exe you just downloaded and follow the instructions given for installing the software.

(3) **For Mac**

(a) Click on the **KyngChaos QGIS download page**

(b) Click on **GDAL Complete 1.11 framework package**

(i) Click on the **GDAL 1.11 Complete [39.0 MiB] 2015-3-4** version (or current version) to download the file. Open the file you just saved.

(ii) Double click on the **GDAL Complete.pkg** file and follow the instructions to install it on your computer.

(c) Go back to the **KyngChaos QGIS download page**

(d) Click on **Matplotlib Python module**

(i) Go down the page and click on **matplotlib 1.3.1-2 [35.8 MiB] (Lion+)** to download the file. Open the file you just saved.

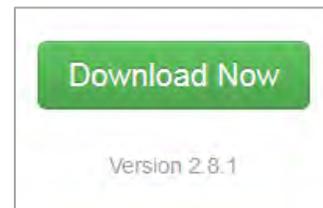
(ii) Double click on the **matplotlib.pkg** file and follow the instructions to install it on your computer.

(e) Go back to the **KyngChaos QGIS download page**

(i) Click on **QGIS 2.8.1-1 [163.8 MiB]** to download the file. Open the file you just saved.

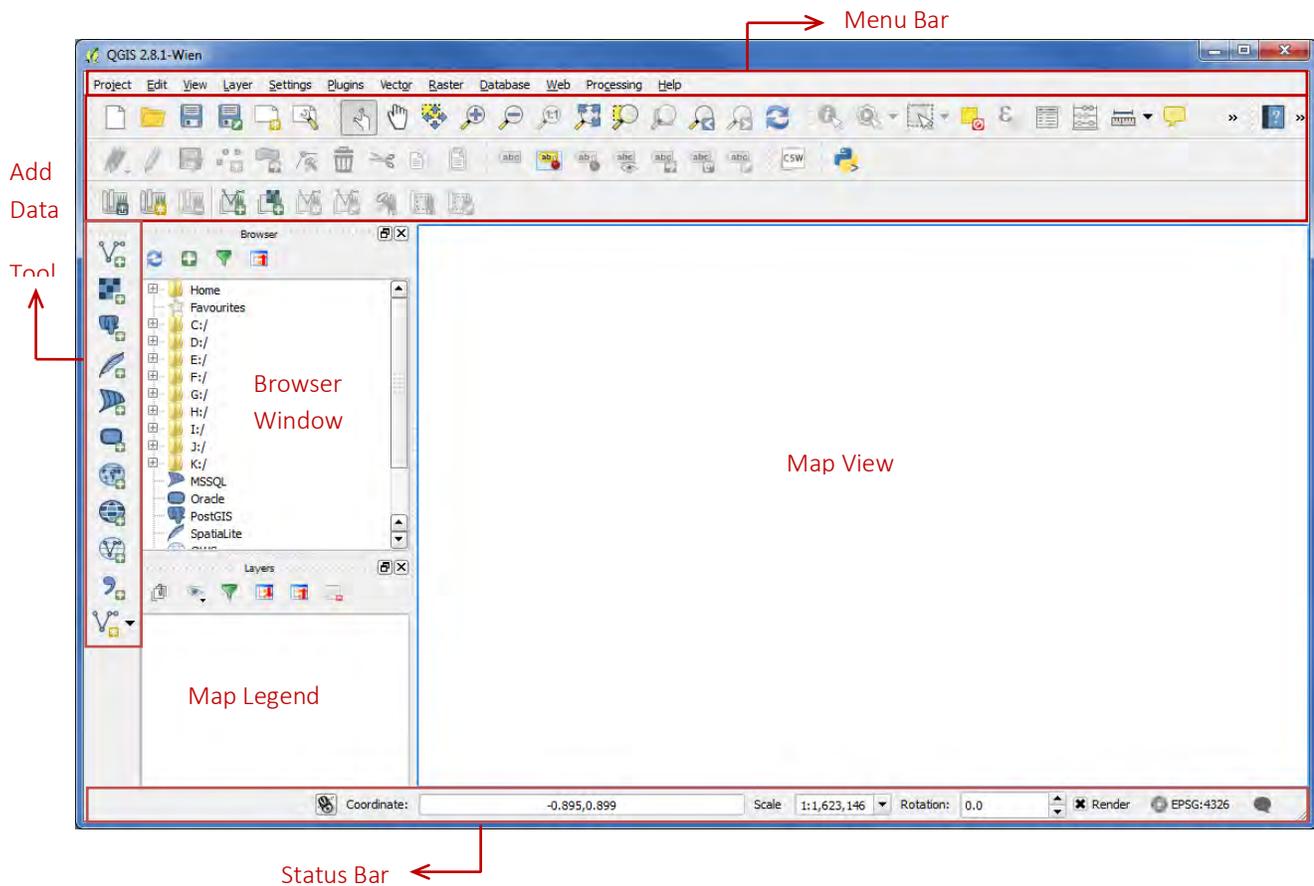
(ii) Double click on **Install QGIS.pkg** and follow the instructions to install it on your computer.

Visit the following YouTube link for a step by step video guide for installing QGIS on a Mac: <https://www.youtube.com/watch?v=AocxUop1RTE>

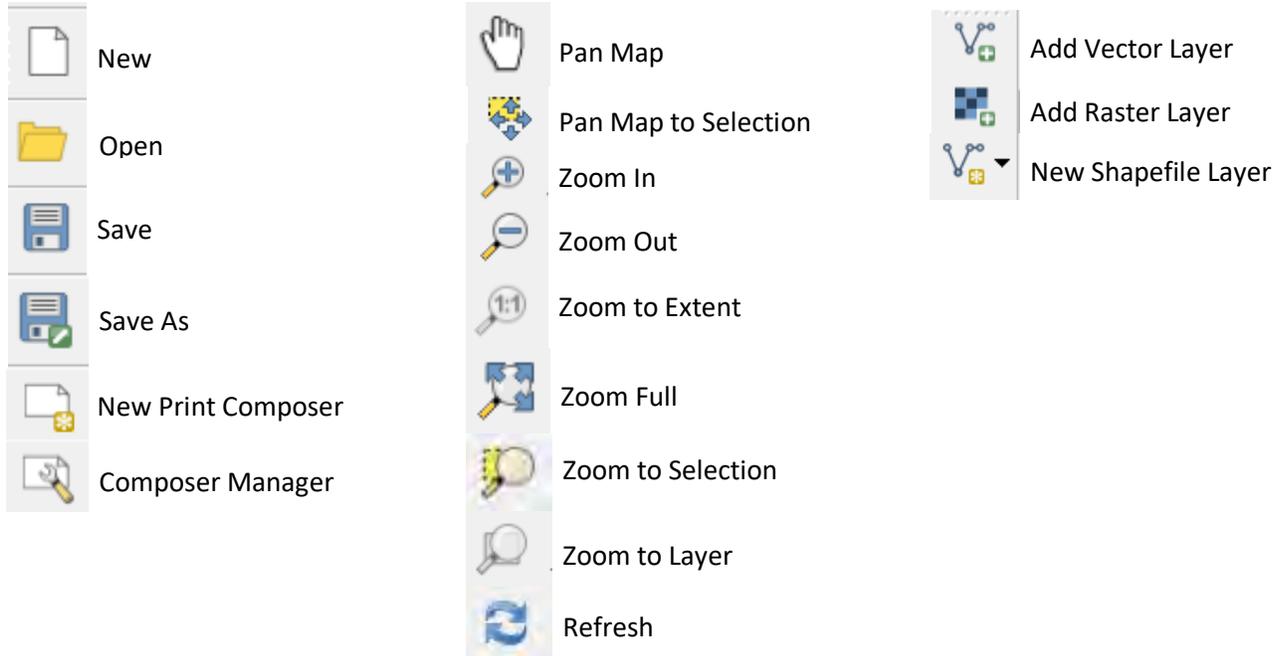


Section 4. Introduction to QGIS Interface

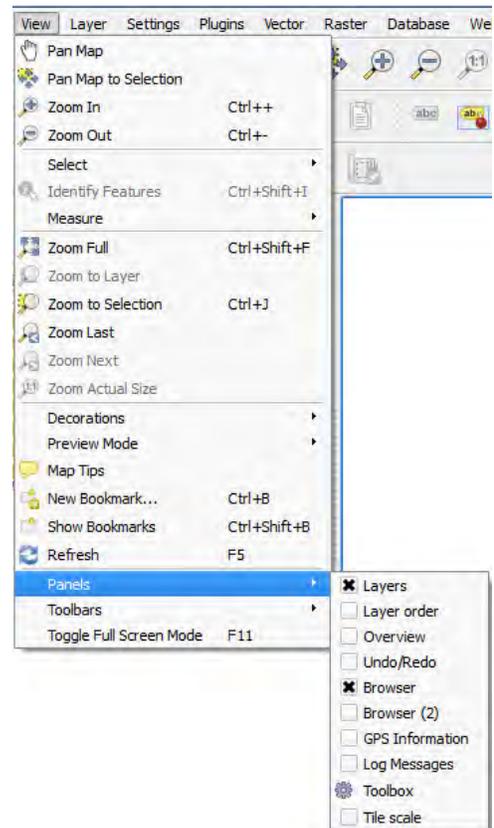
- 1) Open **QGIS Desktop** by clicking the icon added to the desktop, through the Start menu in Windows, or through the Application folder on the Mac. *Note: All screen grabs in this guide are from the Windows platform, there may be some minor differences in the Mac version of the software.*
 - a) Components of the Geographic User Interface (GUI) when you open QGIS.



b) Some important and useful tool icons.



c) The pull-down menus in the **Menu Bar** have the same tools as listed above plus more. If, however, you happen to close the **Map Legend** or **Browser Window**, you can reopen them by going to **View/Panels** and then click on the box next to the panel you would like to open.



Section 5. Data File Organization

For the remainder of the user guide, you will be using data which is provided with this guide. You will want to unzip the folder called *QGIS_Training_Data*. Within the *Data* folder you will find an Excel file and two folders, *Rasters* and *Vectors*, containing layers which you will be adding to QGIS Desktop for the final goal of producing a map export. ***Note: Data provided with this guide is for the purpose of teaching the user QGIS and in no way represents sampling locations in Uganda for the PREDICT project. Rasters (land use layers) and shapefiles (Uganda district and protected areas) are publicly available and website addresses can be found in Section 8. Data Download of this guide. The Rodent_Sampling_Sites.xlsx contains 4 points haphazardly chosen and in no way represents sampling locations in Uganda.***

QGIS_Training_Data

Rodent_Sampling_Sites.xlsx

Raster folder:

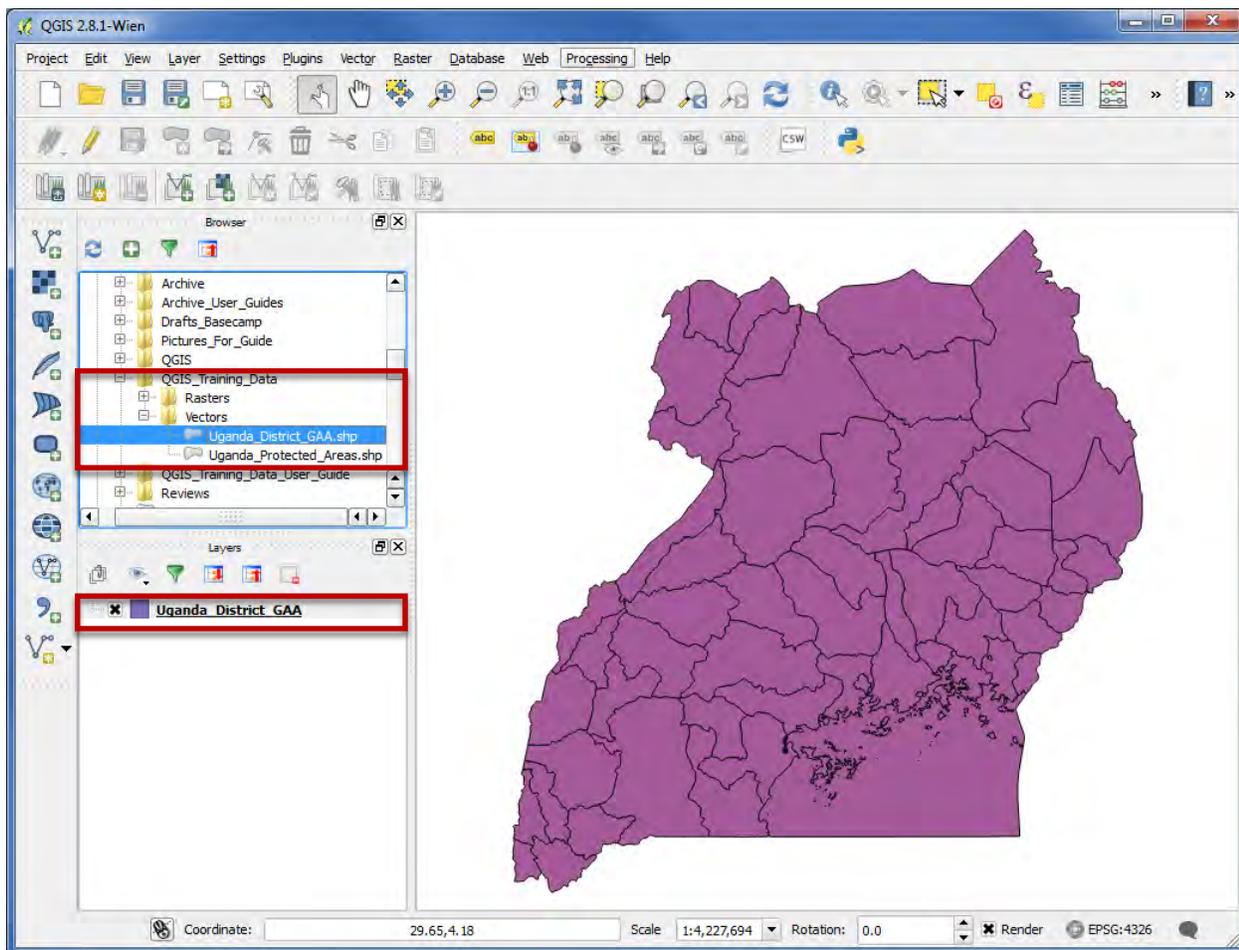
1. Four land use layers (*Africa_LandUse_31, _32, _43, and _44*) for Uganda from the International Steering Committee website. Each layer consists of the following components:
 - a. .tfw: contains the geographic information
 - b. .tif: image file
 - c. .xml: contains metadata for the file
2. *International Steering Committee Website.doc*: explains the symbology of the land use layers

Vector folder:

1. *Uganda_District_GAA* (downloaded from the Global Administrative Areas website) Components of the *Uganda_District_GAA* shapefile includes:
 - a. .dbf – attribute format; columnar attributes for each shape
 - b. .prj – projection format; the coordinate system and projection information
 - c. .sbn – a spatial index of features
 - d. .sbx – a spatial index of features
 - e. .shp – shape format; contains the feature geometry
 - f. .xml – geospatial metadata in XML format
 - g. .shx - shape index format; positional index of the feature geometry
2. *Uganda_Protected_Areas* (downloaded from the Protected Planet website) Components of the *Uganda_Protected_Areas* shapefile includes:
 - a. .dbf – attribute format; columnar attributes for each shape
 - b. .prj – projection format; the coordinate system and projection information
 - c. .sbn – a spatial index of features
 - d. .sbx – a spatial index of features
 - e. .shp – shape format; contains the feature geometry
 - f. .shx - shape index format; positional index of the feature geometry

Section 6. Vectors

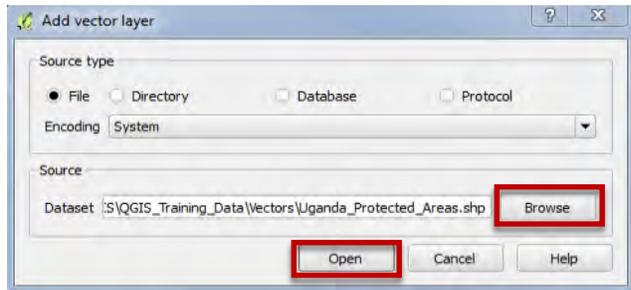
1. **Adding vector layers to your map:** There are two different ways you can add vector layers in QGIS, either by going through the Browser Window or using the Add Vector Layer button on the side of the QGIS screen.
 - a) **Browser window in QGIS** – Browse to where you saved the *QGIS_Training_Data* folder, open the *Vector* folder, and double click on *Uganda_Districts_GAA.shp*. Notice the file is now listed in the **Map Legend** window and visualized in the **Map View** window. *Note: The color of Uganda (or any vector file) will probably be different on your screen. We will discuss how to change the symbology (color) later in this guide.*





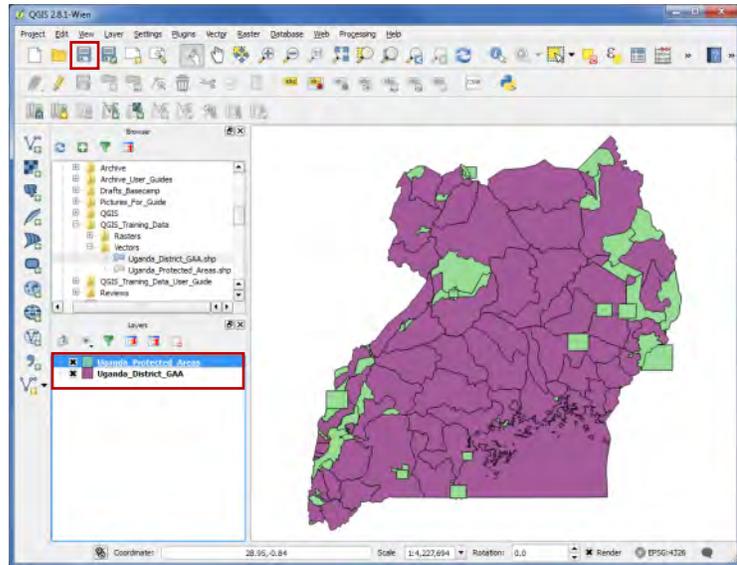
b) **Add vector button:** click the **Add Vector Layer** button *Note: you can also get to the Add Vector Layer interface by going to the **Layer menu, Add Layer, Add Vector Layer**.*

- i) Click the **Browse** button
- ii) In the browse window **Navigate** to the *QGIS_Training_Data* folder, open the *Vector* folder, and select *Uganda_Protected_Areas.shp*
- iii) Hit the **Open** button
- iv) On the **Add Vector Layer**, click the **Open** button.
- v) *Uganda_Protected_Areas.shp* should now be added to the **Map View** and **Map Legend** window.
- vi) Hit the **Save** button to save the changes you have made to the project.



c) **Saving a project:** Now that you've added a layer let's **Save** the project. *Note: It is a good habit to get into saving your project often as you work. QGIS has been known to crash, causing you to lose work you have completed from the last time you saved.*

- i) Click the **Save As** button and browse to the location where you would like to save your project.
- ii) **File Name** – Give your QGIS project a name
- iii) Click **Save**
- iv) After the first time saving your project, if you close QGIS it will ask you if you would like to save any changes you have made.



d) **Opening a project:** Opening an existing QGIS project after closing the project

- i) Click the **Open** icon  in QGIS and
- ii) Or go to the Project drop-down menu and click open
- iii) You can also **double click** on the **project (.qgs)** from within **Windows Explorer**.

2. Making a new vector file

- e) Select the **New Shapefile Layer** button
- f) The **New Vector Layer** window will appear
 - i) Choose the **Type** of file you would like to make (point, line, or polygon). For this example for **Type** choose **Point**. *Note: A vector file can only support one type of data at a time. So, for example, if you make a point file you cannot add lines or polygons within that layer, but will have to make a new layer.*
 - ii) Under **New Attribute**
 - (1) Next to **Name** type “**Name**”
 - (2) **Type** should be “**Text Data**”
 - (3) **Width** should be “**80**”
 - (4) Hit the **Add to Attributes List** button
*Note: Once you do this you will notice that the Name attribute now appears in the list under **Attributes list**. This adds a column in the attribute table so you can name your points when you make them.*



Attributes list

Name	Type	Width	Precision
id	Integer	10	
Name	String	80	

New Vector Layer

Type

Point Line Polygon

File encoding: System

Selected CRS (EPSG:4326, WGS 84)

New attribute

Name: Name

Type: Text data

Width: 80 Precision:

Add to attributes list

Attributes list

Name	Type	Width	Precision
id	Integer	10	
Name	String	80	

Remove attribute

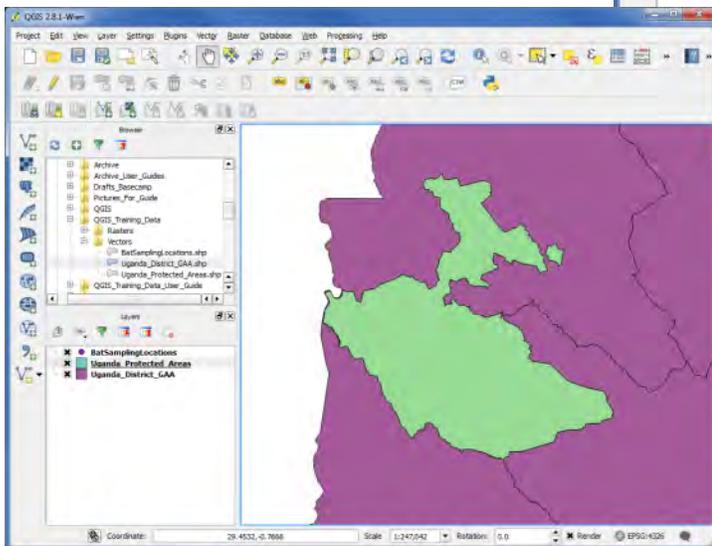
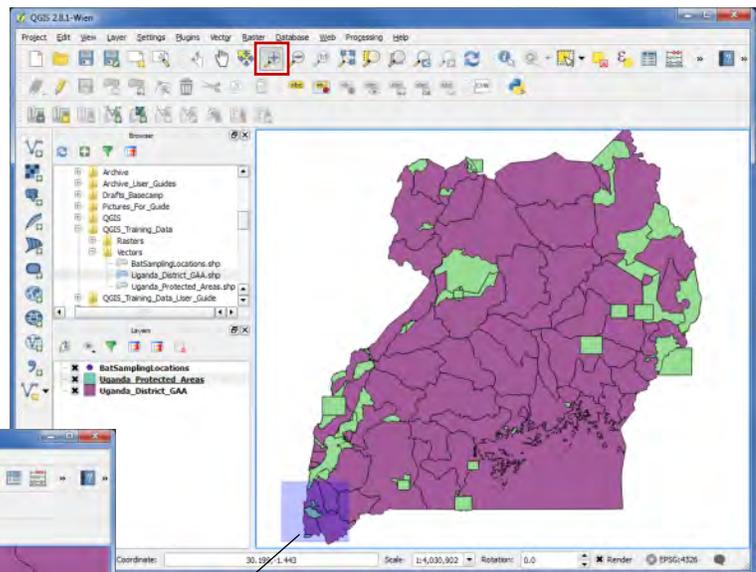
OK Cancel Help

- (5) Now make a column that allows you to input the number of bats sampled at a location. Under **New Attribute**, next to **Name** type “**NumberBats**”
- (6) **Type** should be set to “**Whole number**”
- (7) Accept the default for **Width** which should be “**10**”
- (8) Hit the **Add to Attributes List** button
- (9) Hit the **OK** button
 - (a) Browse to the *Vector* folder in *QGIS_Training_Data* and **Name** the file *BatSamplingLocations*. You have now made a file to which you can add your sampling locations (which we will do in the next section about editing vector files).

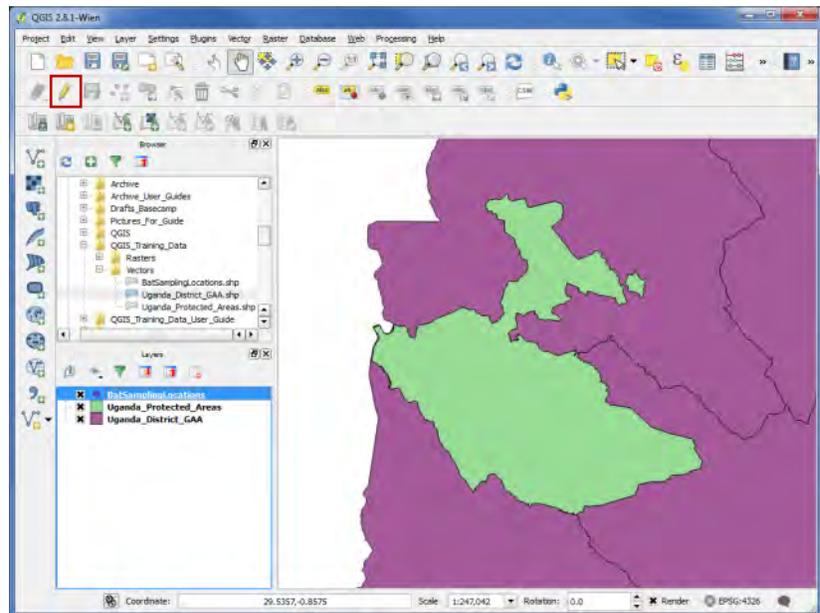
3) Editing a Vector file

- g) Before we start editing, zoom into the southwest corner of Uganda to the Bwindi Impenetrable National Park. There are a couple of different ways to zoom to an area.
 - i) You can use the **Zoom In**  tool which allows you click on the image and the image will zoom into that area at a set interval **OR** you can click and hold down the button to draw a box around the area you want to zoom into.

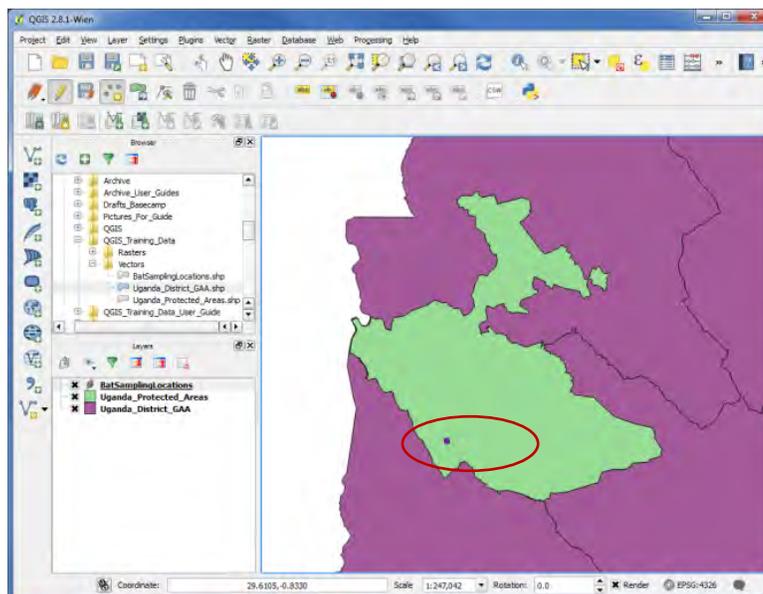
- ii) You can also zoom in and out of the image by using the wheel of the mouse. *Note: The zoom function with the wheel of the mouse is always active, meaning it can happen at any time unintentionally. It seems to happen a lot more easily with the Mac mouse than with the traditional wheel mouse for windows. To turn this function off go to the **Settings** pull-down menu and choose **Options**. Choose **Map Tools** and under **panning and zooming** change **Mouse wheel action** to **Nothing** if you don't want anything to happen or you can choose another option and slow down the zoom by changing the **Zoom Factor** to **1.1** (this is the slowest setting).*
- iii) You can zoom to a specific layer by right clicking on the layer in the **Map Legend** window and choose **Zoom to layer**.



- h) In the **Map Legend** window highlight the point vector file just created (*BatSamplingLocations*) and click the **Toggle Edit** button. 
- i) Select the **Add Feature** button  to the right of the Toggle Edit button
- j) **Click on the map** within the Bwindi Forest where you would like to add a point
- k) A box will appear giving you the opportunity to fill in the attributes for that point (these are the attributes you specified when you created the point file).



- i) **ID:** This should be a unique number for the point, so start with 1 and continue from there as you add points.
- ii) **Name:** Name of the site – For now, name it *Bwindi 1* since the point is inside the park.
- iii) **NumberBats:** Keep this blank for now. We will go in and add the data later.



- iv) Hit the **OK** button and notice the point shows up on the map. If you cannot see it, make sure that the *BatSamplingLocations* layer is the top file in the **Map Legend** window. If it is not the top layer, just select it and drag it up.

v) Add one more point in the park and 2 outside the park. Give the points a unique ID numbers and names. We will enter the number of bats at a later time so for now just click **OK**.



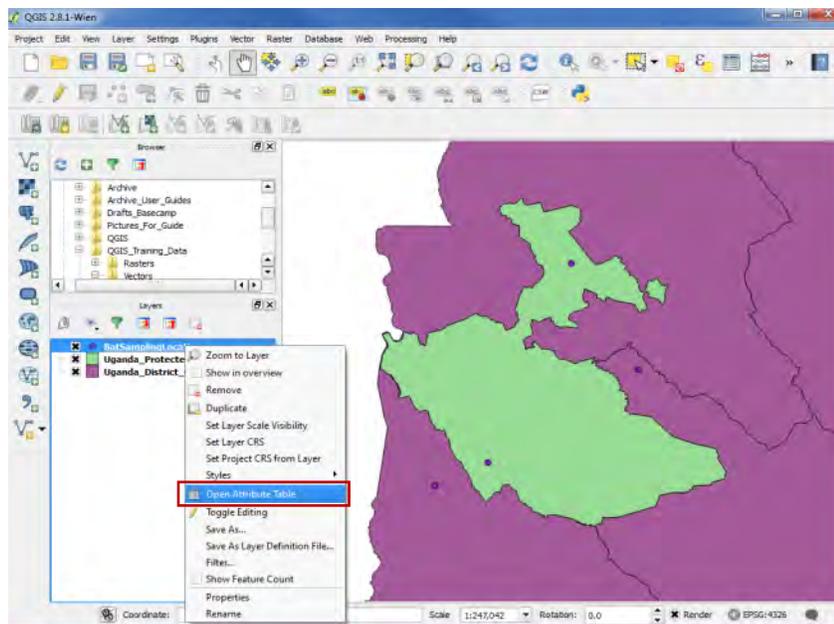
vi) Click the **Save Layer Edits** button (this does not stop the editing but just saves what you have done).

vii) If you realize that a point is in the wrong place, you can move that point using the **Move Feature(s)** button.



(a) After clicking the button, click, and drag the point you wish to move (be sure to save your edits!).

l) To edit data in the attribute table, right click on the name of the file in the **Map Legend** window and select **Open Attribute Table**.

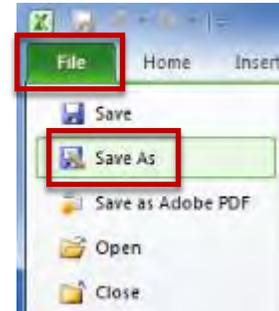


i) Once the attribute table is open you can change or delete any information. Here you can add numbers for **NumberBats** attribute. *Note: You have to be in **Editing** mode to make changes in the attribute table.*

id	Name	NumberBats
0	4 Outside 2	3
1	3 Outside 1	55
2	2 Bwindi 2	10
3	1 Bwindi 1	200

- ii) Be sure to save the changes, and then close the attribute table
 - m) When done editing, click the **Toggle Editing** button again to close the editing tool. When closing the **Toggle Editing** button it should ask you to save your edits if you didn't save before closing. Click **Yes**.
- 4) **Import an Excel file into QGIS:** This section explains how to bring an Excel file containing your data into QGIS. You can use the *Rodent_Sampling_Sites.xlsx* file in the *QGIS_Training_Data* folder for this example.
- a) Before you import an Excel file it must be saved as a comma delimited file (.csv). Steps for that follow:

i) Open the *Rodent_Sampling_Sites.xlsx* Excel file in the *QGIS_TrainingData* folder. Go to the **File** menu and click **Save As**.



ii) The **Save As** window will open,

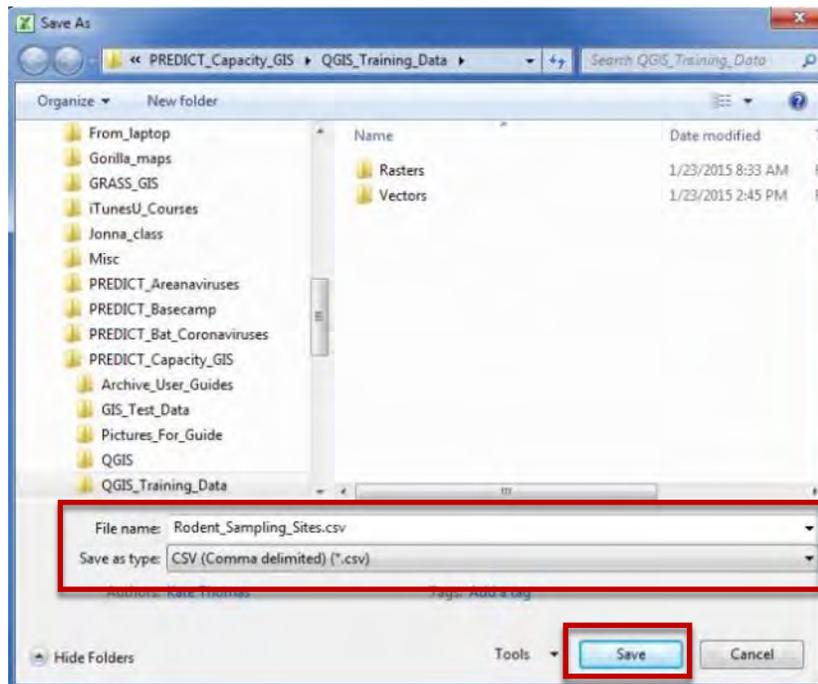
Save as type: Chose CSV (Comma delimited) **Important!** *for Mac choose Windows Comma Separated (CSV).*

(1) **Name:** Name the file if it didn't retain its name (in this case, it is already named *Rodent_Sampling_Sites*).

(2) Hit the **Save** button

(a) Click **OK** to “The selected file type does not support workbooks that contain multiple sheets” warning.

(b) Click **Yes** to the next warning “Rodent_Sampling_Sites.csv may contain features that are not compatible with CSV (Comma delimited). Do you want to keep the workbook in this format?”



(3) Go back into QGIS.

b) Back in QGIS Desktop click the **Add Delimited Text Layer** button.



i) The **Create a Layer from a Delimited Text File** box will pop up.

ii) Browse to the .csv you saved. Once you add the file, double check that information in the other boxes (i.e. X and Y fields, and data from the Excel file) automatically got filled in. If not, activate the circle next to **CSV (comma separated values)**.

(1) Double check that under the heading **Geometry definition**:

(a) **Point coordinates** is activated.

(b) **X Field = Longitude.**

(c) **Y Field = Latitude.**

*Note: If you are using Degrees Minutes Second instead of Decimal degrees be sure to activate the box next to **DMS coordinates**.*

(d) Once you have checked your settings and data, click **OK**.



File Name: D:/PREDICT_Capacity_GIS/QGIS_Training_Data_User_Guide/Rodent_Sampling_Sites.csv

Layer name: Rodent_Sampling_Sites

Encoding: UTF-8

File format: CSV (comma separated values) Custom delimiters Regular expression delimiter

Record options: Number of header lines to discard: 0 First record has field names

Field options: Trim fields Discard empty fields Decimal separator is comma

Geometry definition: Point coordinates Well known text (WKT) No geometry (attribute only table)

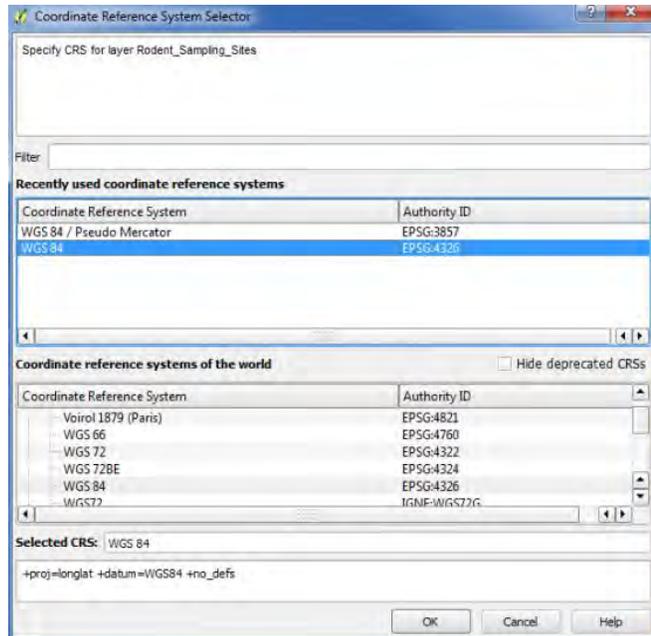
X field: Longitude Y field: Latitude DMS coordinates

Layer settings: Use spatial index Use subset index Watch file

	Name	Longitude	Latitude	Number_Rodents
1	Bwindi rodents 1	29.8117	-1.0772	54
2	Bwindi rodents 2	29.647	-1.0033	3
3	Outside rodents 1	29.6687	-0.9503	25
4	Outside rodents 2	29.7354	-1.1374	48

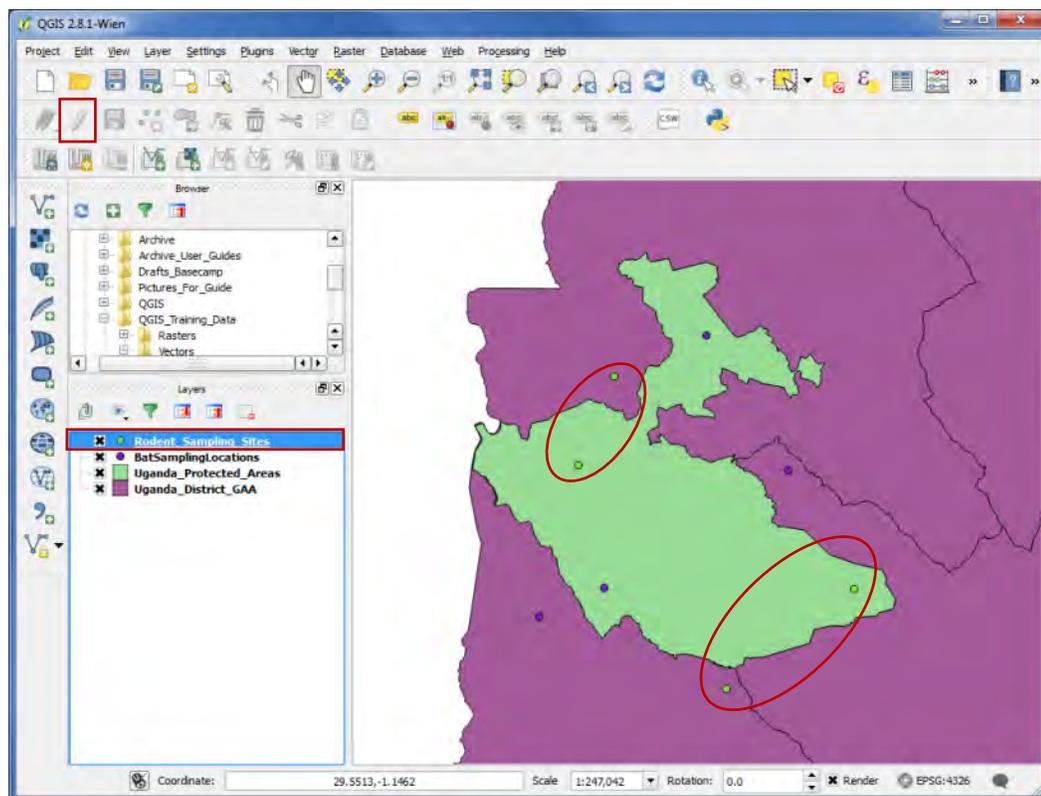
OK Cancel Help

(e) Next, you will be asked to choose your coordinate system for the file. If the coordinate system you are using is under **Recently used coordinate reference systems** you can highlight it there and click **OK**. If not, choose the correct coordinate system under **Coordinate reference systems of the world**. For this exercise, we will use WGS 84.



(f) Click **OK**.

(g) Your data points should show up in the **Map View** window and the file should now be listed in the **Map Legend** window. *Note: This file cannot be edited (toggle edit button is grayed out), and therefore must be saved as a shapefile.*



5) **Convert an imported .csv layer into a shapefile:**

a) Right click on the .csv layer you just imported into QGIS (*Rodent_Sampling_Sites*) and select **Save as**.

b) The **Save vector layer as...** box will appear.

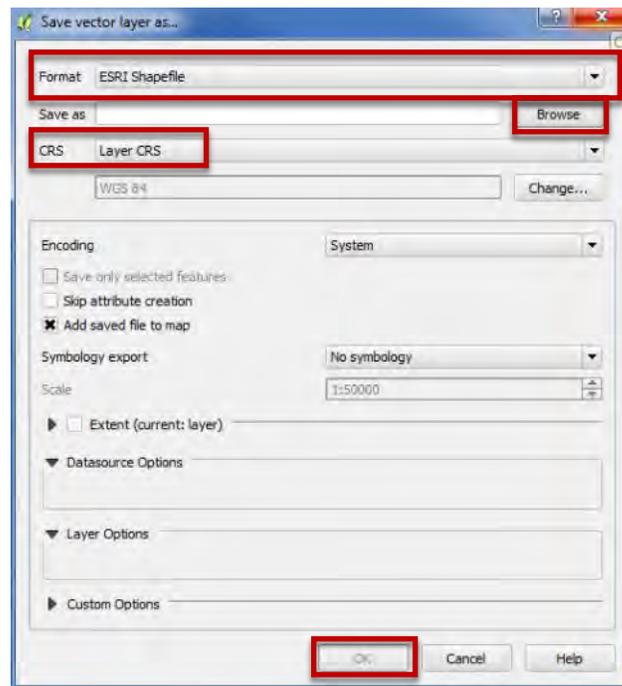
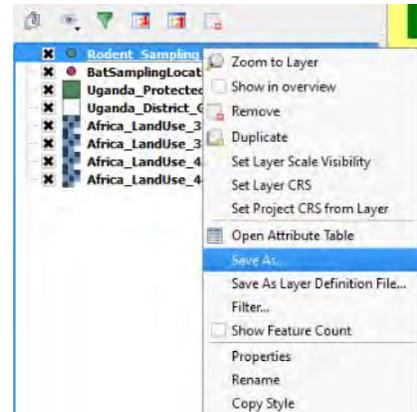
i) **Format = ESRI shapefile.**

ii) **Save as** – Click the **Browse** button and browse to the location you would like to save the file. Be sure to name the file and click **Save**.

iii) **Set the CRS (coordinate reference system) to Layer CRS** if you specified the coordinate system when importing the .csv.

iv) Accept all other defaults and click **OK**. *Note: There are now 2 files called Rodent_Sampling_Sites. The one on the top is the new shapefile. You can remove the second one by right clicking and choosing Remove.*

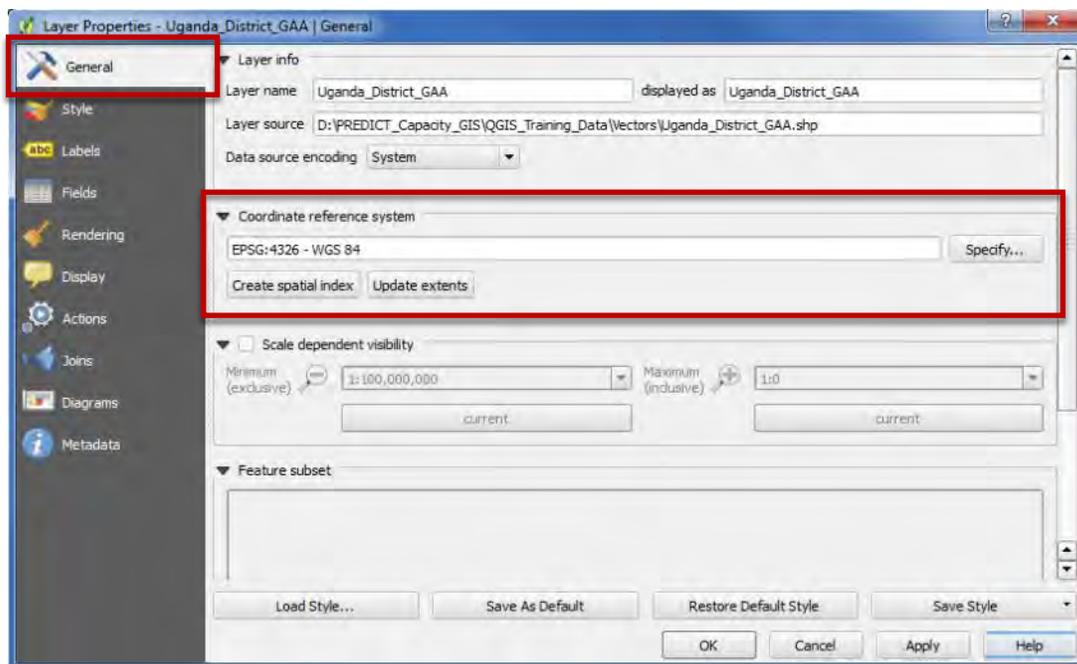
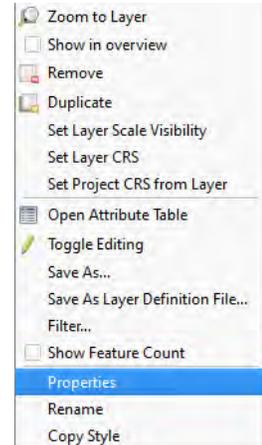
*Notice that when you now click on the shapefile just added to the **Map Legend** window, the **Toggle Edit** button is now selectable. You can now make edits to the point locations and attribute table the same way as listed above.*



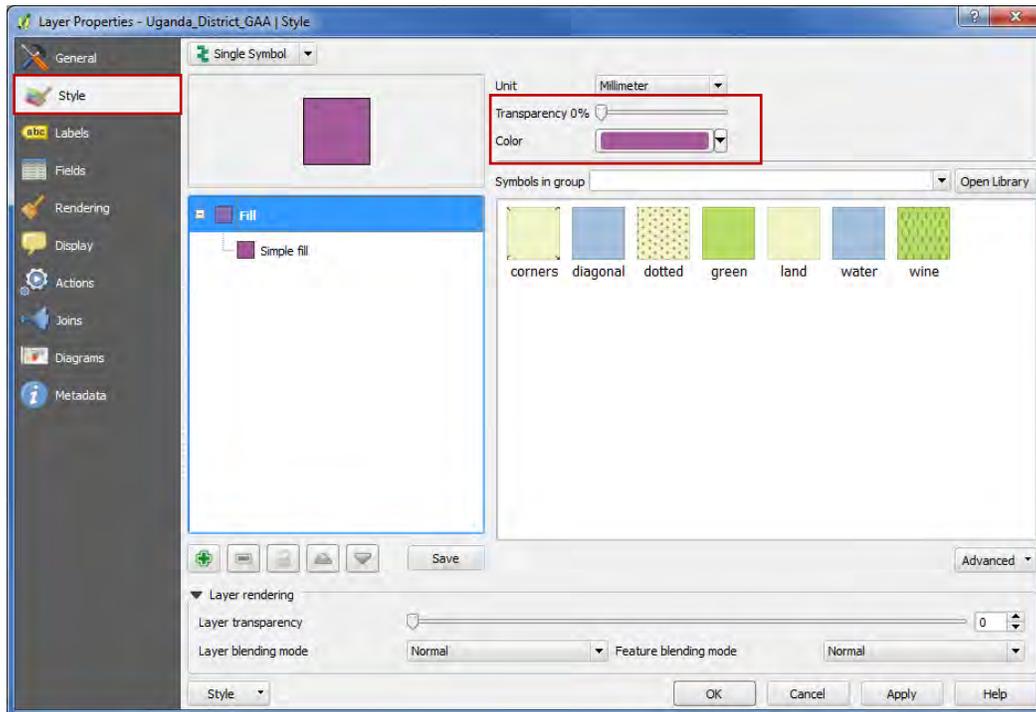
6) **Properties of a vector layer:** Once you add a vector you can change the symbology (i.e. color), coordinate system, and label features for a file by going to the **Properties** menu.

c) In the **Map Legend** window right click *Uganda_District_GAA* and choose **Properties** from the popup menu. Below are descriptions of some of the tabs which might be useful.

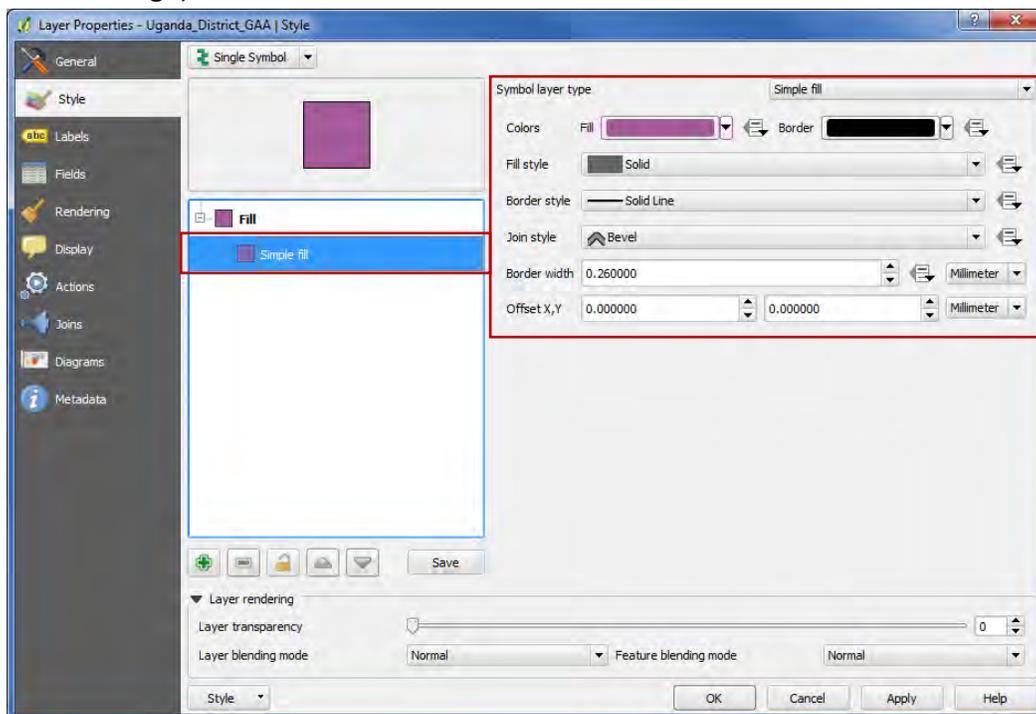
i) **General** tab: Here you can see the layer name, location, and coordinate system (which you can change by clicking **Specify**)



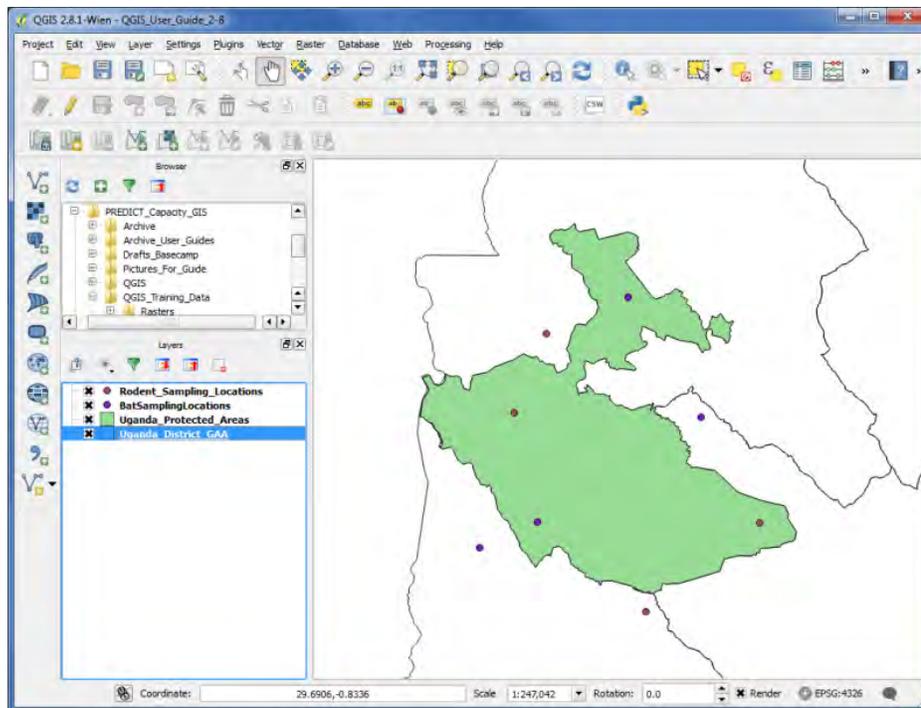
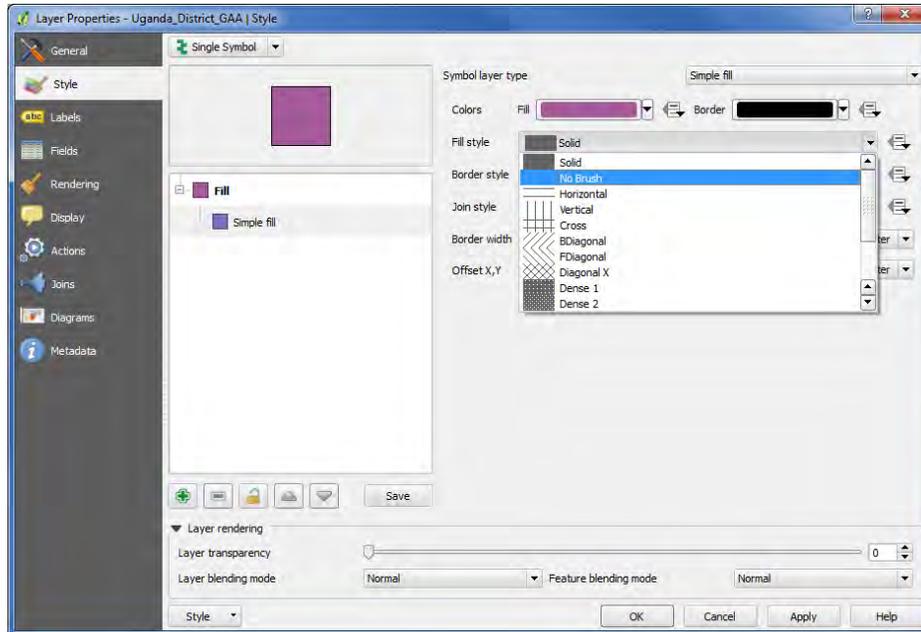
- ii) **Style** tab: Here you can change the symbology (i.e. color and transparency) of the layer. While here we are going to change the **Fill** for this layer.



- (1) Click on **Simple Fill** (you will notice the options on the right side of the screen will change).

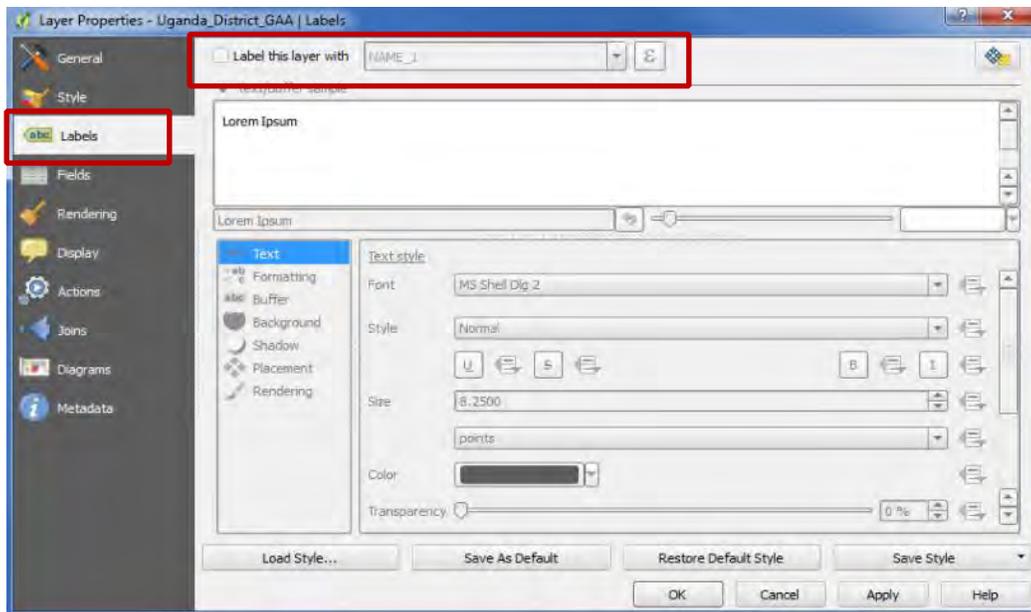


- (2) On the right side of the screen click the arrow next to **Fill style** and choose **No Brush**. Click Ok. Notice in the **Map View** window you now only see the lines from the *Uganda_Districts_GAA* layer; any color is visualizing the *Uganda_Protected_Areas* layer.

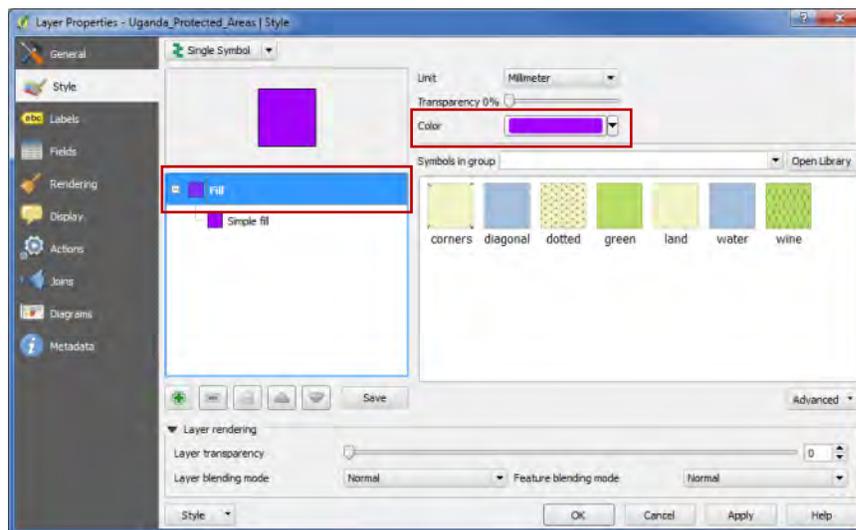


iii) **Labels** tab: Open the Properties tab again for *Uganda_District_GAA* as described on page 21, and select the **Labels** tab. Here you can add labels based on an attribute by activating the **Label this layer with** and then choosing the attribute in the pull down menu.

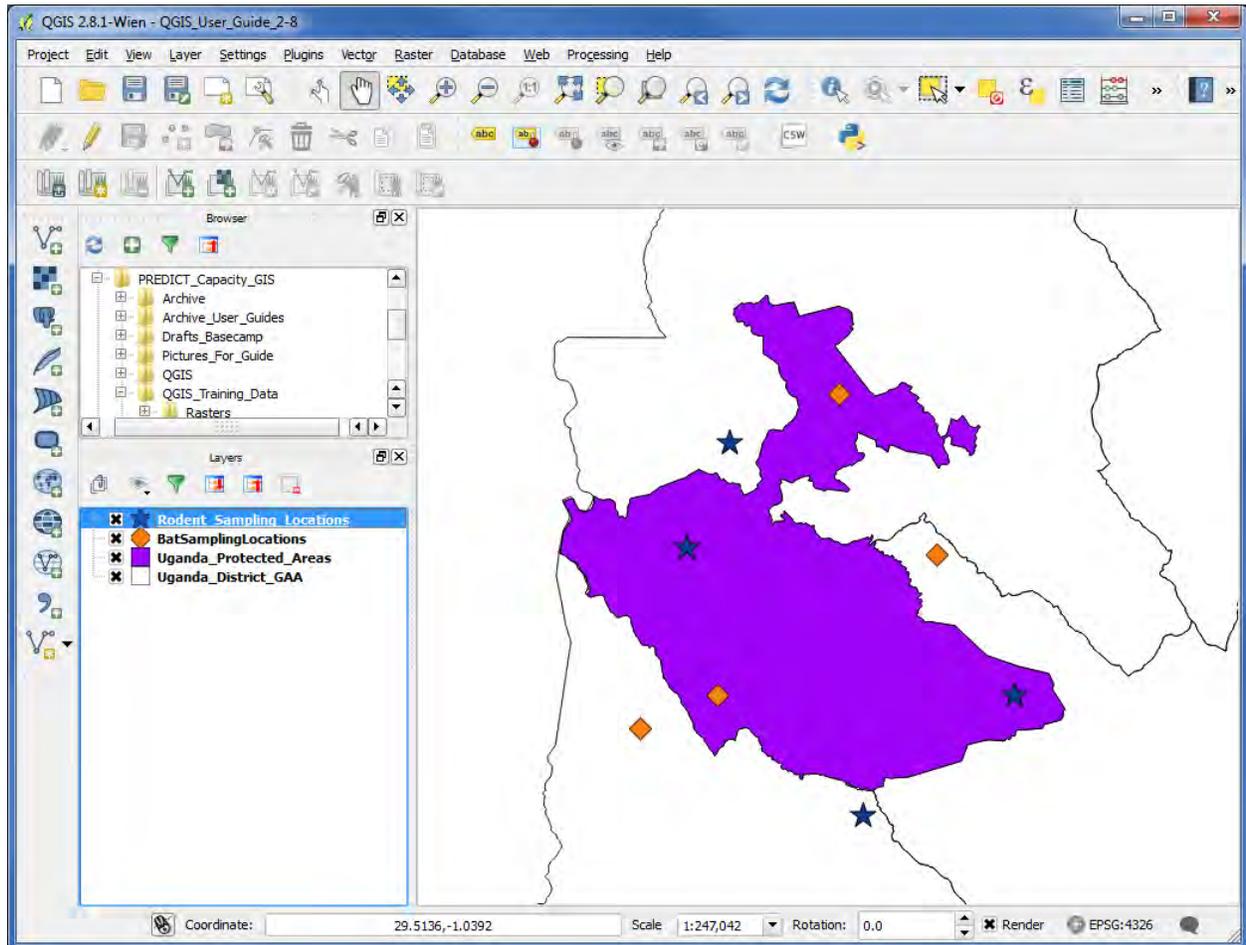
- (1) Click on the box next to **Label this layer with**. Choose **"NAME_1"** from the pull down menu. Hit **Apply**. Notice that the names of the districts are now visualized on the map. You can then change font, color, etc. of the label as well. Deactivate **Label this layer with** by clicking on the box in front of it before proceeding.



iv) Now change the symbology for *Uganda_Protected_Areas*, *BatSamplingLocations*, and *Rodent_Sampling_Locations*. This time, however, since we want the *Uganda_Protected_Areas* and sampling locations to have color you can simply choose a different color without choosing **Simple Fill**.



- v) For the point locations feel free to choose different colors, symbols, and sizes, all of which can be found in the on the Style tab as well.
- vi) Save your project if you haven't recently. *Note: From this point on the images in this guide might differ a little visually depending on the symbols you have chosen to represent your data.*



Section 7. Rasters

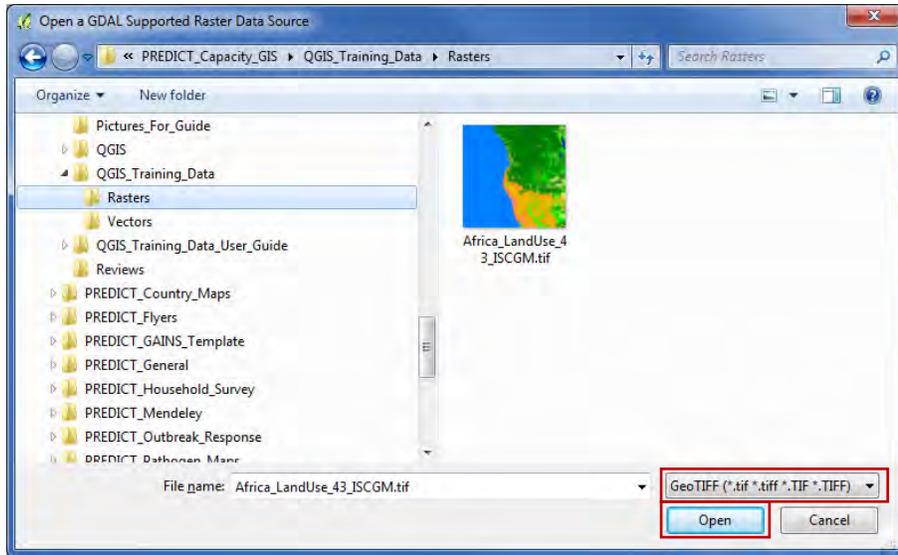
1) **Adding a Raster:** Click the **Add Raster Layer** button

a) We are going to add land use layers for Uganda. Browse to the *Raster* folder within the *QGIS_Training_Data* folder.

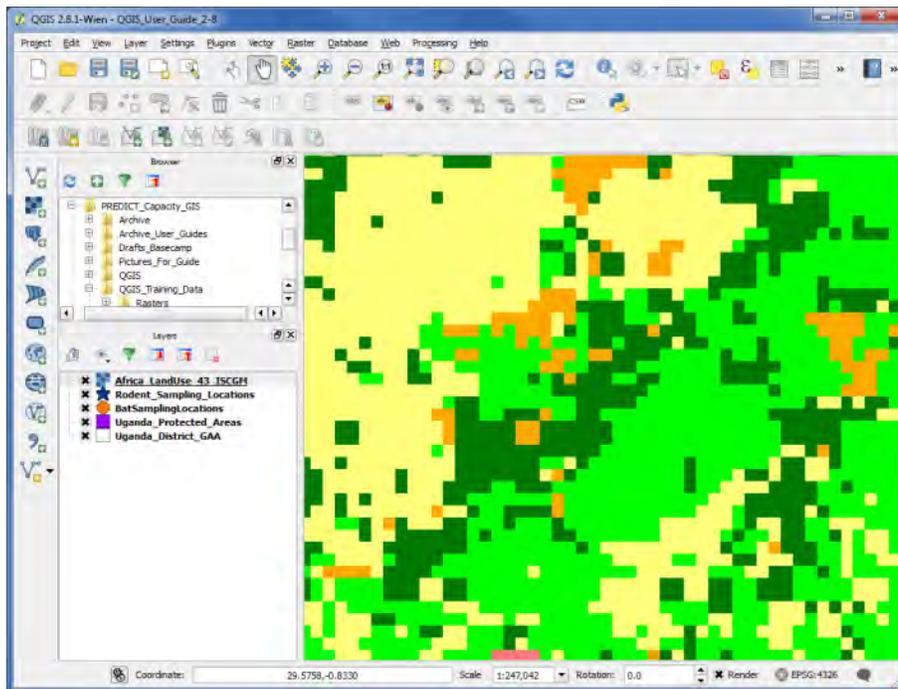
i) Next to **File Name** (where it says **All files**) click the arrow and choose **GeoTIFF**

ii) Select the file *Africa_LandUse_43_ISCGM.tif*

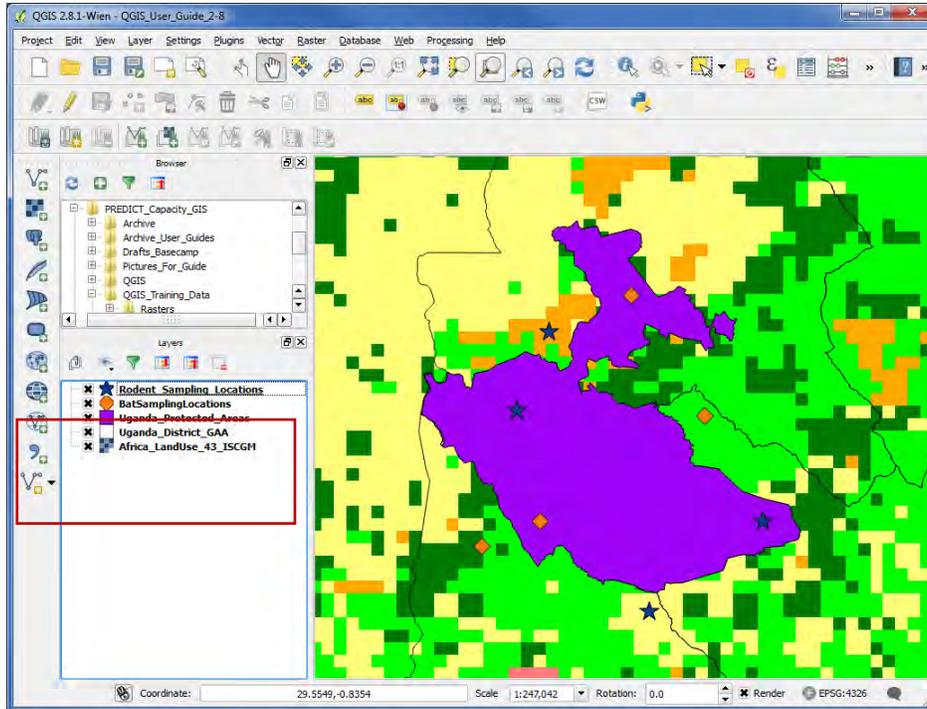
iii) Click **Open**



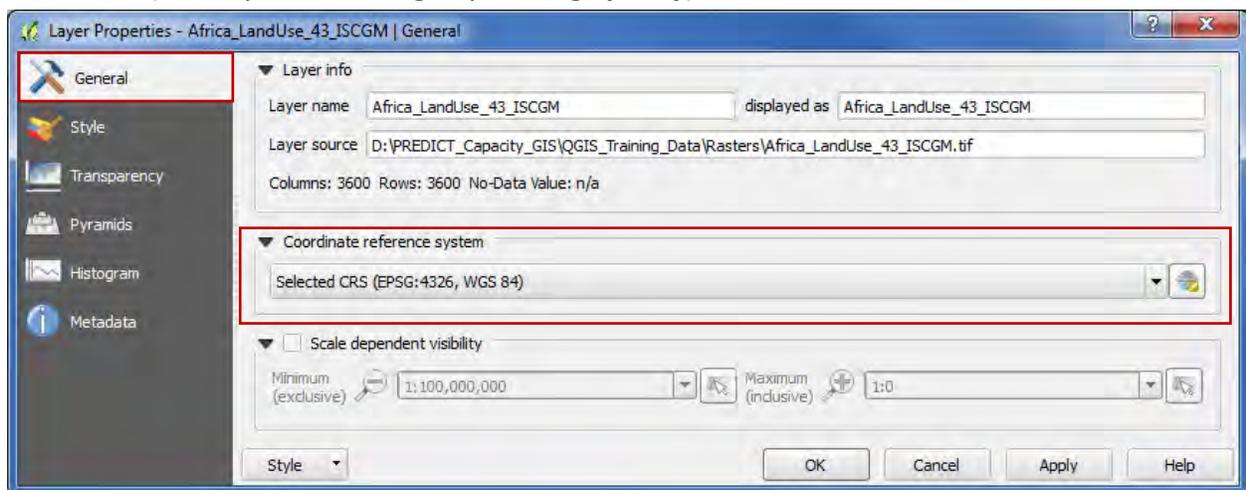
iv) The file should be added to the **Map View** and **Map Legend** windows



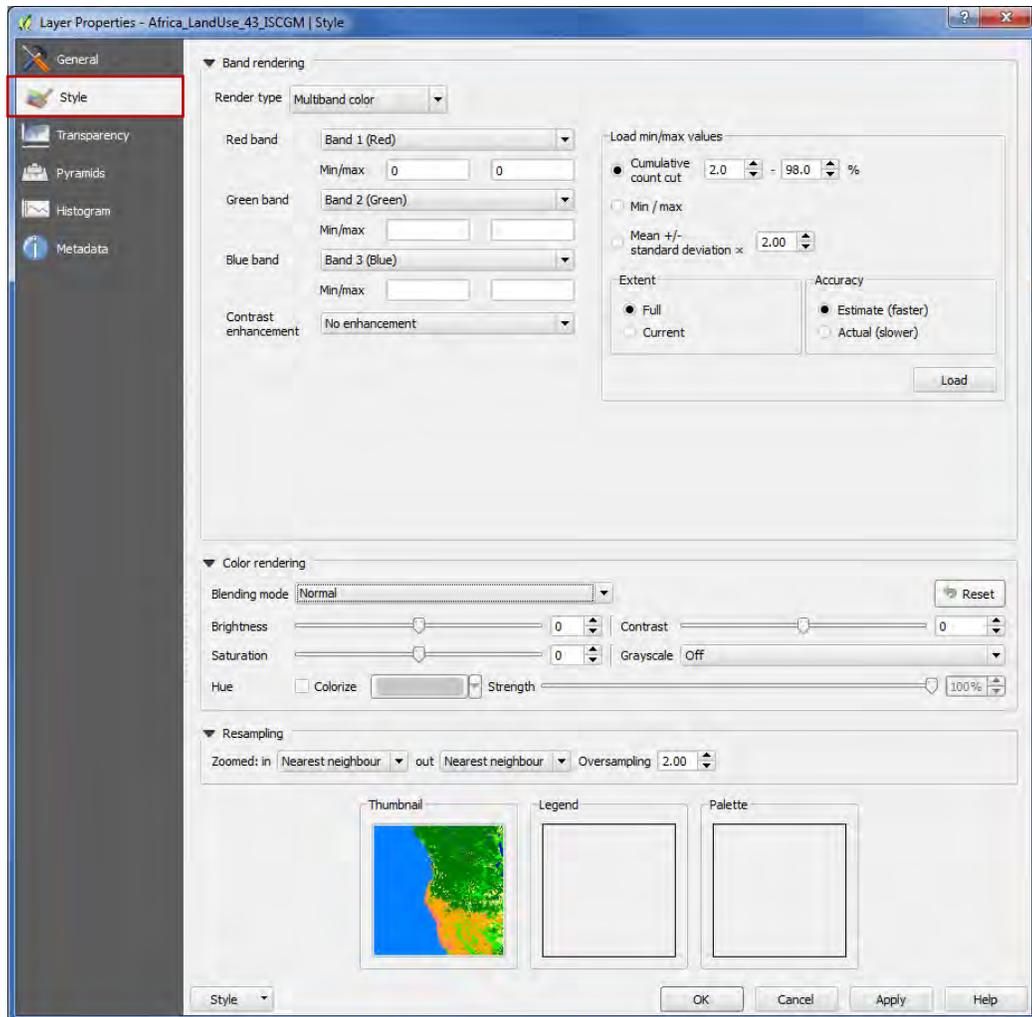
- v) We now want to move the land use raster to the bottom of the list so you can still see all the vectors which you already added. Select the raster in the **Map Legend** window and drag it below the *Uganda_District_GAA* layer. *Note: You can expand the Map Legend window to make it easier to drag the files if needed.*



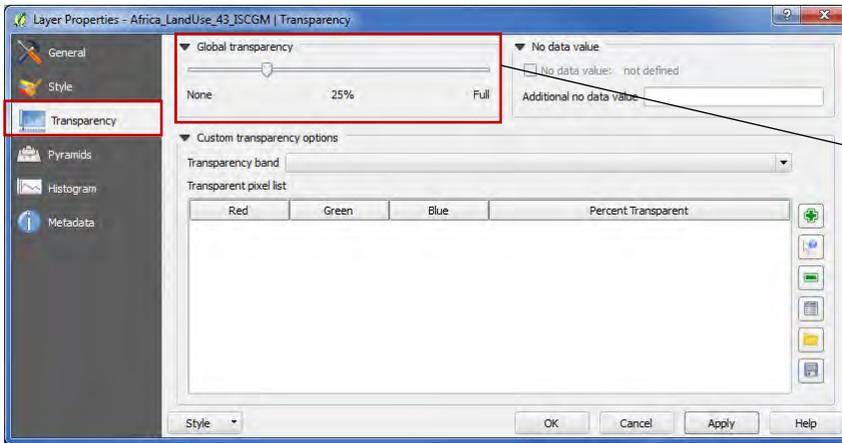
- 2) **Properties of a Raster layer:** Once you add a raster you can change the symbology, coordinate system, and metadata for that file by going to its **Properties**.
- a) Right click on *Africa_LandUse_31_ISCGM* and select **Properties**.
 - i) **General** tab: Here you can see the layer name, location, and coordinate system (which you can change by clicking **Specify**).



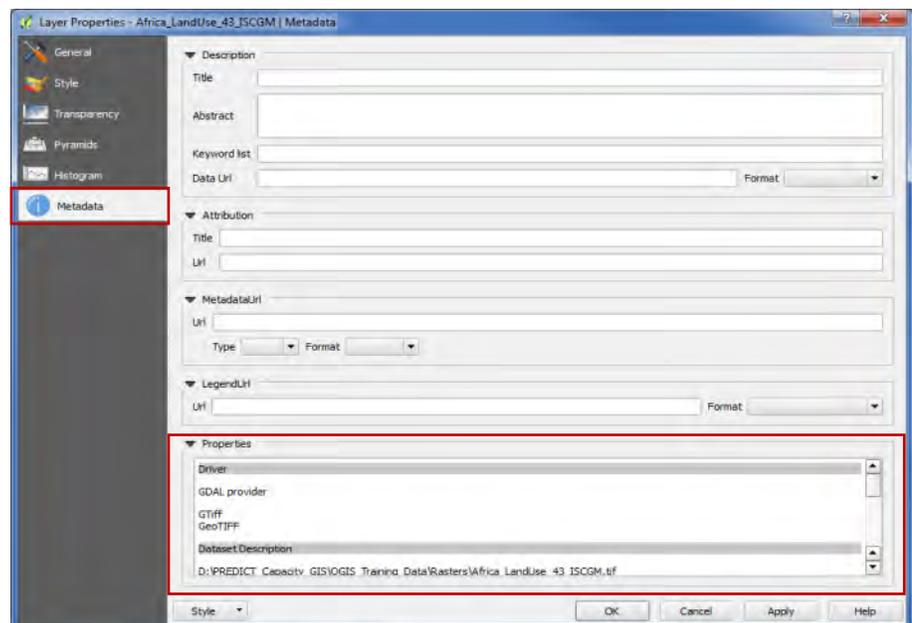
- ii) **Style** tab: Here you can make changes to how the raster is visualized, although we recommend staying with the defaults unless your raster is visualized in only one color.



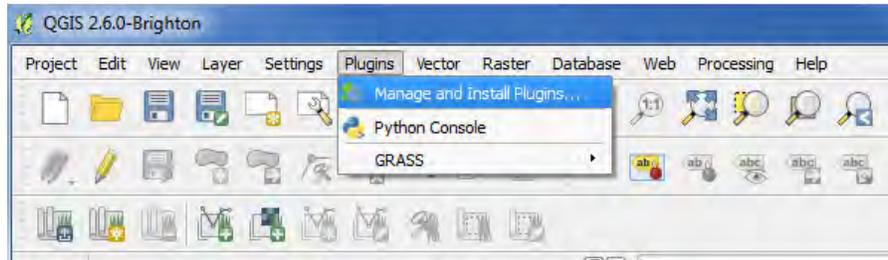
- i) **Transparency** tab – Here you can change the transparency of the layer so you can see more or less of the layers underneath. Change the transparency to 25% and see the difference between the layers.



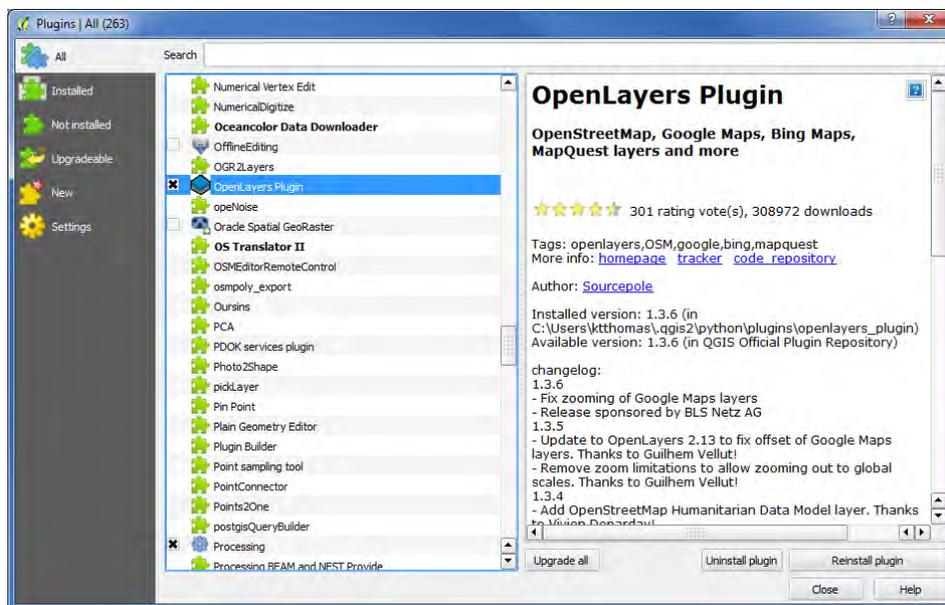
- ii) **Metadata:** Here you can check the metadata for the file and add more information if needed. If you scroll down to the **Properties** tab and scroll in that window you will see the metadata which was entered by the Global Administrative Areas website (GAA) when they made the file. *Note: It's a good idea if you are going to be sharing your file with others that you fill out the metadata with some information so other users know specifics about the file such as coordinate systems, what the data represents, version of the file, etc.* Close the **Properties** box before proceeding.



3. **Adding Background Maps:** In order to add a background map such as a street map or satellite image, you will need to add a plugin called OpenLayers.
- a) Go to the **Plugins** menu.
 - i) Select **Manage and Install Plugins**.

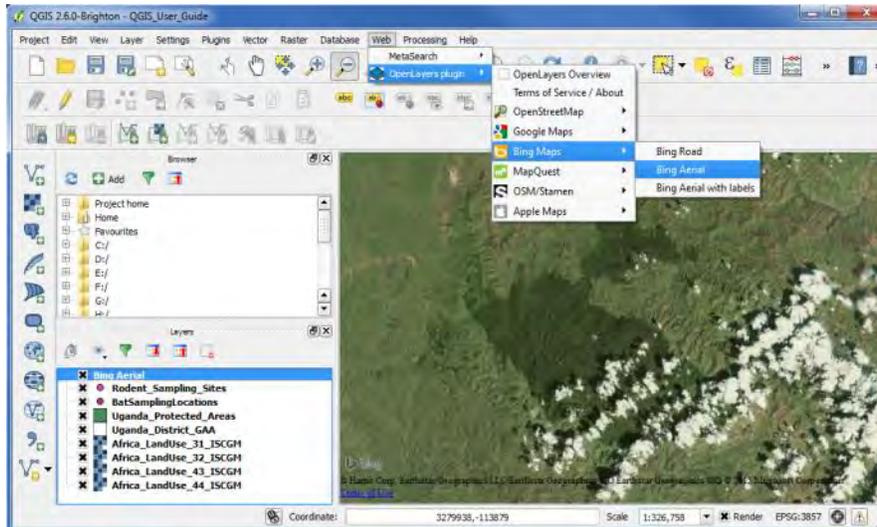


- ii) Scroll down and highlight the **OpenLayers Plugin** and hit the **Install plugin** button.
 - iii) Once installed close the **Plugin** window. *Note: Once you add the Plugin you shouldn't have to add it again.*

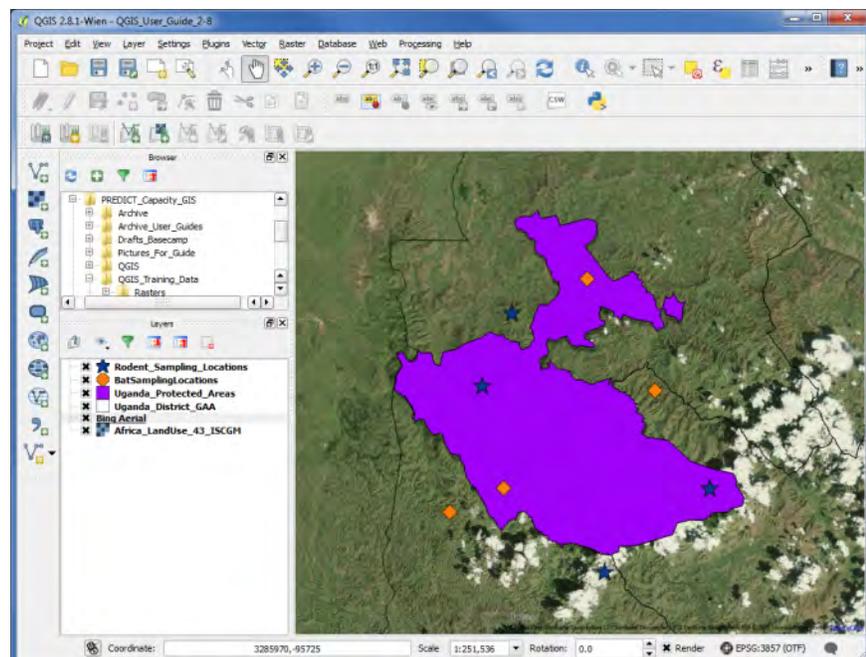


- a) To add a background to your map, go the **Web** menu and select the **OpenLayers** plugin. You will notice that you can select background maps from OpenStreetMap, Google Maps, Bing Maps, etc. For Now let's add the **Bing Aerial Map**.

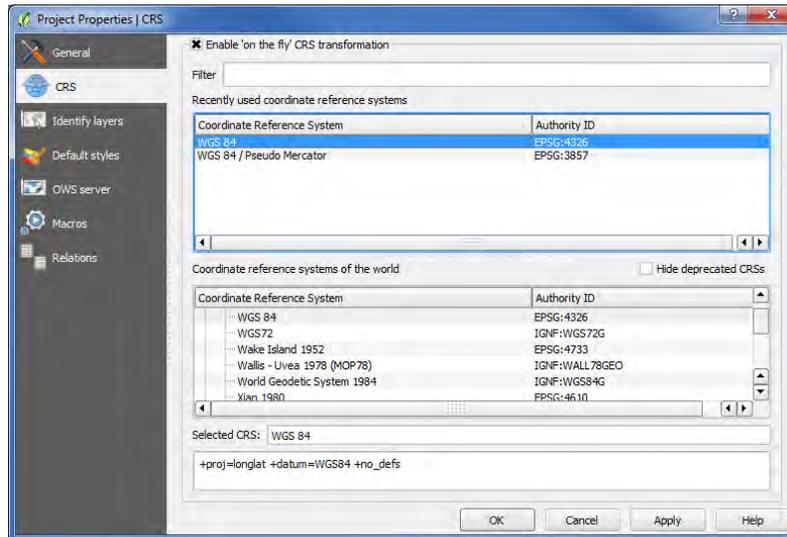
Note: Although you can add the map to your project, not all of these maps can be exported when making maps for presentations or publications. You might have to test which maps you are allowed to export.



- b) When you add a new layer to the map you will notice that the layer will be placed at the top of the list and will cover up all other layers. In the **Map Legend** window click on the background layer and drag it to the bottom of the list. Your data will now be on top of the background map. You will need to deselect the land use layers in order to see the satellite image.

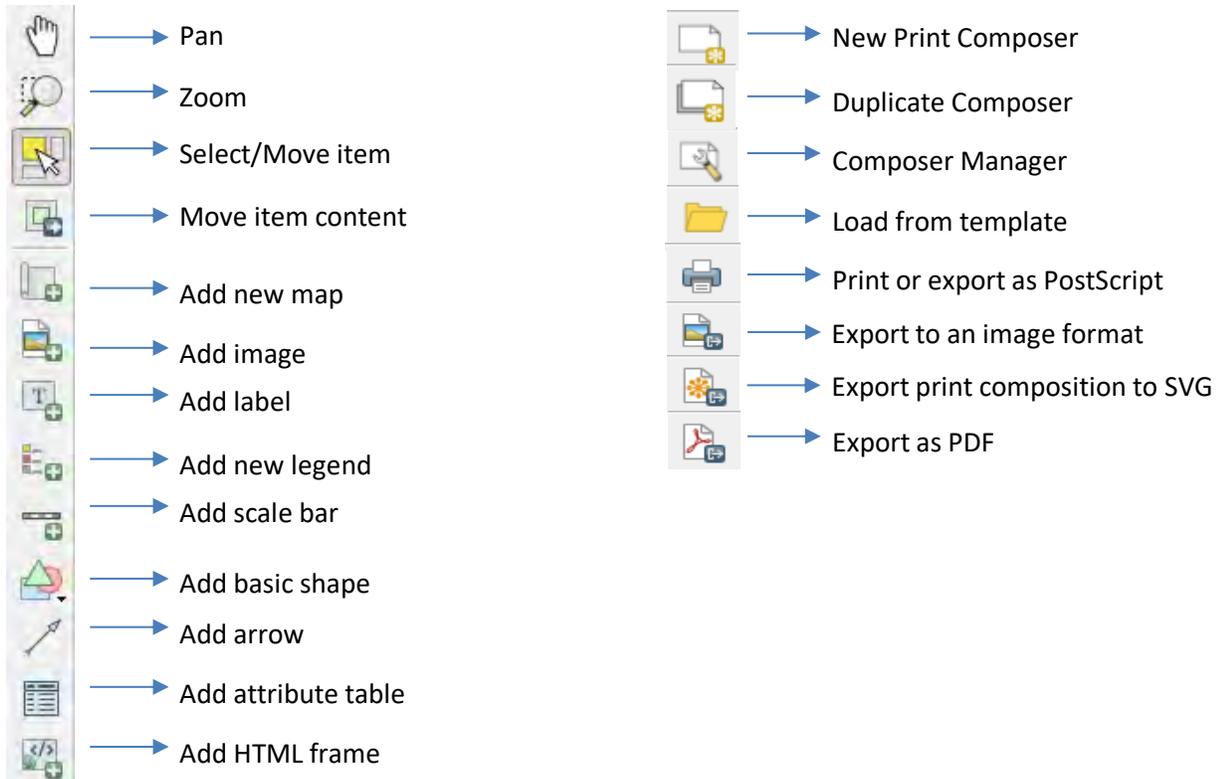


- a) It is possible that by adding the background image it changed your project's coordinate system.
- i) To check this go to the **Project** file menu and choose **Project Properties**.
 - ii) Choose **CRS** and make sure the *WGS 84* and not the *WGS 84/Pseudo Mercator* is chosen.



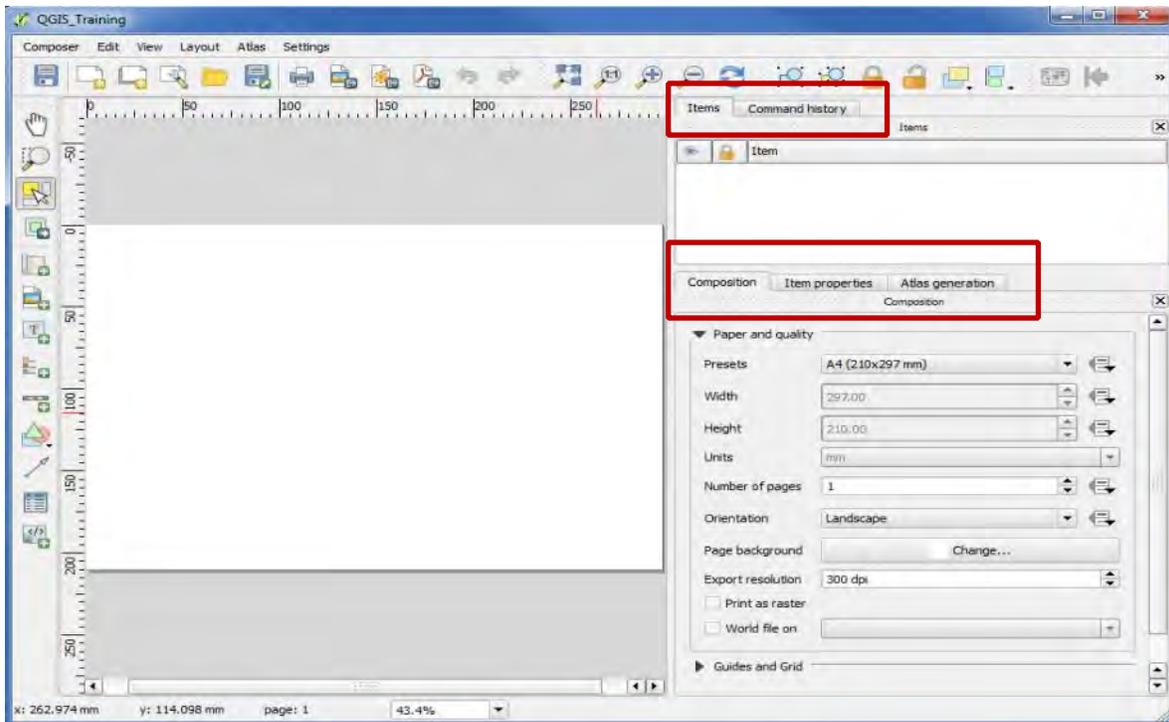
Section 8. Making Maps for Presentations & Publications

1. **Open the New Print Composer button** (you can also do this from the Project menu).
 - a) Give a name to this layout (**Composer title**), click **OK**.
 - b) The **New Print Manager** window will open. Listed below are some useful icons.

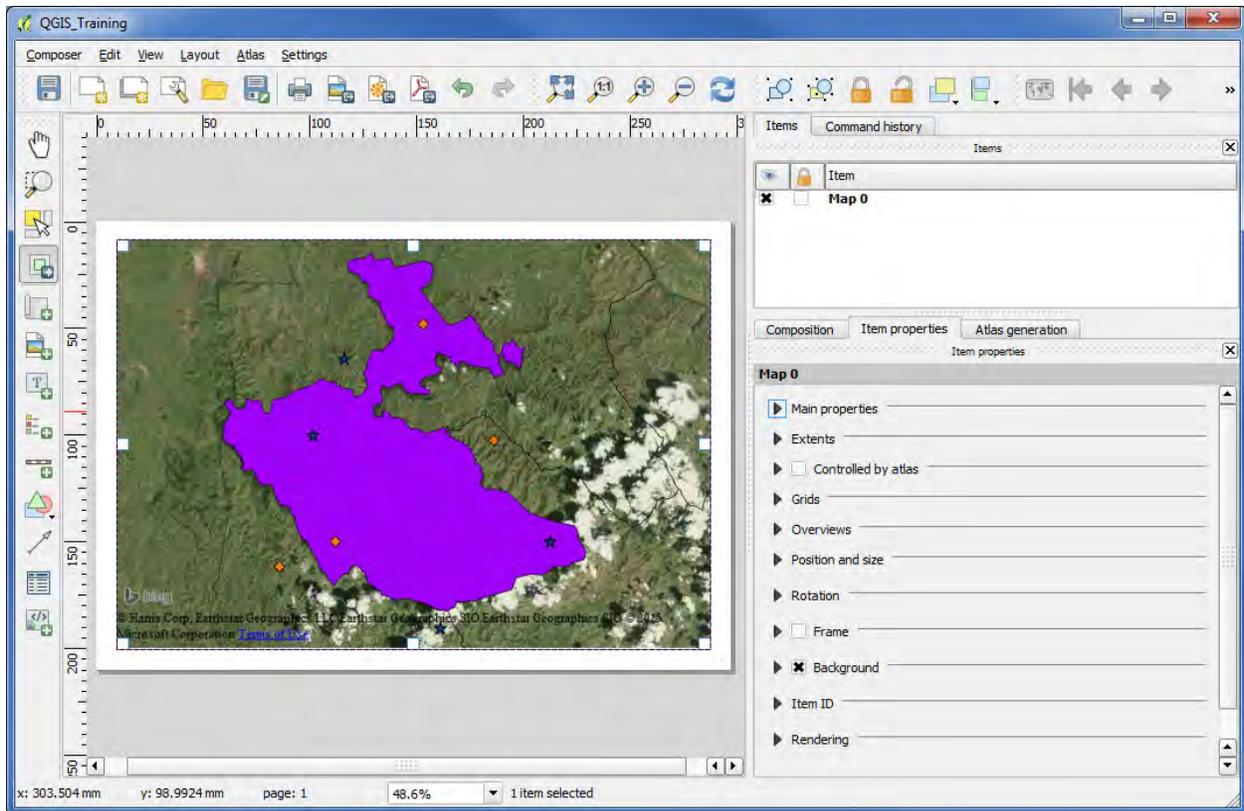


c) On the right side of the window there are 5 tabs. *Note: In the Mac version you may only see the Items, Composition, and Items Properties. If you would like to add the other panels go to the **View** pull-down menu, choose **Panels**, and then **add Command History and Atlas generation**.*

- i) **Items**: provides a list of items added to the canvas.
- ii) **Command history**: history of all changes applied to the Print Composer layout. Here you can undo and redo layout steps performed.
- iii) **Composition**: here you can set paper size, orientation, page background, number of pages, and print quality.
- iv) **Item properties**: displays the properties for the selected item and customizes settings for items like scale bars or labels.
- v) **Atlas generation**: allows you to enable the generation of an atlas (map book) for the current Composer and gives access to its parameters.

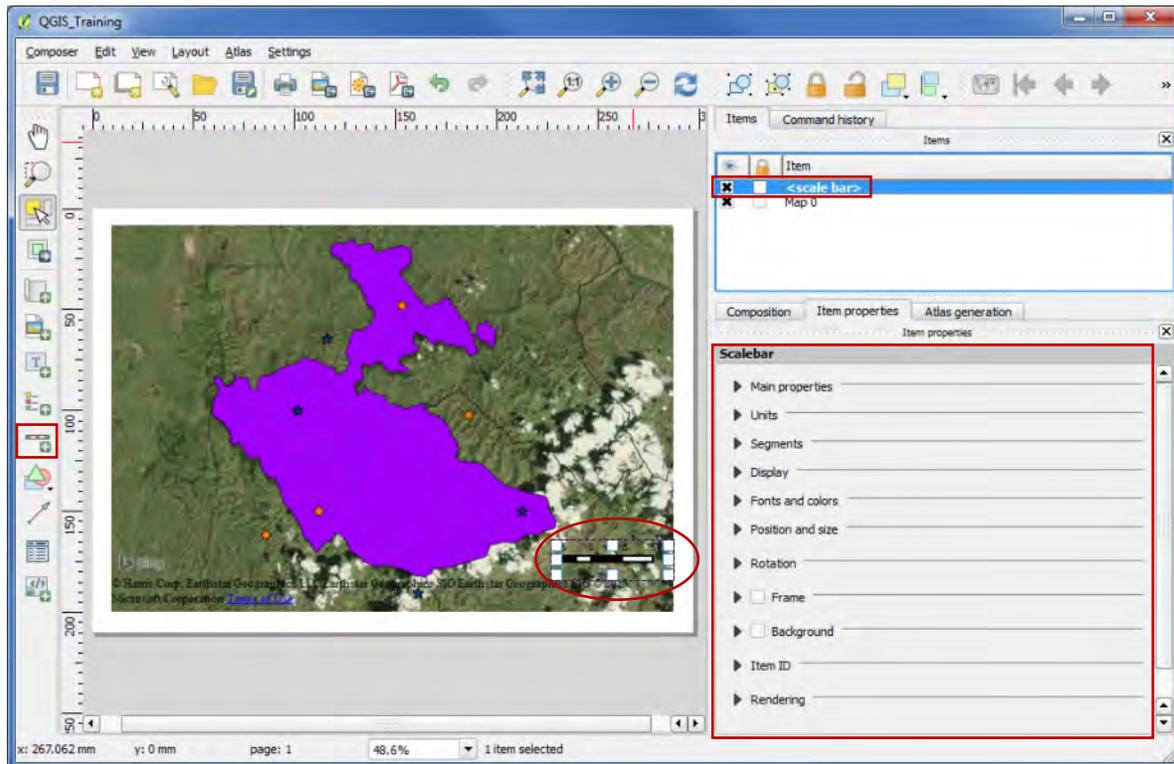


2. **Add New Map:** To add data to the Composer click the Add new map button, crosshairs will appear on the screen. Draw a box covering the area of the page. After you draw the box you will see the map image you just made in QGIS visualized in the composer window.



- 3) **Add a scale bar** by clicking the Add new scalebar button.
 - a) With the crosshairs click on the image and a scale bar will appear.
 - b) To change the properties of the scale bar you can click on **<scale bar>** in the **Items** window.
 - i) Under **Main Properties** the **Style** pulldown menu will allow you to change the style of the scale bar.
 - ii) Under **Units** you can change the units of the scale bar.
 - iii) The **Segment** section allows you to change the number and size of the segments and height of the scale bar.
 - iv) **Display** changes the style of the scale bar such as how far the text is away from the scale bar, size of the box etc.
 - v) **Fonts and colors** allow you to change the color and font used for the scale bar.
 - vi) **Position and Size** changes the location of the scale bar on the page (you can do the same thing by clicking the **Select/Move Item** icon and then select the scale bar and move it.
 - vii) **Rotation** allows you to rotate the scale bar.

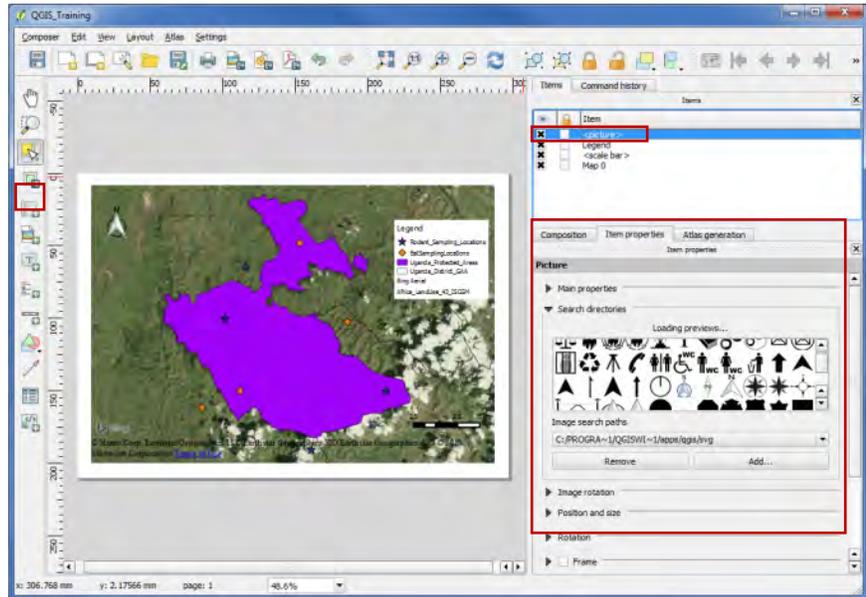
- viii) **Frame** allows you to put a frame around the scale bar.
- ix) **Background** allows you to put a background behind the scale bar.
- x) **Item ID** allows you to add an ID (this will change the name from **<scale bar>** to whatever you type in the ID box in the **Items** window).
- xi) **Rendering** has a function that will allow you to make the scale bar transparent or exclude from exports.



- 4) To add a legend, click the **Add new legend** button
 - a) With the crosshairs click on the map and it will place the legend there (you can always change the location with the **Select/Move Item** tool). *Note: The legend tool will add all the files listed in your QGIS **Map Legend** window even if they are not activated.*
 - b) To change the Legend properties, highlight **Legend** in the **Items** window and go to the **Item Properties** tab.
 - i) **Main properties** allow you to change the legend **title**, the **alignment**, and **wrap text**.
 - ii) **Legend Items** allows you to choose whether or not to auto update.
 - (1) If you do not want to show some of the layer names in your QGIS window then uncheck the **Auto Update** and you will notice that you are now able to select the tools below.
 - (2) The **+** tool will give you a list of layers you can add to the legend.
 - (3) Highlight a layer you would like to delete from the legend and hit the **-** button. You will notice that the layer is removed from the legend.

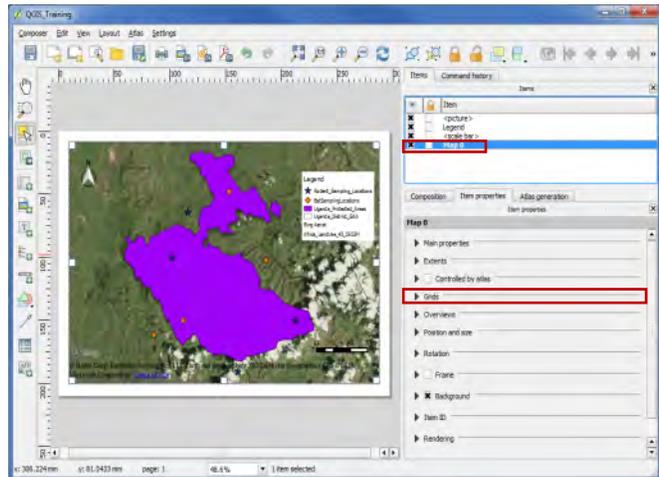
5) **Add a North Arrow:**

- a) Click the **Add Image** button.
- b) Click on the image and draw a box where you would like the north arrow to be.
- c) Under **Items Properties** tab open **Search directories** by clicking on the arrow next to it. Images will appear in the box below, scroll through and choose the north arrow you would like to use.
Click on the arrow. You should notice that the arrow now appears in the box you drew on the map.
- d) You can resize the arrow by changing the size of the box. This is done by dragging the box's corners to the desired size.

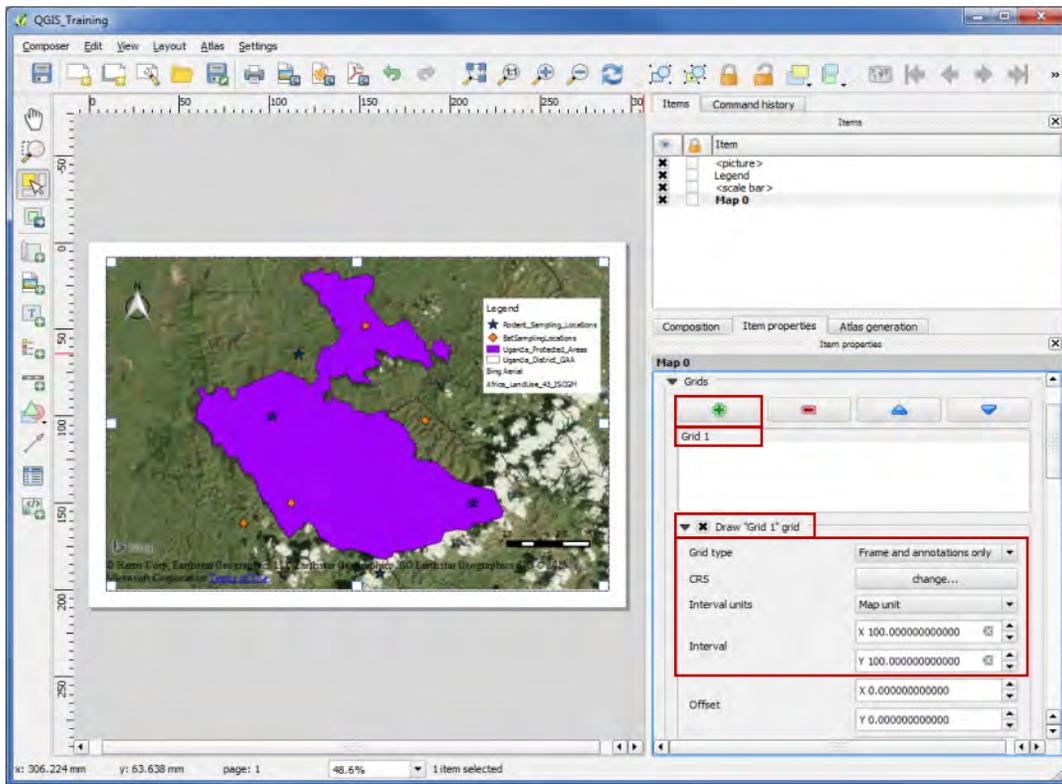


6) **Add a Graticule** (evenly spaced horizontal and vertical lines used to identify locations on a map often using latitude and longitude):

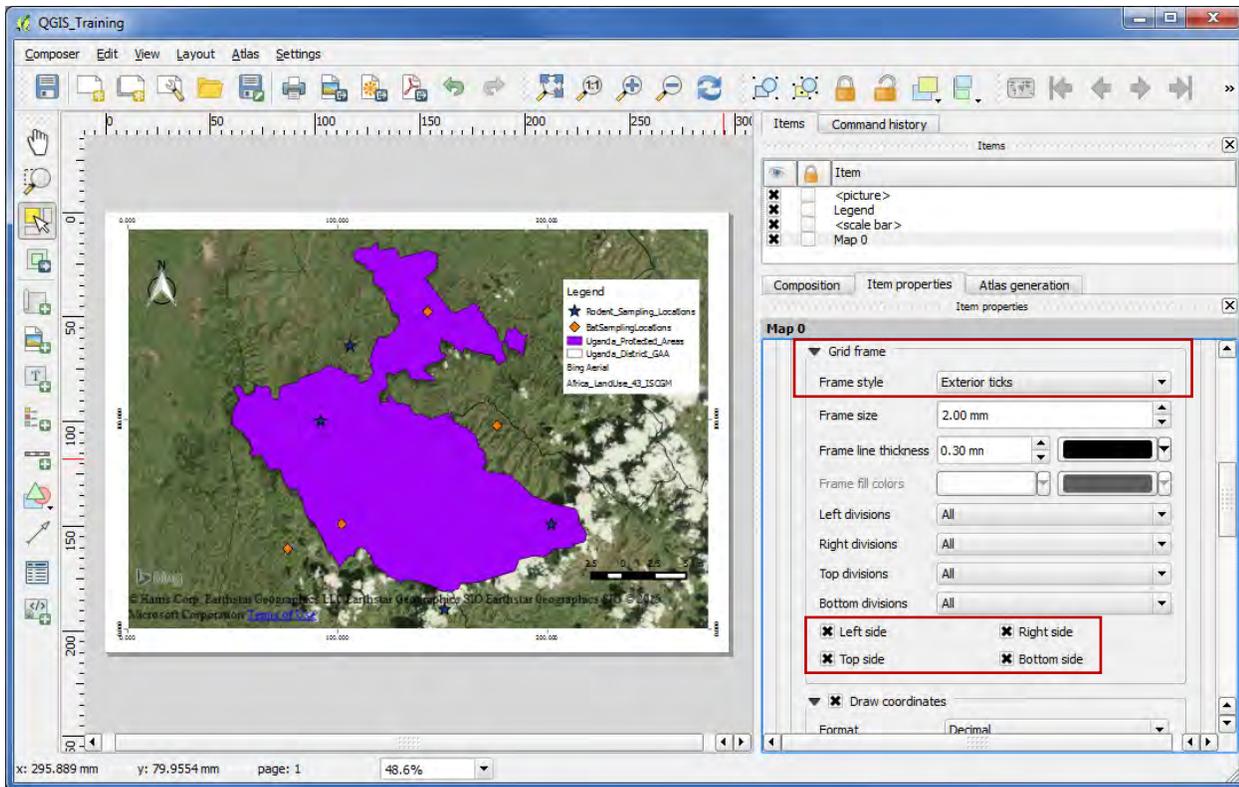
- a) Highlight **Map** under the **Items** window.
- b) In the **Items Properties** window click on the arrow next to **Grids** to expand that section.
- c) Click the + button and you should see **Grid 1** show up in the box below. Highlight **Grid 1** and activate the box next to **Draw "Grid 1" grid**, by clicking on it. This will open the grid properties which you can fill out.
 - i) Choose **Grid Type** as **Frame and annotations only**.
 - ii) For **CRS**, choose **WGS 84** as the coordinate system so that the coordinates are displayed as latitude and longitude.
 - iii) **Interval Units** set to millimeter. *Note: you can keep this set to the default of Map Unit, however, if your coordinates don't show up change to millimeter or centimeter and they will show up.*



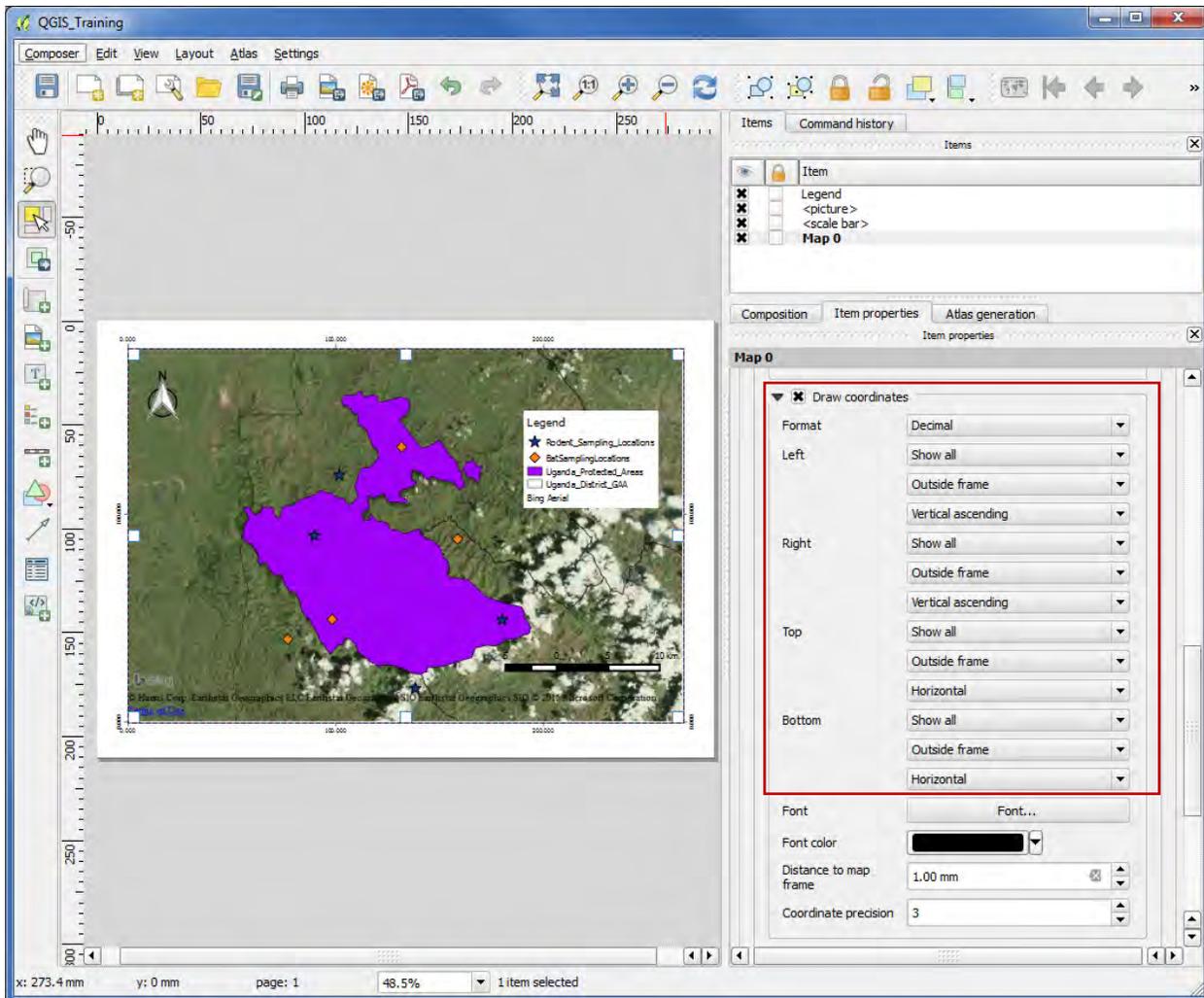
- iv) **Interval** will set the intervals between the numbers displayed on the graticule. For this example set the interval to 100 for both X and Y.



- v) Scroll down to **Grid Frame** and set the **Frame style** to **Exterior ticks** and make sure **Left side**, **Right side**, **Top side**, and **Bottom side** are all checked. This will display the coordinates on all four sides of your map.



- vi) Activate the **Draw coordinates** button by clicking in the box next to it. Choose a **Format**. Here we choose for latitude and longitude to be displayed as **Decimal**. **Left, Right, Top, and Bottom** should all be set to **Outside frame**; and **Left** and **Right** should also be set to **Vertical**. You should now see your graticule around the map. *Note: There have been people using the Mac version where the graticule isn't showing up in QGIS but is there when they export the map. We have not been able to identify the problem yet, but are continuing to look into the problem.*



6) Once you are happy with the layout (feel free to move the items on the map around to make it more pleasing to the eye) you can **Export** the map as an image (.jpg) or .pdf file

a) Export as **Image:** 

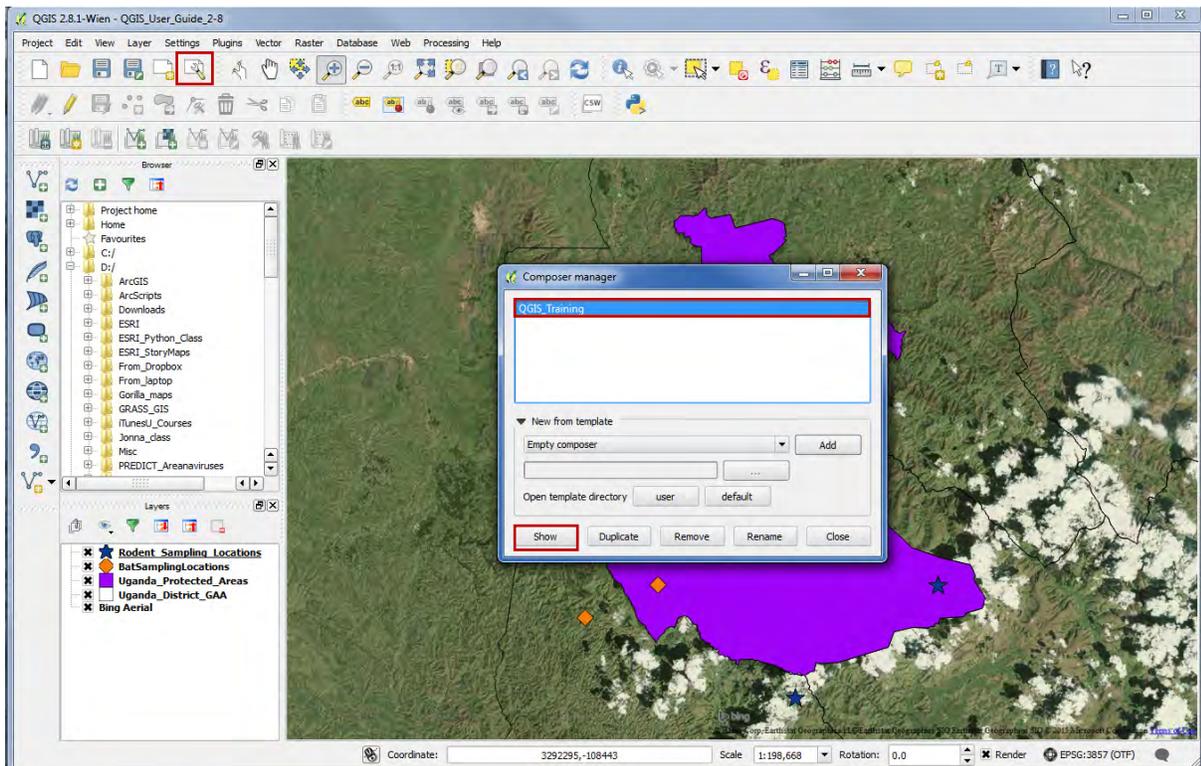
- i) **Browse:** browse to where you want to save the file.
- ii) **File name:** name the file you are saving.
- iii) **Save as type:** choose the type of file you would like to save i.e. jpg or .tif.

b) Export as **pdf:** 

- i) Click **OK** if you get a warning about saving the file as a raster.
- ii) **Browse** to where you would like to save the file.
- iii) **File name:** give the file a name.
- iv) Click **Save**.

7) **Composer Manager:** If you close the New Print Composer window, you can reopen the Print Composer by clicking on the Composer Manager window.

- a) Highlight the template you would like to open and click the **Show** button.

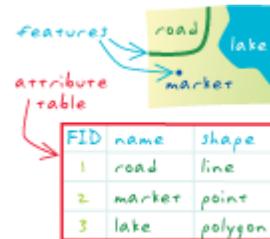


Section 9. Glossary

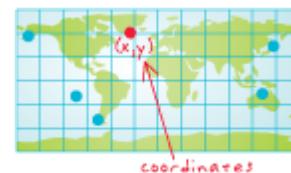
Definitions and pictures are sourced from ESRI unless otherwise stated.

Attribute – Non-spatial information about a geographic feature in GIS, usually stored in a table and linked to the feature by a unique identifier. For example, attributes of a river might include its name, length, and sediment load at a gauging station. In raster datasets, information associated with each unique value of a raster cell.

Attribute tables - A database or tabular file containing information about a set of geographic features, usually arranged so that each row represents a feature and each column represents one feature attribute. In raster datasets, each row of an attribute table corresponds to a certain zone of cells having the same value. In GIS, attribute tables are often joined or related to spatial data layers, and the attribute values they contain can be used to find, query, and symbolize features or raster cells.



Coordinates - A set of values represented by the letters x , y , and optionally z or m (measure), that define a position within a spatial reference. Coordinates are used to represent locations in space relative to other locations.



Coordinate System - Coordinate systems enable geographic datasets to use common locations for integration. A coordinate system is a reference system used to represent the locations of geographic features, imagery, and observations, such as Global Positioning System (GPS) locations, within a common geographic framework.

Datum - The reference specifications of a measurement system, usually a system of coordinate positions on a surface (a horizontal datum) or heights above or below a surface (a vertical datum).

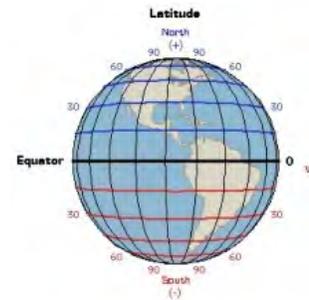
Geographic Coordinate System - A geographic coordinate system uses a three-dimensional spherical surface to define locations on the earth. It includes an angular unit of measure, a prime meridian, and a datum (based on a spheroid). The spheroid defines the size and shape of the earth model, while the datum connects the spheroid to the earth's surface. A point is referenced by its longitude and latitude values. Longitude and latitude are angles measured from the earth's center to a point on the earth's surface. The angles often are measured in degrees. **Note: Because latitude and longitude are based on angles they do not have a standard length throughout the globe. Because of this you cannot use a geographic coordinate system if you plan to do any analysis on your data. Instead you will want to use a projected coordinate system.**

Graticule - A network of lines representing the Earth's parallels of latitude and meridians of longitude on a map.

Layer - The visual representation of a geographic dataset in any digital map environment. Conceptually, a layer is a slice or stratum of the geographic reality in a particular area, and is more or less equivalent to a legend item on a paper map. On a road map, for example, roads, national parks, political boundaries, and rivers might be considered different layers. A layer can also reference to a data source, such as a shapefile, coverage, geodatabase feature class, or raster that defines how the data should be symbolized on a map.

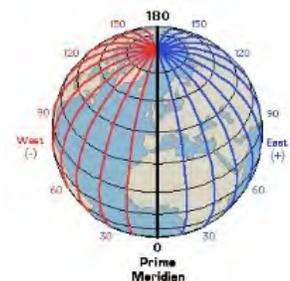
Latitude – Latitude values are measured relative to the equator and range from -90° at the South Pole to $+90^\circ$ at the North Pole. The equator is considered the 0° of latitude and the y value in a coordinate pair. Latitude is an angle measured from the earth's center to a point on the earth's surface. The angles often are measured in degrees.

Image: <http://geographyworldonline.com/tutorial/instructions.html>



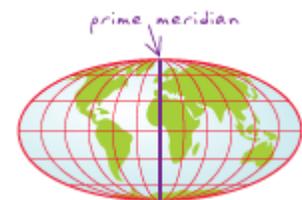
Longitude - Longitude values are measured relative to the prime meridian. They range from -180° when traveling west to 180° when traveling east. The prime meridian is considered the 0° of longitude and the x value in a coordinate pair. Longitude is an angle measured from the earth's center to a point on the earth's surface. The angles often are measured in degrees.

Image: <http://geographyworldonline.com/tutorial/instructions.html>



Metadata - Information that describes the content, quality, condition, origin, and other characteristics of data or other pieces of information. Metadata for spatial data may describe and document its subject matter; how, when, where, and by whom the data was collected; availability and distribution information; its projection, scale, resolution, and accuracy; and its reliability with regard to some standard. Metadata consists of properties and documentation. Properties are derived from the data source (for example, the coordinate system and projection of the data), while documentation is entered by a person (for example, keywords used to describe the data).

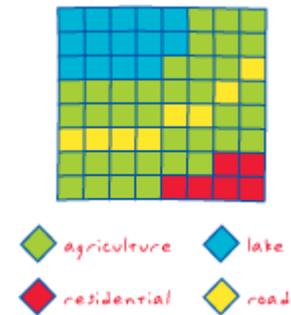
Prime Meridian - In a coordinate system, any line of longitude designated as 0 degrees east and west, to which all other meridians are referenced. The Greenwich meridian is internationally recognized as the prime meridian for most official purposes, such as civil timekeeping.



Projected Coordinate System - A projected coordinate system is defined on a flat, two-dimensional surface. A projected coordinate system has constant lengths, angles, and areas across the two dimensions. It includes a map projection, a set of projection parameters that

customize the map projection for a particular location, and a linear unit of measure. Different projected coordinate systems are useful for different purposes, for example some projected coordinate systems might preserve distance, area, true directions, or shape. Wikipedia has a useful, detailed explanation of coordinate systems at http://en.wikipedia.org/wiki/Coordinate_system

Raster - A spatial data model that defines space as an array of equally sized cells arranged in rows and columns, and composed of single or multiple bands. Each cell contains an attribute value and location coordinates. Unlike a vector structure, which stores coordinates explicitly, raster coordinates are contained in the ordering of the matrix. Groups of cells that share the same value represent the same type of geographic feature.



Shapefile - A vector data storage format for storing the location, shape, and attributes of geographic features. A shapefile is stored in a set of related files and contains one feature class. There are 3 mandatory files (first 3 listed below) that make up a shapefile, however, there may be files for the shapefile containing additional information:

- .shp – shape format; the feature geometry itself
- .shx – shape index format; a positional index of the feature geometry to allow seeking forwards and backwards quickly.
- .dbf – attribute format; columnar attributes for each shape, in dBase IV format
- .prj – projection format; the coordinate system and projection information, a plain text file describing the projection
- .xml – geospatial metadata in XML format
- .sbn and .sbx – a spatial index of features

Sphere vs. spheroid -

- Sphere – a perfectly round geometrical and circular object in three-dimensional space
- Spheroid – a spherelike but not perfectly spherical body

Symbology – The set of conventions, rules, or encoding that define how geographic features are represented with symbols on a map. A characteristic of a map feature may influence the size, color, and shape of the symbol used.

Vector – A coordinate-based data model that represents geographic features as points, lines, and polygons. Each point feature is represented as a single coordinate pair, while line and polygon features are represented as ordered lists of vertices. Attributes are associated with each vector feature, as opposed to a raster data model, which associated attributes with grid cells. Examples of a vector include points representing sampling locations, line representing roads, and polygons representing national parks.

Section 10. Data Download

Global Administrative Areas – Country administrative layers (country, state, county, etc)

<http://www.gadm.org/country>

Choose your **Country**

File format = Shapefile

Click **OK**

On the next page click **Download**

Protected Planet – National parks and protected areas

For this site you have to set up an account but it's free

<http://www.protectedplanet.net/>

International Steering Committee for Global Mapping – National data, Global elevation, Global land cover, and Global vegetation

For this site you will have to register to download data but it's free.

Home page - <http://www.iscgm.org/>

Data download page - <https://www.iscgm.org/gmd/>

IUCN (International Union for Conservation of Nature) Red List of Threatened Species –

Download shapefiles on location information for threatened species.

<http://www.iucnredlist.org/technical-documents/spatial-data>

Natural Earth – Here you can download different data from administrative boundaries to shaded relief rasters.

<http://www.naturalearthdata.com/>

GrassWiki Geodata – This site as a list of data sites. Click on one of the layers you are interested in and it will take you that particular website.

<http://grasswiki.osgeo.org/wiki/Geodata>

United States Geological Survey (USGS) - This website has many links for data worldwide.

<http://landcover.usgs.gov/landcoverdata.php#regional>

Andreas Hamann's website – Climate data for North America, South America, and Europe

<http://www.ualberta.ca/~ahamann/data.html>

Section 11. Helpful Online Tools & QGIS Training Videos

Federal Communications Commission – Site that converts latitude and longitude between Degrees Minutes Seconds to Decimal Degrees

<http://transition.fcc.gov/mb/audio/bickel/DDMMSS-decimal.html>

Geographic/UTM Coordinate Converter – Converts coordinates between latitude and longitude and UTM

<http://home.hiwaay.net/~taylorc/toolbox/geography/geoutm.html>

National Oceanic and Atmospheric Administration (NOAA) Understanding Datums, Coordinate Systems, and Map Projections

http://coast.noaa.gov/digitalcoast/_/elearning/datums/player.html

ESRI About Coordinate Systems and Map Projections

http://webhelp.esri.com/arcgisdesktop/9.3/index.cfm?TopicName=About_coordinate_systems_and_map_projections

There are many videos online which you might be interested in using as a reference. Below are just a few we thought were useful and easy to follow.

- The Interface: <http://qgis-tutorials.mangomap.com/post/79334660226/qgis-video-tutorials-module-1-the-interface>
- Creating a basic map: <http://qgis-tutorials.mangomap.com/post/82295156067/qgis-video-tutorials-module-2-creating-a-basic>
- Classifying vector data: <http://qgis-tutorials.mangomap.com/post/83730031877/qgis-video-tutorials-module-3-classifying>
- Creating a map for print: <http://qgis-tutorials.mangomap.com/post/84321475284/qgis-video-tutorials-module-4-creating-a-map>
- Creating vector data: <http://qgis-tutorials.mangomap.com/#>



FIELD SAMPLING GUIDES

Avian Sampling Methods

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And the PREDICT One Health Consortium

Last updated: 28 November 2016

Objectives: To safely collect biological samples from live and dead wild birds.

Section 1. Confirmation of Knowledge

When you are familiar with the information in this guide, take the PREDICT quiz.

Section 2. Brief Overview of PPE

Minimum PPE Required for Handling Live, Dead, or Samples of Birds

The minimum PPE for bird sampling includes:

1. Designated clothing
2. Closed-toed shoes
3. Nitrile gloves
4. Protective glasses
5. N95 facemask for self-protection and to avoid contaminating samples.

(See the *Biosafety and PPE Guide* for detailed instructions regarding PPE Use)

Section 3. Avian Data Collection

Please refer to the following three templates for required data collection:

1. P2 Animal Data Collection Form
2. P2 Site Characterization Data Collection Form
3. P2 Specimen Data Collection Form

Biometric Measurements

The P2 data templates mentioned above are required to be filled in. Additional biometric measurements may be collected at the discretion of the sampling party.

For many bird species, the sex or age of a captured individual may not be immediately evident with a simple visual inspection. Subtle but significant differences in morphology are often useful for differentiating between sexes and age classes.

Thus collecting biometric measurements can have important applications in disease sampling studies for determining differential infection or exposure rates based on sex or age.

Biometric measurements to be collected (optimal):

- Weight
- Culmen (bill) length and depth
- Tarsus length
- Wing length
- Tail length

Additional important data to establish breeding or physiological status of the bird:

- Presence of brood patches
- Moulting stage

Age class:

- Usually the exact age will not be known and individuals should be assigned to a juvenile or adult age class

For collection methods for biometric measurements refer to FAO manual “Wild Birds and Avian Influenza: an introduction to applied field research and disease sampling techniques”

(<http://www.fao.org/docrep/010/a1521e/a1521e00.htm>)

Photographs for Bird Identification

(Reference: European Commission DG Sanco 2006)

Digital photographs should be taken of each individual. The bird should fully fill the photographic frame, and wherever possible the image should include a ruler or other scale measure.

Photographs should be taken of:

- The whole bird, dorsal side, with one wing stretched out and tail spread and visible;
- The head in profile clearly showing the beak;
- Close-up photos of the tips of wing feathers can often determine whether the bird is an adult or a juvenile (bird in its first year);
- Ventral photographs should show the legs and feet (since leg color is often an important species diagnostic). If any rings (metal or plastic) are present on the legs, these should be photographed in situ as well as recording ring details.
- Any conspicuous markings/patterns should be photographed.

In late summer many water birds, especially ducks and geese, undergo moult and can be especially difficult to identify by non-specialists. At this time of year there is particular need for clear photographs to aid identification. The patch of color on the open wing’s secondary feathers (called the “speculum”) is often especially useful.

Section 4. Avian Sample Collection

Samples are to be collected in duplicate from each animal. One sample must be collected into Trizol and one into viral transport media (VTM). Tubes must be labeled with a unique identifier number. Printed labels should be used.

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two oral swabs** - one in 500 μ L VTM and one in 500 μ L Trizol
2. **Two cloacal swabs** - one in 500 μ L VTM and one in 500 μ L Trizol and/or
Two fecal samples - one with max of 500ul/0.5cc feces in 500 μ L VTM and one with max of 500ul/0.5cc feces in 1 mL Trizol
3. **Two whole blood samples** - one with max of 500 μ L of whole blood in 500 μ L VTM and one with max of 500 μ L of whole blood in 500 μ L Trizol
4. **Two serum samples** - 2 x 0.5ml aliquots frozen without media

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

If there is no **short-term** access (i.e., within 24 hours) to cold chain such as in an emergency situation, then samples can be collected in 500 μ L of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

- 1 day at 37 °C (i.e., ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80°C for storage until analysis

Details on Sample Collection and Storage Media

1. **Two oral swabs:** Using sterile, polyester-tipped swabs with either an aluminum or plastic shaft, rub the swab tip gently but thoroughly against the back of the animal's throat, saturating the swab with saliva (see Figure 1).



Figure Avian 1: Oral swab sample collection from a bird (Photo credit: Taronga Zoo/Karrie Rose from *FAO Wildlife bird highly pathogenic avian influenza surveillance manual*)

Place 1 swab in a cryovial filled with 500 μ L Trizol and use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the shaft of the swab above the tip. [Note: If the plastic shaft can be snapped, then scissors are not necessary and the risk of cross-contamination is reduced. To snap the swab, lift the swab a little above the bottom of the vial then snap it. This will ensure the swab will not block the cap]. Place the other swab into 500 μ L of VTM (= maximum final ratio of 1:1) in a cryovial.

Store in a liquid nitrogen dry shipper or dewar and transfer to -80°C freezer when possible.

2. **Two cloacal swabs/fecal samples:** Gently and slowly insert the head of the swab into bird's cloacal cavity (cloacal cavities of small birds can be very shallow; thus the swab head should not be inserted very far into the cloaca). Gently twirl or rotate the swab back and forth 2-5 times to exfoliate (collect) cells from the cloacal wall (see Figure 2). Remove the swab from the cloacal cavity and place in a cryovial filled with 500 μ L Trizol and use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the shaft of the swab above the tip. [Note: If the plastic shaft can be snapped, then scissors are not necessary and the risk of cross-contamination is reduced. To snap the swab, lift the swab a little above the bottom of the vial then snap it. This will ensure the swab will not block the cap]. Repeat above process with second swab, and place into 500 μ L of VTM (= maximum final ratio of 1:1) in a cryovial.

For fecal samples, add 500 μ L or pea-sized piece of feces directly into 2 cryovials, one containing 1 mL Trizol (= maximum final ratio of 1:2) and one containing 500 μ L VTM (= maximum final ratio of 1:1) and mix each tube well.

Store in a liquid nitrogen dry shipper or dewar and transfer to -80°C freezer when possible.



Figure Avian 2: Cloacal swab sample collection from a bird (Photo credit: Taronga Zoo/Karrie Rose from *FAO Wildlife bird highly pathogenic avian influenza surveillance manual*)

- 3. Whole blood and serum samples:** Blood can be collected from the jugular vein (right side of the bird's neck; see Figure 3), brachial/ulnar vein (wing vein) or medial metatarsal vein (leg vein; see Figure 4) using a 22G, 23G, 25G, or 27G hypodermic needle hypodermic needs or butterfly needle and a 12 mL, 10 mL, 6 mL, 3 mL or 1 mL syringe, depending on the size of the bird and the amount of blood to be collected.

In general, it is safe to collect 0.3-0.6 cc of blood per 100 g of body mass from live birds, however, it is always best to collect as little blood as is necessary to conduct the testing required. If you plan to do hematology tests in addition to disease surveillance, it is recommended that you use a 22G through 25G needle as a 27G needle or smaller damages cells as they pass through this narrow diameter needle. After blood is collected, cover the venipuncture site with gauze and apply digital pressure until bleeding stops (30-60 seconds).

Add up to 500 μ L of whole blood directly into 2 cryovials, one containing 500 μ L Trizol and one containing 500 μ L VTM (= maximum final ratio of 1:1) and mix each vial well.

Note: *If animals are too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in VTM after serum separation.*

For serum samples, collect blood using a non-heparinized syringe and place blood into a serum vacutainer (red-top) tube containing serum-clotting factor. After allowing the blood to clot, either spin tube in a centrifuge or allow tube to stand vertically on ice as much as possible. Use a sterile pipette tip and pipette gun to draw off serum and collect 2 x 0.5ml aliquots (no Trizol or VTM).

Store in a liquid nitrogen dry shipper or dewar and transfer to -80°C freezer when possible.



Figure Avian 3: Blood sample collection from the jugular vein. (Photo credit: FAO, 2007)



Figure 4: Blood sampling from the medial metatarsal vein. (Photo credit: FAO, 2007)

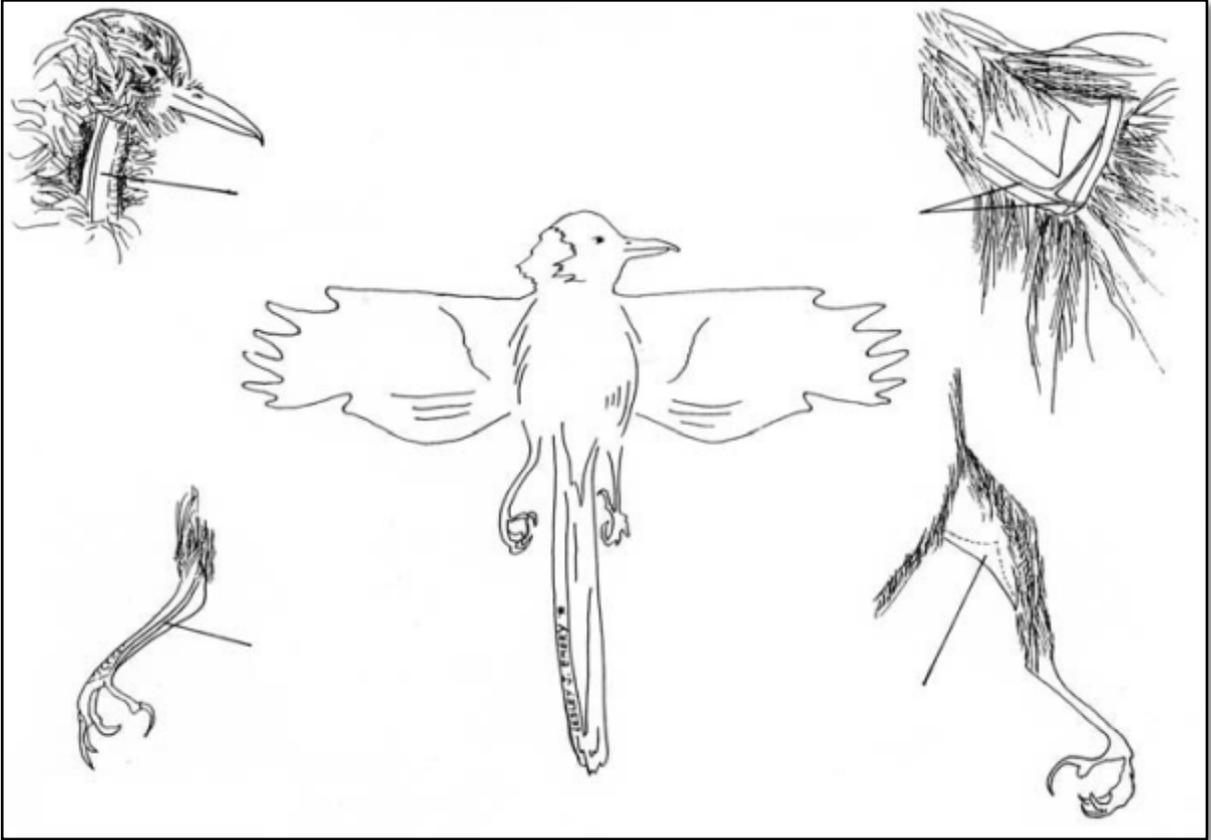


Figure Avian 5: Common sites of venipuncture and administration of subcutaneous fluids in birds (Photo credit: FAO, 2006)

Sampling of Dead or Euthanized Birds

If carcasses are not whole, the PREDICT guide for ***Bushmeat Sampling*** may be more applicable. If bodies are relatively whole and fairly fresh then blood, organ tissues and urine should be collected.

As discussed throughout this protocol, all wildlife should be considered potentially infectious for a wide variety of dangerous pathogens and dead animals in particular should be sampled only following all safety measures including proper use of PPE, proper work station decontamination and proper carcass disposal as outlined here and in other PREDICT documents (***Section: Biosafety and PPE Use, Section: Safe Disposal of Carcasses and Infectious Waste Guide, and Section: General Field Sampling Station Setup***).

Thorough necropsy procedures can be very beneficial and might pertain to some animals (e.g., valuable or known individuals, suspicious deaths, etc.); these procedures are addressed in separate documents. Time and skill permitting, when full necropsies are performed, following any Association of Zoos and Aquariums/AZA (or similar) necropsy protocol is recommended. Most of these protocols can be adjusted for application to other species. (Note: properly following extensive necropsy procedures and collecting and measuring all samples can require 4-6 hours for a single animal.)

Post-Mortem Blood Collection

In recently dead animals, it may be possible to collect whole blood (often clotted) from the right side of the heart, where the largest volume of blood is available. Blood may also be found in the chest cavity. If the animal died recently and the blood has not yet clotted, collect whole blood into 1 lavender top tube containing EDTA and add up to 500 μL of whole blood directly into 2 cryovials, one containing 500 μL Trizol and one containing 500 μL VTM. Also collect whole blood separately to obtain serum. Collect available blood into an appropriate sized container (typically one or more blood tubes) and allow it to sit undisturbed for at least 30 minutes. Then centrifuge at high speed (2000 x G for 20 minutes), remove the serum (clear, yellow or red-tinged fluid at the top), transfer clots into cryovials containing 500 μL Trizol and 500 μL VTM, and freeze the samples. If a centrifuge is not available, allow clots and cells to settle as much as possible and then collect serum into 2 x 0.5ml aliquots and blood clots into cryovials containing 500 μL Trizol and 500 μL VTM.

Tissue Collection from Dead Birds

Collect three, adjacent, approximately 200mg (pea-sized) samples of the following tissues:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Testes
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas
- Other, if required

One specimen should be frozen in 500 µL VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

It will usually require experience to identify abnormal tissues, but potentially recognizable gross lesions include masses, discolored areas, ulcerations, etc. Samples for histopathology (i.e., in formalin) should be collected at the abnormal margins to include both normal and abnormal sections in the same piece of tissue. Collection of any obvious internal parasites in ethanol is also recommended.

Section 10e. Health and Welfare of Birds during Capture and Handling

The health and well-being of the birds is the primary concern during all phases of capture. There are multiple methods for trapping and handling varying bird types, and examples can be found in the FAO Animal Production and Health Manual No. 5 (“Wild Bird HPAI Surveillance: sample collection from healthy, sick and dead birds”, available at:

<http://www.fao.org/avianflu/en/animalhealthdocs.html>)

The following principles should be adhered to, to ensure birds are captured and handled correctly, safely and with minimum disturbance (FAO, 2007):

- Wild bird capture is strictly controlled in most countries; those engaged in capture activities should be aware of and comply with local and national laws and obtain all required local, state, provincial & federal permits well in advance.
- Capture techniques and equipment that expose birds to foreseeable risk of injury should be avoided at all costs.
- Approved restraint techniques and handling guidelines should be used e.g., those described by FAO (2007); consult with experienced wildlife veterinarians and biologists if modifications to restraining and handling techniques are required.
- Those conducting capture efforts should take all precautions to avoid disturbing nesting birds at breeding sites or enhancing vulnerability to nest site predation following human intrusion.
- Monitor weather forecasts prior to conducting capture efforts to ensure birds are not captured during extreme climatic conditions that would expose them to an increased risk of hypothermia or hyperthermia.

- Always have a sufficient number of experienced personnel (at least four) available before undertaking any capture operation.
- Check operative traps and nets at appropriate time intervals; birds should not remain in traps or nets any longer than is necessary. This is capture technique and weather dependent, and could be as short as every 15 minutes to twice a day.
- Close or dismantle traps and nets that are inoperative and not checked regularly.
- Maintain a calm and quiet environment at the bird-handling site.
- Conditions at the bird-processing site should be appropriate for the environmental conditions: in cold, wet conditions, birds should be kept warm and dry; in hot, sunny conditions, birds should be processed in a sheltered, shaded and cool site.
- Processing stations should be located as near as possible to the capture site to avoid holding birds for transportation any longer than is absolutely necessary.

Bird Welfare (FAO, 2007)

There is always the risk of distress or injury when handling wild birds. Preferably, an appropriately trained veterinarian will be available to examine and treat any injured or distressed bird, but, at the very minimum, a basic first aid kit should be included in the equipment list of every field study. In no instance should a seriously injured bird be released into the wild without first being examined and treated by a veterinarian. If euthanasia is required see the AAZV and [AVMA guidelines \(Section 8.5.2\)](#).

Common Maladies and Treatments

Scratches, cuts, and abrasions

These may be unavoidable during capture and confinement and simple treatment by rinsing the injury with clean water or sterile saline before releasing the bird should suffice for most minor injuries. More serious injuries should be brought to the attention of a veterinarian.

Shock/Inertia

Birds are susceptible to the stress of capture and handling and may suffer a physiological (shock) or neurological (inertia) reaction where birds become unresponsive to external stimuli to the point that they appear “frozen”. Shock may be accompanied by rapid breathing (not evident in inertia).

Birds should be allowed to recover in a quiet, sheltered and well-ventilated area, well away from any human activity. Limiting time in captivity, maintaining a calm and quiet captive environment, and working at a site appropriate for the environmental conditions will help prevent shock and inertia.

Hypothermia and Hyperthermia

Capturing, transporting and handling birds during extreme temperatures, rain or foul

weather makes them vulnerable to hypothermia or heat stress (hyperthermia) and should be avoided where possible.

Hypothermia can occur in cold conditions when feathers become wet and lose their insulating properties. Signs of hypothermia include shivering, lethargy and skin that is cold to the touch. Birds suffering from hypothermia should be dried and placed near a heat source such as a heating lamp (compact fluorescents bulbs should be at least 4-6" (10-15.25cm) from the animal's head and UV bulbs 12-20" (30.5-50.8cm)) or a hot water bottle (non-insulated). Holding wet birds in dry airy crates, at sufficiently low density and away from human disturbance usually allows them to preen themselves dry.

Handlers should avoid use of petroleum-based lotions (e.g., common in hand-creams and moisturizers) that may cause plumage to lose its insulating properties.

Hyperthermia can occur in hot conditions when birds are held in direct sunlight, at high ambient temperatures, or in overcrowded crates without adequate ventilation or water. Hyperthermia may also occur if birds are subject to a prolonged chase during capture. Signs of hyperthermia include panting, wings held away from the body, lethargy, seizures or prostration.

Birds suffering from hyperthermia should not be handled, but should be placed in a well-ventilated box/crate, moved to a cool, shaded area and provided with abundant drinking and swimming water. It may be beneficial to mist the bird with water or apply alcohol or water to the bird's feet to accelerate heat dissipation.

Section 10f. References

European Commission, DG SANCO (2007).

EU Guidelines for AI surveillance in wild birds and poultry in 2007.

FAO 2006. Wild Bird Highly Pathogenic Avian Influenza Surveillance: Sample collection from healthy, sick and dead birds. Edited by K. Rose, S.H. Newman, M. Uhart and J. Lubroth. FAO Animal Production and Health Manual, No. 4. Rome

FAO. 2007. Wild Birds and Avian Influenza: an introduction to applied field research and disease sampling techniques. Edited by D. Whitworth, S.H. Newman, T. Mundkur and P. Harris. FAO Animal Production and Health Manual, No. 5. Rome. (also available at www.fao.org/avianflu)

Bat Sampling Methods

Prepared by

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Last updated: 28 November 2016

Objective: To safely collect biological samples from bats.

Section 1. Confirmation of Knowledge

When you are familiar with the information in this Guide, take the PREDICT quiz.

Section 2. Brief Overview of PPE

All staff handling bats or their blood products should be vaccinated for rabies. If possible, ensure that they have a protective antibody titer, and be prepared to institute appropriate post exposure prophylaxis measures in the event that a bat bite or scratch occurs. It is recommended that personnel with frequent bat contact follow CDC and WHO guidelines of checking titers every two years

(www.who.int/rabies/WHO_Guide_Rabies_Pre_Post_Exposure_Prophylaxis_Humans_2013.pdf?ua=1).

Minimum PPE required for handling, capturing, or sampling live bats

The minimum PPE for handling bats during capture and sampling includes:

1. Eye protection
2. N95 or P100 respirator
3. Nitrile gloves¹
4. Tyvek-type suits ***
5. Washable shoes or shoe covers

***Tyvek-type suits are required for ALL sampling activities that involve direct contact with live wild bats and/or situations where contact with any fluids or excreta from live wild bats could soil clothing (eg, caves, roosts). If there is risk of being bitten or scratched by a live wild bat, other protective gear should be employed as appropriate, such as **heavy gloves (e.g. leather, sterilizable), long-sleeve clothing and pants should be worn underneath Tyvek-type suits**. As always, field teams should take necessary precautions to avoid additional routes of exposure by wearing eye protection, fitted respirator (N95 or P100), and nitrile gloves.

(See the *Biosafety and PPE Guide* for detailed instructions regarding PPE Use)

¹ Nitrile gloves are recommended for handling bats, in the absence of nitrile gloves and allergies to latex, double latex gloves could be considered.

First aid protocol for a bite, scratch, or needlestick

1. The injured person must notify other research staff and work must stop.
2. The bite, scratch, or needlestick site should be washed well with water and betadine (povidone-iodine) or benzalkonium chloride (this is known to kill rabies virus) for a full 15 minutes. It is recommended that benzalkonium chloride be kept readily available in a first aid kit for such purposes.
3. If the injury (bite or scratch) is from a bat, the post-exposure rabies vaccination should be obtained as soon as possible. It is recommended that the field team develop a post-exposure vaccination plan with their physician prior to fieldwork if working in a remote location so that a booster dose can be administered soon after exposure. Otherwise, exposed personnel should immediately report to a medical clinic for administration of the booster doses. See WHO guidance for post-exposure prophylaxis at:
<http://www.who.int/rabies/human/postexp/en/>
<http://www.who.int/rabies/human/prevvaccperson/en/>

Section 3. Data Collection

Please refer to the **required data collection templates** for data to collect. These include:

1. P2 Animal Data Collection Form
2. P2 Site and Event Characterization Data Collection Form
3. P2 Specimen Data Collection Form

Section 4. Bat Capture, Handling, and Sampling

Capture techniques will vary based on the species being targeted and the location where the samples are being collected and details of the main techniques including mist nets, harp traps, and hand capture are available in other documents such as the FAO guide *Investigating The Role Of Bats In Emerging Zoonoses* (www.fao.org/docrep/014/i2407e/i2407e00.pdf). Note that not all the sample collection techniques in that guide are recommended for PREDICT2 and field staff should use the PREDICT2 guide for specimen collection guidance.

Note: *The PPE requirements for handling animals during capture or during processing are the same. All animal capture, handling and sampling should be done in accordance with current IACUC protocols.*

Handling Procedures

1. Each bat should be placed into a porous cotton bag (with draw-string mouth), hung from a sturdy line over a polyethylene sheet (to catch urine), and kept in a cool dry place until sampling time.
2. Bats should be weighed (in grams) in bags using a Pesola hanging scale or a tabletop scale with or without a container (such as a cup). The container should be tared and both bat and bag should be weighed together. Once the bat is removed from the bag for sampling, the bag should be re-weighed and subtracted from previous total.
3. The bat should be removed from the bag and the samples below collected. The order of sampling may vary. For example, urine may be expelled on initial handling and urine would then be the first sample collected.

Note: check bag for fresh feces before continuing. If fresh feces are available, these may be used as a sample and then a rectal swab is not necessary. The sampler must be certain that the feces belong to the bat being sampled. Bags should be either discarded after first use or washed/disinfected between uses.

4. Bats will not be held longer than 6 hours. Frugivorous and nectivorous bats will be give 100% fruit juice or sugar water prior to release.

Sampling Procedures

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two oral swabs** - one in 500 μ L VTM and one in 500 μ L Trizol
2. **Two fecal samples** - one with max of 500 μ L/0.5cc feces in 500 μ L VTM and one with max of 500 μ L/0.5cc feces in 1 mL Trizol

Or

- Two rectal swabs** - one in 500 μ L VTM and one in 500 μ L Trizol
3. **Two blood samples** - 2 x 500 μ L aliquots, one in 500 μ L VTM and one in 500 μ L Trizol
4. **Two serum samples** - 2 x 500 μ L aliquots (only if more than 2ml of blood available), frozen without media. A minimum of 100 μ L serum (single aliquot) should be collected to be useful for PREDICT diagnostic testing

Note: *If animals are too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in VTM after serum separation*

5. **Two urogenital swabs/urine samples** - one with max of 500 μ L of urine in 500 μ L VTM and one with max of 500 μ L of urine in 500 μ L Trizol

Freeze all samples in liquid nitrogen immediately in the field and transfer to -80°C lab freezer.

If there is no **short-term** access (i.e., within 24 hours) to cold chain, such as in an emergency situation, then samples can be collected in 200 μ L of RNAlater instead of Trizol and VTM.

Storage times and temperatures for samples in RNAlater are as follows:

- 1 day at 37 °C (i.e. ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80 °C for storage until analysis

Collection of samples from bats:

- a. **Two oral swabs: 1 in Trizol, 1 in VTM:** Using sterile, polyester-tipped swabs with either an aluminum or plastic shaft, rub the swab tip gently but thoroughly against the back of the animal's throat, saturating the swab with saliva (recommend Puritan® Small Tapered Polyester-Tipped Swab from VWR, Catalog No.: 89133-756).
 - i) Place 1 swab in a 1mL screw-top cryovial filled with 500 µL VTM and use alcohol-wiped (or ethanol-wiped), flame-sterilized scissors to cut the shaft of the swab about 1cm above the tip. Swabs should be cut on the shaft as close as possible to the end-swab without touching it. Scissors should be wiped with ethanol or isopropyl alcohol and flame sterilized after cutting each swab.
 - ii) Place the other swab into 500 µL Trizol in a cryovial and cut the shaft as above.
 - iii) Store in a liquid nitrogen dry shipper or dewar and transfer to -80°C freezer later.
- b. **Two rectal swabs: 1 swab in VTM, 1 swab in a tube with Trizol.**

***DO NOT USE TRIZOL AS A LUBRICANT – IT IS HIGHLY IRRITATING TO TISSUE.
DO NOT FORCE TIP OF SWAB INTO RECTUM, IF IT WON'T ENTER EASILY, DO NOT COLLECT THIS SAMPLE.***

Gently insert the sterile swab tips, one at a time, into the animal's rectum. Place 1 swab in a cryovial filled with 500 µL of VTM and using isopropyl alcohol-wiped (or ethanol-wiped), flame-sterilized scissors cut the shaft of the swab above the tip (or snap as mentioned above). Place the other swab into a cryovial with 500 µL of Trizol. Store in a dewar or dry shipper with liquid nitrogen and transfer to -80°C freezer when possible.

- c. **Alternatively, collect fresh feces:** Add 500 µL or pea-sized pieces of feces directly into two vials, one containing 500 µL VTM (= maximum final ratio of 1:1) and one containing 1 mL Trizol (= maximum final ratio of 1:2) and mix each tube well. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.
- d. **Whole blood in VTM and Trizol, and serum divided into two aliquots;**
 - i) Manually restrain bats during blood collection. For larger bats, two or preferably three people are required for these manipulations: one person to safely restrain the bat, one to take samples, and a third to manage the tubes (i.e. unscrewing the lids, holding them up to the sample taker, making sure the lids are replaced tightly and kept in order) and record samples. Smaller insectivorous bats may be restrained and sampled by a single person. Anyone sampling bats should have had previous training in bat venipuncture to avoid injury to the animal. In addition:
 - It is recommended that large fruit bats (*Pteropus*, *Aceradon*, and other large species) be anesthetized using either injectable medetomidine (50 µg/kg) + ketamine (5 mg/kg) or gas anesthesia (isoflurane 4-5% induction, 2% maintenance).
 - The person restraining the bat is responsible for monitoring respiration and communicating respiratory status appropriately.

- ii) Bats must be bled with caution to maintain a ratio no greater than 10 μL of collected blood to 1 g of bat body weight (equivalent to 1% bodyweight).
NOTE: for bats <100g we use the maximum amount of 6 μL per gram of body weight.
- iii) **For bats > 100 g:** Use a non-heparinized syringe to collect blood (not to exceed 1% of the total body weight). Recommended venipuncture sites include the propetagal (cephalic) vein, the uropetagal (saphenous) vein, or the brachial vein (Figure 1.) If volume allows, place some blood in an EDTA (lavender top) tube and some in a serum vacutainer (red-top) tube containing serum-clotting factor. From the lavender tubes collect 500 μL of whole blood and place in 500 μL VTM, and 500 μL of whole blood in 500 μL Trizol. After allowing the blood to clot in the red top tubes, either spin tube in a centrifuge or allow tube to stand vertically on ice overnight. Use a sterile pipette tip and pipette gun to draw off serum and place even aliquots into 2 cryovials (minimum 60 μL).



Figure 1. Preferred venipuncture sites for large (>100g) bats. Note: Apply pressure with a cotton ball to ensure that hemostasis is achieved after blood draw, especially with the brachial vein or artery, which are closely associated, and higher pressure vessels compared to the cephalic or saphenous vein. (From Newman, Field, de Jong and Epstein, FAO 2011)

- v) **For bats <100 g:** Use a 75 µL heparinized glass hematocrit tube to collect blood. Bat is restrained in one hand and the wing is gently extended by the wrist. The radial artery or vein is punctured using the tip of a sterile 25 G (gauge) needle and a droplet of blood is allowed to form. Collect up to 0.6% body mass of blood (e.g., 6µL per gram) using hematocrit tubes. Use a bulb to expel the whole blood in a cryotube with 500 µL of VTM. **Apply pressure to site of bleeding using a cotton ball until bleeding ceases (approximately 1 minute).** Hematocrit tubes can be centrifuged using a portable hematocrit centrifuge to separate serum. Score glass tube (using a razor blade or X-acto knife) where the serum meets the red cell fraction and carefully snap the tube. Use a bulb to expel serum into a micro-cryovial and freeze. If two or more capillary tubes are filled, collect two aliquots of serum. Preserve the remaining red cell clots in a separate cryovial and freeze.
- vi) **Do not recap needle.** Place needle in sharps container and syringe in biohazard bag. Deliver medical waste to an incinerator or other secure medical waste disposal where possible.
- vii) Bats must be fully recovered from anesthesia before release to prevent injury.
- e. **Urogenital swabs/urine** - When handling bats, collect two urogenital swabs and place one into 500 µL VTM, one into 500 µL Trizol. If the bat urinates, collect two 500 µL urine samples at an optimal ratio of 1 part urine: 1 part VTM; and 1 part urine: 1 part Trizol (e.g., ~500 µL bat urine in 500 µL Trizol). Store samples in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.
Note: Larger fruit bats tend to urinate as they are removed from the cotton bag. Urine may be collected at this point using a pipette or tube. Urine may also be collected using a pipette from a surface however contamination is more likely and this should be avoided.
- f. **Necropsy sampling** - In case of accidental death before or during animal sampling, or where dead animals are available for opportunistic sampling, **collect tissue samples** – three, adjacent, approximately 200mg (pea-sized) pieces of each tissue type: one frozen in 500 µL VTM at -80°C, one frozen in 1 mL Trizol at -80°C, and one stored at room temperature, in a small vial or jar, in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment). In these cases also collect as much blood as possible. Cardiac puncture is recommended.

Collect approximately 200 mg (pea-sized) samples of the following tissues:

- Adrenal
- Cecum
- Colon
- Duodenum
- Heart
- Kidney
- Liver
- Lung
- Lymph node
- Spleen
- Ovary
- Pancreas
- Testes
- Other, if required

If euthanasia is required see the AAZV and **AVMA guidelines**.

Additional Data To Collect-Updated March 2018:

Additional identification and biometric measurements may be collected at the discretion of the sampling party, although they are not mandatory (unless they are needed for species identification).

- Whole body photograph
- Identifying characteristic photographs
- Age class*
- Sex
- Body weight
- Body condition **
- Biometric measurements (see Biometrics section below for details)
- Additional morphometric measurements
- Reproductive status

***Age classes:** For some bat species it will be possible to classify bats into one of five age classes. Most bat biologists will be familiar with the bat age classifications given below. However, for the PREDICT-2 project, we had to create generalized age classifications that would be appropriate across all of the taxa that are included in the eBook. For data that will be entered into EIDITH specifically, follow the EIDITH Age Classification rather than the Bat Age Classification. For clarity, both are listed in the table below.

Note: Follow this equivalency table to correctly classify bats using the same EIDITH definition.

7. PREDICT SOP Bat Sampling

Bat Age Classification	Cross-taxa EIDITH Age Classification
Fetus (in utero)	fetus (in utero)
Neonate (most microbats: hairless with eyes closed; megabats: thick hair and open eyes)	neonate (newborn)
Juvenile (pup is still clinging to dam and suckling or in a maternal colony)	juvenile (dependent on dam)
Subadult (pup is independent from dam, may be adult sized. There is an absence of secondary sexual characteristics (e.g. an absence of elongated teats, not gravid at the time of capture, and for male Pteropodid bats, absence of tiny barbules present on the glans penis. Incomplete fusion of phalangeal symphysis)	subadult (immature, independent)
Adult (secondary sexual characteristics present, pregnant or lactating, adult size. Complete fusion of phalangeal symphysis.)	adult (reproductive age)

**** Body Condition:** For larger fruit bats, it is also useful to evaluate body condition based on pectoral muscle mass--a quick and subjective measurement of nutritional status and robustness, which is a useful when assessing health in the context of infection. Record pectoral muscle mass as one of three categories: “Poor” (emaciated, prominent sternum), “Fair” (flat across pectoral muscles and sternum), “Good” (pectoral muscles are rounded and extend/bulge past the sternum).

Species identification:

1. The following digital photographs* should be taken of each bat where there is uncertainty about species identification:

- a. Full body in anterior-posterior presentation and wings extended with identification card displaying unique identifying number
- b. Full anterior facial (macro setting)
- c. Full lateral facial/head (macro setting)
- d. View of parted pelage on ventrum and dorsum (macro setting)

**Proper PPE should be worn at all times while holding animals, including while holding animals for photos or measurements.*

2. The biometric measurements (in millimeters) listed below should be taken. However, collecting these measurements adds time to the sampling effort. For micro-bats these are common measurements and they are valuable for identification; nevertheless the specific needs vary by species and by region. If you are in doubt of an identification look at reference texts for the genus or family and try to determine which the characteristics that are relevant for that group. Someone with experience in identifying Microchiroptera in the area is usually required for this.

Microchiroptera biometric measurements (as per Menzel et al., 2002)

- a. Forearm/radius length ('elbow to wrist')
- b. Ear length (most distal tip of ear to middle of the base)
- c. Tragus length (top of tragus to base of ear)
- d. Body length (measured with the bat in ventral recumbancy from the tip of nose to the base of tail).
- e. Hind foot length ('ankle to toe')
- f. Tail length (from base to tip)
- g. Tibia length ('knee to ankle')

Megachiroptera biometric measurements (as per Menzel et al., 2002)

- a. Forearm/radius length ('elbow to wrist')
- b. Head length
- c. Body length

For larger fruit bats, it is also useful to evaluate body condition based on pectoral muscle mass—a quick and subjective measurement of nutritional status and robustness, which is useful when assessing health in the context of infection. Record pectoral muscle mass as one of three categories: “Poor” (emaciated, prominent sternum), “Fair” (flat across pectoral muscles and sternum), “Good” (pectoral muscles are rounded and extend/bulge past the sternum).

3. Based on these morphometrics and other appropriate unique characteristics, identify bats to genus, species (where possible), age class, and sex. For female bats, determine pregnancy status by gently palpating the abdomen and lactation status by gently attempting to express milk from the teats.
4. Release bats as close to their site of capture as possible.
5. If a sonic recording device is available, for Microchiroptera record the bat’s calls upon release. These recordings can assist with identification of the specimens and with compiling resources for identifying bats in the area.

Section 5. References

Food and Agriculture Organization of the United Nations. 2011. Investigating the role of bats in emerging zoonoses: Balancing ecology, conservation and public health interests. Edited by S.H. Newman, H.E. Field, C.E. de Jong and J.H. Epstein. FAO Animal Production and Health Manual No. 12. Rome. <http://www.fao.org/docrep/014/i2407e/i2407e00.pdf>

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Menzel M.A., J. M. Menzel, S. B. Castleberry, J. Ozier, W.M. Ford and J.W. Edwards (2002) Illustrated Key to Skins and Skulls of Bats in the Southeastern and Mid-Atlantic States. USDA Forest Service, PA. 10p (Accessed 25.04.09 : http://www.fs.fed.us/ne/newtown_square/publications/research_notes/pdfs/2002/rnne376.pdf)

Smith,C., C. DeJong and H.E. Field (2010) Sampling small quantities of blood from microbats. *Acta Chiropterologica*. 12(1): 255-258.

Section 6. Appendix I. Supply and Equipment List

Note: Supply details, availability, and vendor sources may vary.

PPE

- Tyvek-like suits
- Flexible face shield or other eye protection
- N95 or P100 respirator
- Nitrile examination gloves
- Washable shoes

First Aid

- Betadine or (or benzalkonium chloride)
- First aid kit (with post-exposure prophylactic vaccine if working in remote areas where vaccine is not rapidly accessible)

Data Collection

- Datasheets (or EIDITH tablet for direct data entry)
- Pencils
- GPS

Capture and Handling

- Mist nets, poles and ropes
- Flagging tape
- Leather gloves
- Holding bags
- Spring/electronic balance
- Dial/digital caliper
- Stainless steel wing rulers
- Large ziplock bag
- Chemical restraint requirements
- Camera
- Identification guides

Sampling

- Processing trays
- Permanent lab markers for tube numbering
- Cryotubes
- Needles 25G, 27G
- Needles and syringes for blood draws
- Sterile swabs (dacron/polyester)
- Cryo resistant tube labels
- Cryovial rack
- Cryoboxes and dividers

- 75 μ L glass hematocrit tubes (heparinized)
- Plastic vacutainers (EDTA and dry)
- Pipettors and disposable tips
- Portable centrifuge for vacutainers
- Portable centrifuge for hematocrit tubes
- Cryo gloves
- Fine point forceps
- Scissors
- Dissection kit
- Trizol reagent
- Viral Transport Medium (VTM)
- RNAlater reagent
- Buffered formalin
- 95% ethanol
- Lighter
- Liquid nitrogen shipper/liquid nitrogen

Waste Disposal and Decontamination

- Paper towel
- Sharps containers
- Bleach
- 95% ethanol
- Biohazard bags
- Sprayers

Bushmeat Sampling Methods

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Last updated: 28 November 2016

Objective: Guidance on how to collect biological samples from hunted wildlife and wildlife byproducts in the context of the PREDICT project.

Section 1. Confirmation of Knowledge

When you are familiar with the information in this guide, take the PREDICT quiz.

Section 2. Brief Overview of PPE

Minimum PPE Required for Bushmeat Sampling

The minimum PPE for sampling small carnivores includes:

- Double gloves
- Protective glasses
- N95 facemask for self-protection and to avoid contaminating samples

Note: *Wear appropriate PPE according to species and pathogen-associated risk level.*

(See the ***Biosafety and PPE Guide*** for detailed instructions regarding PPE Use)

Section 3. Bushmeat Sample Collection

Samples to Collect

Duplicate specimens are to be collected from each animal (if feasible). If only one sample can be collected, then place into VTM.

- 1) Blood
 - a) **Fresh kill:** collect whole blood and serum.
 - i) **Whole blood: Collect as much blood as possible. Cardiac puncture is recommended.** Collect whole blood into 1 lavender top tube containing EDTA. Transfer a max of 500 μ L of whole blood to cryovial containing 500 μ L VTM and another 500 μ L of whole blood to cryovial containing 500 μ L Trizol. Freeze in liquid nitrogen or -80°C freezer.
 - ii) **Serum:** collect blood in at least 1 serum separator tube. Allow blood to clot and store a minimum of 2 x 0.5mL serum aliquots, frozen without media.

Note: *If blood volume recovered is too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in 500 μ L VTM after serum separation.*

- b) **Carcass:** collect blood clot. Place in at least one cryovial containing 500 μ L VTM, and freeze in liquid nitrogen or -80°C freezer.
- 2) **Swabs** (if fresh kill – x2 of each swab type): Collect 2 oral and 2 rectal swabs placing 1 of each sample into 500 μ L VTM and Trizol, respectively.
- 3) **Tissue:** Collect three, adjacent, approximately 200mg (pea-sized) samples from each of the following organs:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Testes
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas
- Other, if required

One specimen should be frozen in 500 μ L VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

If there is no **short-term** access (i.e., within 24 hours) to cold chain such as in an emergency situation then samples can be collected in 500 μ l of RNeasy instead of Trizol and VTM. Storage times and temperatures for samples in RNeasy are as follows:

- 1 day at 37 °C (i.e. ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80 °C for storage until analysis

Section 4. Bushmeat Sample Collection Methods

Sample Collection Technique

1. Ensure all sample collection tubes or vials are pre-labeled with appropriate information pertaining to sample ID (unique sample ID, or barcode and/or date).
2. **Wear appropriate PPE** according to species and pathogen-associated risk level (see above for minimum requirements).
3. Sample methods:
 - a. Use sterile, disposable sample collection utensils (tweezers/scalpels/needle and syringe) or wipe and flame with ethanol or isopropyl alcohol any metal instruments (e.g. scissors and tweezers) before collecting each sample type.
 - b. **For whole blood and serum** (fresh kill only):
 - i. Label vacutainer and prop tube upright in tube holder.
 - ii. If possible, perform cardiac puncture (laterally between ribs or longitudinally under sternum) using 3 ml or 5 ml syringe and adequate (largest possible for size of species) size needle to reach heart and draw blood (e.g. 19G for larger

- animal) without opening the carcass. Alternatively, open thoracic cavity to reach the heart.
- iii. Transfer blood (retaining ~1 ml in the syringe if volume permits) from syringe to a serum separator or red top vacutainer by disposing of the needle to the sharps box, and uncapping the vacutainer. Do not contaminate outside of blood tube with blood (If this occurs lightly clean outside of tube with ethanol-moistened gauze prior to moving on). Place labeled vacutainer in rack in shade for up to 2 hours before following instructions below on “Blood clot”.
 - iv. Transfer up to 500 μ L of the blood remaining in the syringe to a cryotube with 500 μ L of VTM and an additional 500 μ L of remaining blood to a cryotube with 500 μ L of Trizol (maximum final ratio of 1:1 in both cases).
- c. **For blood clot** (carcass where collection of whole blood is not feasible):
 - i. Using a sterile scalpel blade or forceps, collect blood clot ensuring no contamination from the external environment. Blood clot should be placed directly into 500 μ L VTM and frozen.
 - d. **For swabs (fresh kill only):** Using sterile polyester or Dacron-tipped (aluminum or plastic shaft – not wooden) swabs, collect 2 oral and 2 rectal swabs. Place one oral and one rectal swab in separate cryovials filled with 500 μ L VTM. Place one oral and one rectal swab in separate 2 ml cryovials with filled with 500 μ L Trizol. After placement into the tubes, cut swab tips (with ethanol-flamed scissors) on the shaft as close to the swab tip without touching/contaminating it. Scissors should be wiped with ethanol or isopropyl alcohol and flamed between each sample. Alternatively, snap swab shafts above the tip. After closing tubes, mix each tube well. Sealed, labeled vials with samples are to be immediately stored in liquid nitrogen (dry shipper or dewar) until transfer to -80°C freezer.
 - e. **For muscle tissue:** Using a sterile scalpel blade, dissect beneath the exposed surface to take three $\sim 0.5\text{ cm}^3$ (small pea-sized) samples of muscle tissue ensuring no contamination. Take muscle samples from most fresh area available (raw tissue preferable). Place one sample in a labeled cryovial with 500 μ L VTM and recap, and place another in a labeled cryovial with 1 mL Trizol and recap. Store immediately in liquid nitrogen (dry shipper or dewar) until transfer to -80°C freezer. Place third sample in labeled jar or vial with 10% buffered formalin at a volume of fixative 10 times the volume of the tissue, and store at room temperature.
 - f. **For organ tissue:** Using sterile/clean scalpel blade, take three 0.5 cm^3 samples of each organ tissue (see recommended list of organs above), ensuring no contamination from external environment. Organ samples should each be placed in individual, labeled cryovials. Place one sample in a cryovial with 500 μ L VTM and another sample in a cryovial with 1 mL Trizol, and freeze samples at -80°C (or liquid nitrogen in the field). Place third tissue sample in a labeled jar or vial with 10% neutral buffered formalin at a volume of fixative 10 times the volume of the tissue, and store at room temperature.

Additional sampling considerations:

In many bushmeat market or hunter-killed sampling situations, it may not be acceptable to traders for you to take organ samples. Remember that under PREDICT ethical guidelines, you CANNOT pay or trade anything for the samples. If allowed, intestinal/lymph node samples can often easily be obtained by inserting long hemostats into rectum and pulling out a sample of colon tissue. If the animal is to be butchered, you may also ask the owner/hunter to cut small samples of liver, lung, small intestine, large intestine, spleen, and kidneys. From these hunter samples, collect a small part of each organ tissue (~0.5 cm³) while maintaining sterility to the extent possible (i.e. avoiding surface of original hunter-taken tissue and asking the trader to clean her/his knife between cuttings of samples of various organs). Remember that (legal or not) bushmeat is intended for human consumption so, during sampling be very careful not to contaminate carcasses with hazardous chemicals (e.g., Trizol or formalin) or to touch bushmeat with potentially contaminated gloved hands or non-sterile utensils.

The researcher must consider quality of specimens and the pathogens of interest when deciding whether or not to sample a carcass for pathogens. Tissue from animals that have been smoked, dried, or dead longer than 24 hours are much less likely to harbor live pathogens or detectable RNA viruses, and are more likely to contain contaminating agents and bacterial overgrowths. Other pathogens, such as DNA viruses, may be detectable in tissues for an extended period of time. Most types of tissue (including skin or hair) can be used for genetic analysis (species identification), even from specimens that are of lesser quality (dried, processed, etc.).

Required sample storage conditions:

- Store all collected specimens immediately in liquid nitrogen or -80 °C freezer.
- Keep all samples frozen in liquid nitrogen in a dry shipper or dewar until transfer to -80°C freezer for long-term storage.
- Do not allow samples to thaw once frozen.
- Tissues in buffered formalin must be kept at room temperature.

Section 5. References

CDC and WHO (Centers for Disease Control and Prevention and World Health Organization). 1998. Infection Control for Viral Haemorrhagic Fevers in the African Health Care Setting. Atlanta, Centers for Disease Control and Prevention, pp. 198.

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Livestock Sampling Methods: Camel, Cattle, Goats, Sheep, and Swine

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Last updated: 28 November 2016

Objectives: To safely collect biological samples from livestock.

Section 1. Brief Overview of PPE

Minimum PPE Required for Livestock Sampling

The minimum PPE for livestock (including camels, cattle, sheep, goats, and swine) sampling includes:

1. Dedicated clothing
2. Nitrile (recommended) exam gloves
3. Safety glasses or other eye protection

(See the PREDICT *Biosafety and PPE Guide* for detailed instructions regarding PPE Use)

Standard disinfection procedures for equipment and clothing should be followed when moving between animal enclosures or properties.

Section 2. Livestock Handling and Welfare

Performance standards during handling include careful, considerate, respectful, calm, human interactions with animals in as positive a manner as is possible. Animals handled in a respectful manner will be calmer and easier to handle than animals handled in a rough or disrespectful manner. PREDICT field staff should be familiar with the correct techniques and the anatomy of each livestock species before attempting sampling procedures. At all times, observe animals for signs of excessive distress. If animals are unwell, stop all procedures, provide adequate support care, and release upon recovery.

While most veterinarians are familiar with handling livestock, we recommend that PREDICT staff visit the following guidelines as a refresher.

http://www.dardni.gov.uk/safe_cattle_handling_guidance.pdf

For more information on Animal Handling and Transport, see:

<http://www.fass.org/docs/agguide3rd/Chapter05.pdf>

For more information on welfare considerations for cattle handling, see:

<http://www.animalwelfarestandards.net.au/files/2011/02/Cattle-Standards-and-Guidelines-for-Endorsement-May-0807141.pdf> (Section 5, pages 13-16) and

Beef cows: <http://www.fass.org/docs/agguide3rd/Chapter06.pdf>

Dairy cows: <http://www.fass.org/docs/agguide3rd/Chapter07.pdf>

For more information on welfare considerations for sheep handling, see:

<http://www.animalwelfarestandards.net.au/files/2011/02/Sheep-Standards-and-Guidelines-for-Endorsement-May-2014-080714.pdf> (Section 5, pages 14 and 15) and

<http://www.fass.org/docs/agguide3rd/Chapter10.pdf>

For more information on welfare considerations for blood collection from cattle, see:
<http://www.dpi.nsw.gov.au/agriculture/livestock/animal-welfare/general/livestock/sop/cattle/blood-collection>

For more information on welfare considerations for swine handling, see:
<http://www.fass.org/docs/agguide3rd/Chapter11.pdf>

For more information on welfare considerations for camels handling, see:
<http://www.publish.csiro.au/Books/download.cfm?ID=5204>

Section 3. Sample Data Collection

Introductions and informed consent

Upon arriving to a household or farm, introduce yourselves (team members, purpose of the visit) to the acting head of household responsible for the livestock. Explain the purpose of the study, allow time for questions, and clarify any issues that may arise. If local regulations require it, obtain informed consent per project guidelines and protocols.

Animal Handling and Sampling Procedures

Note: For all food animals, manual restraint will be used. If drugs are used for sedation in a food animal, that animal will not be allowed to return the human food chain unless it is specifically labeled for use in that species and withdrawal periods are observed

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two nasal swabs** - one in 500 μ L VTM and one in 500 μ L Trizol
2. **Two fecal samples** - one with max of 500 μ L/0.5cc feces in 500 μ L VTM and one with max of 500 μ L/0.5cc feces in 1 mL Trizol

Or

- Two rectal swabs** - one in 500 μ L VTM and one in 500 μ L Trizol
3. **Two whole blood samples** - one with max of 500 μ L of whole blood in 500 μ L VTM and one with max of 500 μ L of whole blood in 500 μ L Trizol
4. **Two serum samples** - 2 x 1.0 ml aliquots frozen without media
5. **Two urogenital swabs or urine samples** - one with max of 500 μ L of urine in 500 μ L VTM and one with max of 500 μ L of urine in 500 μ L Trizol

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

If there is no **short-term** access (i.e. within 24 hours) to cold chain such as in an emergency situation, then samples can be collected in 500 μ L of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

- 1 day at 37 °C (i.e. ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80 °C for storage until analysis

Collecting Nasal Swabs

Using sterile, polyester-tipped swabs with a plastic shaft, rub the swab tip gently but thoroughly against the walls of the animal's nares, about 1-2" from the opening, saturating the swab with mucus. **Place 1 swab in a cryovial filled with 500 µl of VTM and the other swab into 500 µL of Trizol in another cryovial.** Mix each tube well. Store both cryovials in a liquid nitrogen dry shipper or dewar & transfer to -80°C freezer when possible.

Bleeding Collection Techniques

1. Cattle

Blood can be collected from the jugular vein in cattle of all ages or from the tail (coccygeal) vein of older cattle.

A variety of collection devices may be used - vacutainers, bleeding tubes, syringe and needle. Restraint should ensure quick, easy and safe collection of the sample causing minimal distress. This may involve use of a bail, race, or crush for tail bleeding. For jugular bleeding the animal may require minimal restraint (e.g. halter) or may need to be restrained in a crush with head bail and the employment of a halter or nose grips. Use of nose grips should be avoided wherever possible.

Operators should use gloves and disinfect or replace them between animals to prevent the transmission of blood-borne diseases. Equipment such as vacutainer holders should also be cleaned between animals. An antiseptic must be applied to clean skin surface prior to venipuncture.

For a visual guide see the following online tutorials:

Cattle

<https://www.youtube.com/watch?v=luNbsTMrlul> (tail and jugular)

<https://www.youtube.com/watch?v=ZEsHMwKFbKg> (tail)

<https://www.youtube.com/watch?v=812CskWCqGQ> (jugular)

Procedure for Jugular Venipuncture Using Vacutainer Needle and Tubes:

1. Identify and georeference the study site and document the signalment of the animal on the data collection sheet.
2. Before sample collection, ensure that the animal is effectively and humanely restrained to avoid injury to the animal and/or study personnel.
3. Using the halter, position the animal's head so that it is slightly elevated and drawn to the side opposite the jugular vein to be sampled.
4. Disinfect venipuncture area with alcohol
5. Occlude the vein by applying digital pressure in the jugular groove located in the lower neck.
6. Place a vacutainer needle attached to a vacutainer holder into the distended jugular vein at a 45° angle cranial to the jugular groove.
7. Once needle is positioned in the vein, insert a vacutainer into the needle to collect the blood.
8. When the desired volume has been collected (5 ml minimum suggested) remove the occluding pressure from the vein.
9. Detach the tube from the needle and withdraw the needle from the jugular vein.
10. You can collect more than 1 tube by repeating steps 7 and 8.
11. Label the vacutainer tubes with the sample ID.

Procedure for Jugular and Coccygeal Venipuncture Using Syringe and Needle

Jugular bleeding

1. Restrain cow with the head elevated and the jugular groove exposed.
2. Raise the jugular vein by placing pressure at the base of the jugular groove.
3. Pass the needle through the skin and into the vein by a firm thrust directed at an angle of 20° to the skin surface.
4. Withdraw the blood sample.

Tail Bleeding

1. Restraint should prevent the cow from moving away during the procedure.
2. Raise the tail vertically with one hand until it is horizontal with the ground.
3. Approximately 150 mm from the base of the tail, locate the groove lying in the ventral midline of the tail.
4. Midway along the body of a coccygeal vertebra, insert the needle perpendicularly to the surface of the skin to a depth of a few millimeters.
5. Withdraw blood sample.
6. Apply pressure to the venipuncture site after withdrawal of the needle until the bleeding stops.

Once blood is collected, place the needle into a sharps container. Open red-top and purple top vacutainer tubes. Place approximately 2.5cc in each tube, then discard the syringe into a biohazard container. Invert each tube several times to mix.

2. Sheep/Goats

Blood should be collected from the jugular vein. The procedures for blood collection are identical to those described for cattle, with the exception of the amount of restraint needed and the possibility of shearing the bleeding area on the neck for easier viewing of the vein and minimizing the chance of introducing dirt or bacteria into the vein with the needle.

In sheep and goats, blood sampling can be done with assistance or alone. If you are not proficient at drawing blood alone, work with an assistant. The assistant should restrain the sheep/goat's body and turn the head to the side, at a 30-degree angle, by holding the animal under its jaw to allow for easy access to the jugular vein.

Restraining a sheep or goat without assistance is better for those who have become proficient at drawing blood. The handler should straddle the sheep/goat, place his or her knees behind the animal's shoulders, and back the sheep/goat into a corner or against a wall to help control their hindquarters. The sheep/goat's head should be turned opposite to the side of collection, once again at a 30-degree angle. Restraint of the head is accomplished by using the elbow and the upper arm to keep it held off to the side. This leaves both hands available for the blood collection.

The easiest way to locate the vein is to draw an imaginary line from the middle of the sheep/goat's eye down the side of the neck. The vein can be located by applying pressure with the thumb or fingers in the groove on either side of the trachea. The pressure will cause the vein to pop up and be easy to feel or see if the area has been shaved. Proceed as with cattle, using a vacutainer collection system or syringe and needle.

For a visual guide see the following online tutorials:

Sheep/goats (small ruminants) <https://www.youtube.com/watch?v=47tlmqXX3eE>

Blood sample processing and storage:

Whole Blood

- Collect whole blood into 1 lavender top tube containing EDTA, and allow another tube to clot for collection of serum.
- Add up to 500 μL of whole blood (from EDTA tube) directly into 2 vials, one containing 500 μL Trizol and one containing 500 μL VTM (= maximum final ratio of 1:1) and mix each vial well.

Serum

- After clotting is complete, use a plastic pipette to take 1 ml of serum and transfer into 2 cryovial tubes, 0.5 ml each.
- If a centrifuge is available, centrifuge samples for 15 minutes and then collect 1 ml serum and transfer into 2 cryovial tubes, 0.5 ml each.
- Label the cryovial tubes with the same label information used on vacutainer tube.
- You can harvest additional serum for serum bank as appropriate.
- Freeze all samples in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

3. Camels

Because of the risk of MERS CoV exposure, sample collectors should wear gloves, a respirator, and eye protection when handling camels.

Blood can be collected from the jugular vein in camels of all ages, though it is recommended that this be undertaken on animals while they are in sternal recumbency (kush position), well-restrained, or sedated. The lateral thoracic vein or caudal epigastric (“milk”) vein may be used but should only be targeted in animals where physical or chemical restraint prevents kicking.

A vacutainer needle (18G or 19G) with purple top (EDTA) tubes and red-top (with serum clot activator) tubes may be used, or a 5cc syringe and 18G or 19G needle. Restraint should ensure quick, easy and safe collection of the sample causing minimal distress.

Equipment such as vacutainer holders should be cleaned between animals.

Procedure for Jugular Venipuncture Using Vacutainer Needle and Tubes

1. Identify and georeference the study site and document the signalment of the animal on the data collection sheet.
2. Before sample collection, ensure that the animal is effectively and humanely restrained to avoid injury to the animal and/or study personnel.
3. Using the halter, elevate the animal’s head and draw it to the side opposite the jugular vein to be sampled.
4. Disinfect venipuncture area with alcohol
5. Occlude the vein by applying digital pressure in the jugular groove located in the lower neck. Alternatively, a rolled towel affixed with a rope over the withers can be applied at the same level to act as a temporary incomplete tourniquet.
6. Place a vacutainer needle, attached to a vacutainer holder, into the distended jugular vein at a 45° angle cranial to the jugular groove.
7. Once the needle is positioned in the vein, insert a vacutainer into the needle and collect the blood.
8. When the desired volume has been collected (5 ml minimum suggested), remove the occluding pressure.

9. Detach the tube from the needle.
10. Detach the needle from the jugular vein and apply pressure to the venipuncture site after withdrawal of the needle until the bleeding stops.
11. If more than one tube of blood is required, repeat steps 7 through 9 with occluding pressure.
12. Label the vacutainer tubes with sample ID.

Note: If vacutainer needles are unavailable, a 5cc syringe and 18G or 19G needle can be used. Once blood is collected, place the needle into a sharps container. Open red-top and purple top vacutainer tubes. Place approximately 2.5cc in each tube, then discard the syringe into a biohazard container. Invert each tube several times to mix.

Whole blood can be aliquoted into cryotubes with VTM and Trizol using a pipette gun. Serum tubes can either be centrifuged (if available) or placed vertically in a cooler with ice bricks and allowed to stand undisturbed overnight (~12 hours) for clean serum separation. Serum can then be aliquoted into cryotubes.

Procedure for Jugular Venipuncture Using Syringe and Needle

Jugular bleeding

1. Restrain camel with the head elevated and the jugular groove exposed.
2. Disinfect venipuncture area with alcohol
3. Raise the jugular vein by pressure at the base of the jugular groove.
4. Pass the needle through the skin and into the vein by a firm thrust directed an angle of 20° to the skin surface.
5. Withdraw blood sample.
6. Apply pressure to the venipuncture site after withdrawal of the needle until the bleeding stops.

Lateral Thoracic/Caudal Epigastric Vein Bleeding

1. Restraint should prevent the camel from moving away or kicking during the procedure.
2. Identify the lateral thoracic vein, caudal to the point of the elbow's olecranon process.
3. Pass the needle through the skin and into the vein by a firm thrust directed an angle of 20° to the skin surface.
4. Withdraw blood sample.
5. Apply pressure to the venipuncture site after withdrawal of the needle until the bleeding stops.

4. Swine

All personnel handling or sampling pigs should wear appropriate PPE and practice appropriate biosafety practices to avoid spreading infection from one animal to another and from one herd, farm or property to another. This includes wearing dedicated clothing (e.g. coveralls and rubber boots) that can be removed and disinfected once work at a site has been completed. Recommended PPE includes nitrile gloves, a respirator and safety glasses.

Restraint: Manual restraint is recommended, without the use of anesthesia. Pigs to be sampled should be constrained to a separate pen, if possible. The use of a snout snare (see appendix) by the animal restrainer is recommended for pigs over 20 kg, but should only be used by experienced personnel and for short term restraint to avoid injury to the pig's snout. Pigs will be restrained for a maximum of three minutes and then released. If blood collection is unsuccessful, then the pig will be allowed to calm down for five minutes before a second attempt is made.

Blood can be collected from the external jugular vein, or the cranial vena cava, using a 1", 20G needle and a 5cc syringe. This technique requires the head to be restrained and elevated parallel to the ground, typically using a snout snare. In pigs weighing less than ~50 kg, blood can be collected further caudally (and more medially) in the jugular groove, nearer the manubrium from anastomose of internal and external jugular vein. For pigs weighing less than ~20 kg, a technician will manually restrain the pig on his lap, holding the forelegs in one hand, and the animal's head in the other. Then a max of 5.0 to 10 ml may be collected from the jugular vein. Venipuncture should only be performed by experienced personnel.

The marginal ear veins are the only veins that are easily visible on pigs of any size. Usually there are three prominent veins. The lateral or central vein is usually the largest of these. These veins may also be punctured for blood collection. An alternative venipuncture site is the caudal auricular ("marginal ear") vein, though this typically yields low (<1 mL) blood volumes. A smaller, 22G or 23G needle should be used for this vein.

See also <http://oslovet.norecopa.no/teaching/pig/pigbleed/> for more details on blood collection from pigs.

Collecting Fecal Samples

Ensure the animal is properly restrained prior to sampling. Fresh fecal samples should be collected, preferably from the rectum. If freshly passed, feces can be collected off the ground. Only the top part of a freshly passed fecal pat should be collected using a disposable spoon or scooped up in a gloved hand, plastic bag or plastic vial.

For Collection from the Rectum in Cattle and Camels

- The operator places an obstetrical sleeve on one arm
- The arm is formed into a cone and the animal's tail held to one side with the opposite gloved hand.
- Gentle pressure is applied to the anal sphincter until penetration into the rectum is obtained.
- A fecal aliquot of sufficient size for the intended laboratory procedure is scooped with the sleeved hand and removed from the animal.
- The fecal sample is placed in a separate container or the obstetrical sleeve is inverted off the arm such that the fecal sample is trapped inside.

Small calves, sheep, goats, and swine: restrain manually. Gently pass a gloved, lubricated finger through the anus and massage the rectal wall to stimulate rectal evacuation. If feces are not produced, collect feces with finger.

Place two ~200 mg (pea size) samples of fresh feces into 2 vials, one containing 500 µL VTM (= maximum final ratio of 1:1) and one containing 1 mL Trizol (= maximum final ratio of 1:2). Homogenize by shaking. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

If feces are not available, collect 2 rectal swabs- 1 in VTM and 1 in Trizol: Place 1 swab in a cryovial filled with 500 µL of VTM. Place the other swab into a tube with 500 µL of Trizol. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80°C freezer when possible.

Collecting Urine/Urogenital Swabs

Many animals will urinate as a fear reaction while they are handled. Urine can be collected free catch in plastic vials. Add up to 500 µL of urine directly into 2 vials, one containing 500 µL VTM and one containing 500 µL Trizol (= maximum final ratio of 1:1) and mix each tube well. Store in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

If urine is not available, collect 2 urogenital swabs: 1 in VTM and 1 in Trizol. Place 1 swab in a cryovial filled with 500 µL of VTM. Place the other swab into a tube with 500 µL of Trizol. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80°C freezer when possible.

Section 4. Sample Collection from Dead or Euthanized Livestock

PREDICT's primary approach to sample collection in livestock is to collect specimens from living animals. In the event that an animal has died of natural causes or been euthanized due to humane or veterinary care reasons, the guidelines below for necropsy sampling may be followed. If bodies are relatively whole and fairly fresh, then sample as described above. The *American Veterinary Medical Association guidelines* in the PREDICT Operating Procedures ebook provides information on animal euthanasia that may be useful to PREDICT veterinarians called upon to euthanize an animal.

As discussed throughout this protocol, all animals should be considered potentially infectious for a wide variety of dangerous pathogens, and dead animals in particular should be sampled only following all safety measures, including proper PPE use, proper workstation decontamination, and proper carcass disposal, as outlined here and in other PREDICT documents.

Though not required for PREDICT sampling, thorough necropsy procedures can be very beneficial and relevant for some animals (e.g., suspicious deaths). Time and skill permitting, when full necropsies are performed, following any Association of Zoos and Aquariums/AZA (or similar) necropsy protocol is recommended and most can be adjusted for application to livestock species. Necropsy protocols are also addressed in the Non-Human Primate Sampling protocol, Appendix V.: AAZV's Occupational Primate Disease Safety Guidelines for Zoological Institutions: Standardized Necropsy Report for Non-Human Primates Work Sheet; most of the information and worksheets in this document can be utilized for sampling of livestock. (*Note that properly following extensive necropsy procedures and collecting and measuring all samples can require 4-6 hours for a single animal.*)

Duplicate blood samples are to be collected from each animal; one sample must be collected into Trizol and one into viral transport media (VTM). If only one sample can be collected, then place the sample into VTM.

Tissue specimens should be collected in triplicate. One specimen should be frozen in 500 μ L VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

Post-Mortem Blood Collection

From recently dead animals, it may be possible to collect whole blood (often clotted) from the right side of the heart where the largest volume of blood is available. Collect all available blood into an appropriate size container (typically one or more blood tubes). Allow the tubes to sit undisturbed for at least 30 minutes, and then centrifuge at high speed (2000 x G for 20 minutes). Transfer the serum (clear, yellow or red-tinged fluid at the top), preferably via pipetting, to

appropriately labeled cryovials. Transfer the remaining blood clots to separate cryovials. Refrigerate or freeze both the serum and blood clots.

If a centrifuge is not available, allow the clots and cells to settle as much as possible, and then collect the serum and clots as described above. If the animal's death is recent enough that the blood has not yet clotted and a centrifuge is not available, invert the blood tubes after the blood has been collected to allow the clot to form on the rubber stopper. After the blood has clotted, turn the tube right side up and carefully remove the stopper with the adhered clot, thereby leaving a clean serum sample in the tube.

At a minimum, as many of the following blood samples as possible should be collected:

- 2 samples of 500 μ L (**whole blood**) placed in 2 vials, one containing 500 μ L **Trizol** and one containing 500 μ L **VTM** (= maximum final ratio of 1:1). Mix each vial well.
- 2 or more aliquots (0.5 ml) of **separated serum**, frozen

Tissue Collection

Collect three, adjacent, approximately 200mg (pea-sized) samples of the following tissues:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Testes
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas
- Other, if required*

*It will usually require experience to identify abnormal tissues, but potentially recognizable gross lesions include masses, discolored areas, ulcerations, etc. Samples for histopathology (i.e., in formalin) should be collected at the abnormal margins to include both normal and abnormal sections in the same piece of tissue. Collection of any obvious internal parasites in ethanol is also recommended.

Section 5. References

Higgins, A. J., & Kock, R. A. (1984). A guide to the clinical examination, chemical restraint and medication of the camel. *The British Veterinary Journal*, 140(5), 485–504.

Fowler, M. E. (2010). Chapter 4 Clinical Diagnosis: Examination and Procedures in *Medicine and Surgery of Camelids*. (3 edition). Ames, Iowa: Wiley-Blackwell.

http://www.dardni.gov.uk/safe_cattle_handling_guidance.pdf

<http://www.fass.org/docs/agguide3rd/chapter05.pdf> =

<http://www.dpi.nsw.gov.au/agriculture/livestock/animal-welfare/general/livestock/sop/cattle/blood-collection>

<http://www.biotracking.com/goats/biopryn/use>

Section 6. Appendix I. Dentition Age Determination for Cattle, Sheep, and Goats

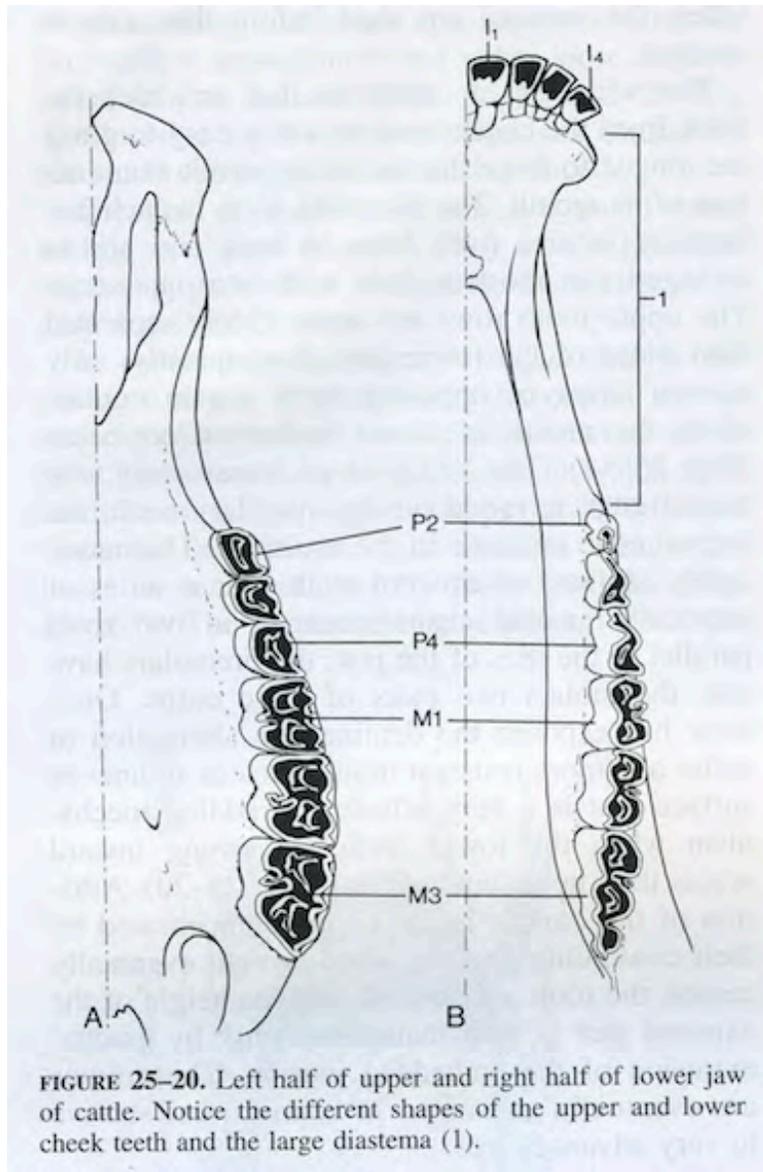


Figure 1: From Dyce, Keith M., Wolfgang O. Sack, and Cornelis Johannes Gerardus Wensing. *Textbook of Veterinary Anatomy*. Elsevier Health Sciences, 2009.

Table 1: Eruption dates of the teeth of cattle

Teeth	Deciduous Teeth	Permanent Teeth
Incisor 1	Birth to 2 weeks of age	18 – 24 months
Incisor 2	Birth to 2 weeks of age	24 – 30 months
Incisor 3	Birth to 2 weeks of age	36 months
Incisor 4	Birth to 2 weeks of age	42 – 48 months
Premolar 2	Birth to 1 week	24 – 30 months
Premolar 3	Birth to 1 week	18 – 30 months
Premolar 4	Birth to 1 week	30 – 36 months
Molar 1		12 – 18 months
Molar 2		24 – 30 months
Molar 3		18 – 24 months

Table 2: Eruption dates of the teeth of sheep and goats.

Teeth	Deciduous Teeth	Permanent Teeth
Incisor 1	Birth to 1 weeks of age (at birth)	12 – 18 months
Incisor 2	Birth to 1 weeks of age (at birth)	18 – 24 months
Incisor 3	Birth to 1 weeks of age (at birth)	30 – 36 months
Incisor 4	1 to 3 weeks	36 – 48 months
Premolar 2	3 weeks	18 – 24 months
Premolar 3	3 weeks	18 – 24 months
Premolar 4	3 weeks	18 – 24 months
Molar 1	3 – 4 months	
Molar 2	8 – 10 months	
Molar 3	18 – 24 months	

Section 7. Appendix II. Snares



Figure 1: A commercial snout snare (left) and use of a modified snout snare, made from local materials, to restrain a pig during sampling in Bangladesh (right).

Section 8. Appendix III. Checklist for Supplies

General equipment and supplies

- Animal handling equipment – Halters and animal restraining ropes
- Data Collection forms
- Rubber stamp ink and pad
- GPS
- Camera
- Field Notebook
- Pen/Pencil
- Permanent markers
- Cryomarkers
- Protective clothing – Waterproof rubber boots, overalls, facemask, and nitrile gloves
- First aid kit
- Ice box containing ice packs (for short term storage and transport)
- Sharps bin
- Sturdy garbage bags
- Field centrifuge (portable 12vt)
- Liquid nitrogen dewar

Blood sample collection equipment and supplies

- EDTA vacutainer tubes – 9ml (lavender top)
- Serum separator vacutainer tubes – 9ml (red/gray top)
- Vacutainer needle holders
- Vacutainer needle: Cattle and Camels, 1½” 18 or 19 gauge; Sheep, Goats, and Swine, 1” 20G
- Syringes: 20, 10 and 5 ml
- Needles: Cattle and Camels, 1½” 18 or 19 gauge; Sheep, Goats, and Swine, 1” 20 gauge for jugular or 22 or 23 gauge for auricular vein
- Alcohol (squirt bottle or vaporizer)
- Gauze
- Vacutainer tube rack
- Cryovial tubes
- Cryovial rack
- Centrifuge
- VTM
- Trizol

Fecal Sample Collection Equipment and Supplies

- Obstetrical Sleeve
- Disposable Spoons
- Plastic bags or vials
- Cryovial Rack
- Cryovials with VTM and Trizol

Urine Sample Collection Equipment and Supplies

- Plastic vials
- Plastic pipettes

- Cryovial Rack
- Cryovials with VTM and Trizol

Swab Collection Equipment and Supplies

- Plastic handle, polyester tip swabs
- Cryovial Rack
- Cryovials with VTM and Trizol

Tissue Collection Equipment and Supplies (in case of animal necropsy)

- 21 Gauge needles for cardiocentesis
- 1 mL Syringe for cardiocentesis
- Scalpel and surgical blades
- Forceps
- Sharp and blunt tip scissors
- Cryovial Rack
- Cryovials with VTM and Trizol
- Small Vials or Jars
- 10% Buffered Formalin

Section 9. Appendix IV. Additional Permit Requirements for Livestock Samples Imported into the United States

In addition to all other permits, livestock samples require special import permits from the USDA.

http://www.aphis.usda.gov/publications/plant_health/2012/fs_imp_food_ppq.pdf

http://www.aphis.usda.gov/wps/portal/aphis/resources/permits!/ut/p/a1/jZDLDoIwFES_hi0dKmJ1VyVCfUVjjNiNQYOVBKgpKL8vGjfG5-

[zuzTnJZlgkEZFFfEIVXKW6iLPbLb2tKxilLVDBlgsPYjiftQeUOgi8Btg0wCDgoduZAHAZhfD7od_pTgHh_efjQzh--Wsin5HA4X7jLSezRTgExs4D-FbxDnzpMCJSZxp332PDi12LKSJNckhMYuyzad7HqjqVPQsW6rq2ldYqS-y9zu3YWHhnHXVZkegFJqd8FSGd52tW8ivrt9DV/?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_animal_health%2Fsa_import_into_us%2Fct_animal_imports_home](http://www.aphis.usda.gov/wps/portal/aphis/resources/permits!/ut/p/a1/jZDLDoIwFES_hi0dKmJ1VyVCfUVjjNiNQYOVBKgpKL8vGjfG5-zuzTnJZlgkEZFFfEIVXKW6iLPbLb2tKxilLVDBlgsPYjiftQeUOgi8Btg0wCDgoduZAHAZhfD7od_pTgHh_efjQzh--Wsin5HA4X7jLSezRTgExs4D-FbxDnzpMCJSZxp332PDi12LKSJNckhMYuyzad7HqjqVPQsW6rq2ldYqS-y9zu3YWHhnHXVZkegFJqd8FSGd52tW8ivrt9DV/?1dmy&urile=wcm%3apath%3a%2Faphis_content_library%2Fsa_our_focus%2Fsa_animal_health%2Fsa_import_into_us%2Fct_animal_imports_home)

Non-Human Primate Sampling Methods

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Objective: To safely collect biological samples from non-human primates.

Section 1. Confirmation of Knowledge

When you are familiar with the information in this Guide, take the PREDICT quiz.

Section 2. Brief Overview of PPE

Minimum PPE Required for Handling Live, Dead, or Samples of NHP

The minimum PPE for NHP sampling includes:

1. Eye protection (goggles or face shields)
2. N95 or P100 respirator
3. Tyvek-type suits (Duct tape can be wrapped around the overlapping Tyvek suit and gloves at the wrist to avoid skin exposure) ***
4. Nitrile gloves (double gloving is preferred, especially if sampling dead NHP)
5. In the rare cases where it is acceptable (see below), anyone hand-restraining NHP for sampling should wear disinfected¹, heavy-duty leather (or similar) gloves to protect against bites
6. In order to protect both human handlers and sampled NHP, all personnel handling NHP should be tuberculosis (TB) tested beforehand as described below

***Tyvek-type suits are required for ALL sampling activities that involve direct contact with live wild non-human primates and/or situations where contact with any fluids or excreta from live wild non-human primates could soil clothing (eg, caves, roosts), *this does not include non-invasive sampling (i.e. rope technique)*. If there is risk of being bitten or scratched by a live wild animal, other protective gear should be employed as appropriate, such as **heavy gloves (sterilizable), long-sleeve clothing and pants should be worn underneath Tyvek-type suits**. As always, field teams should take necessary precautions to avoid additional routes of exposure by wearing eye protection, fitted respirator (N95 or P100), and nitrile gloves.

Macaque Handling

Due to the risk of infection with *Cercopithecine herpes 1* ('B virus'), which can be fatal in humans, handling macaques (or other potential B virus carriers such as other NHP in close contact with macaques) requires special preparation. When handling macaques, it is imperative that before animals are handled all precautions are taken to minimize the risk of exposure to B virus and to minimize the risk of infection in the event of an accidental exposure. Please note that human to macaque transmission of herpesviruses may also occur. Protective measures include:

¹ Because they are porous, leather gloves cannot easily be disinfected. Spraying, wiping, or soaking in the best available disinfectants (e.g., 10% bleach) and allowing to sit or dry for >10 minutes can help destroy many potential pathogens. Likewise, wearing and changing over-sized disposable gloves over protective leather gloves can help to minimize cross contamination between handled animals. More easily cleaned protective gloves made from synthetic materials (heavy duty nitrile, Kevlar) can also be used. Some PREDICT teams have had good success with Hexarmor Hercules 400R6E gloves.

- Wearing a full-face shield (not just goggles) along with an N95 (or better) respirator.
- Having sufficient and immediately available eyewash (1 liter of saline if working in remote location) for a 15-minute continuous flush of any exposed mucous membranes.
- Having water and detergent soap (chlorhexidine or povidone-iodine) immediately available and in sufficient quantity to allow a 15 minute scrub of any exposed skin.
- Preferably also having freshly prepared 0.25% hypochlorite/Dakin's solution (1:20 dilution of household bleach) for initial wash of skin - but NOT mucous membranes.
- Keeping extra swabs, viral culture media, and serosampling materials available for post-exposure sampling of handler and macaque.
- Carrying medical alert cards.
- Consider having a cage ready for short term (2-3 week) captivity of suspect macaques for post-exposure sampling in the event of accidental exposure.

First Aid Guidance for a Bite, Scratch, Needlestick, or Facial Splash

The injured person must notify other research staff and work must stop immediately (with the possible exception of other workers ensuring the safety and containment of any live animals).

All NHP - Any bite, scratch, or needlestick site should be immediately washed well with soap and water for a full 5 minutes and then with betadine (povidone-iodine) or benzalkonium chloride (if available and especially if rabies virus exposure is suspected).

Macaques (or other possible B virus carriers) - Any possible exposure to B virus is potentially life threatening and must *immediately* trigger activation of the B Virus Emergency Exposure Protocol detailed in ***Appendix II. B Virus Exposure Emergency Protocol.***

Suspect Ebola cases (e.g., ape carcasses) - Any possible exposure to Ebola virus is potentially life-threatening and should immediately trigger activation of the Ebola Virus Emergency Exposure Protocol detailed in ***Appendix III. Ebola Virus Exposure Emergency Protocol.***

Section 3. Special Considerations for Handling NHP

Note: This training guide supplements the *Safe Animal Capture and Sampling* which contains general information on working with wildlife species. This training guide also complements information in the *Bushmeat Sampling Methods*.

Handling NHP involves a number of special considerations.

1. Regardless of their specific status (e.g., endangered, threatened, protected or not), NHP are often high-profile species that engender special attention. Anyone handling NHP should strictly adhere to all regulations and follow all protocols and guidelines.
2. All primate species, regardless of size, are capable of inflicting serious injuries to their handlers; particularly bite wounds. Unlike most other taxa, many NHP have grasping hands and feet and are likely to grab (and then bite) rather than scratch or push their handlers during procedures. Heavy-duty leather gloves should be worn by anyone handling conscious (unanesthetized or unседated) NHP. Hand restraint is discouraged as a primary means of NHP immobilization. Chemical, rather than physical, restraint should be employed with a few exceptions. Hand restraint may be considered in rare instances when it can be done safely and without significant added stress or risk to the animal, such as when handling infants, severely debilitated individuals, or during the process of chemically immobilizing very small NHP with hand injections.
3. NHP are typically very social animals and are likely to protect and defend other individuals in their group. Care must be taken, particularly during capture and immobilization, to protect against attacks, injuries, or disruptions from non-target individuals and especially from defensive adult males. Using visual blinds to hide activities and/or employing personnel fully dedicated to watching for aggressive or approaching animals can help minimize these risks.
4. Due to their size, considerable strength, and in some cases habituation to human visitors, great apes (and some larger monkeys) should be considered very dangerous. Even without aggressive intentions, field staff should be aware that great apes often grab, kick, strike, and drag humans for play and/or display behavior purposes.
5. If NHP need to be tracked for capture, or are opportunistically sampled as individuals or in low numbers, it may not be feasible or practical to set-up proper sampling stations as described below. In such situations, sampling station guidelines should be followed as closely as possible for both field collection sites and any later sample processing sites.
6. PREDICT personnel should already understand that due to their close genetic relationship to humans, NHP are considered to be more likely to share infectious agents (zoonoses) with humans. This means that they are more likely to transmit infections *to* their human handlers, and they are also more susceptible to acquiring infections *from* their handlers.
 - a. Proper use of PPE and related biosafety measures as described will help protect both handlers and the sampled NHP.
 - b. To protect both staff and any handled NHP, all people working closely with NHP should be tuberculosis (TB) tested every 6 months with negative results documented

and available before handling NHP. **Any staff suspected of being infected with TB must not work with NHP.** TB testing is typically done by intradermal tuberculin skin test (TST). Workers who have been vaccinated with BCG (Bacillus Calmette-Guerin, standard vaccine for many Europeans) should still be tested and the possibility of false positive results from vaccination needs to be discussed with their health care provider (see relevant information at:

<http://www.cdc.gov/tb/publications/factsheets/testing/diagnosis.htm>). Personnel vaccinated with BCG and positive skin test should work with their health care provider to have additional confirmatory tests performed.

- c. To protect NHP from human infections, no persons with any current or recent (within a few days²) clinical signs of illness (coughing, sneezing, fever, diarrhea, rash, cold sores, etc.) should handle or have close contact (<5 m) with any NHP. It must be remembered, however, that many agents are infectious to other animals *before* the infected individual becomes clinically ill (or after recovery). Ideally, personnel working regularly with NHP should participate in some level of an employee health program, and be up to date on all available vaccinations (especially measles, polio, hepatitis A, influenza(s), meningococcal meningitis, rabies, and tetanus). This helps to ensure their health and to protect their co-workers and any animals they may handle.
7. NHP are not typical sources of rabies virus transmission to humans, but like any mammal must be considered a risk, especially in areas where they might be regularly exposed to common, high-risk rabies reservoirs (e.g., domestic dogs in many countries). If there is any suspicion of rabies exposure (e.g., handler is bitten by or exposed to nervous tissues from a primate exhibiting neurologic signs), their physician should be contacted and post-exposure rabies vaccination should be obtained as soon as possible. Rabies symptoms in primates are variable (irritability, self-mutilation, paralysis, malaise).
8. NHP are also not typical sources of anthrax exposure in humans, but are known to suffer and even die from anthrax, including in atypical forest environments. Proper PPE use and appropriate disposal of suspect carcasses are the most effective measures of preventing anthrax exposure. For additional information see <http://www.bt.cdc.gov/agent/anthrax/>.
9. Two particularly important and dangerous pathogens that workers may be exposed to by handling NHP are Ebola virus and *Cercopithecine herpes-1* (B virus). **EXPOSURE TO THESE PATHOGENS IS LIFE-THREATENING AND REQUIRES IMMEDIATE ACTION.**

² There are no distinct time rules because pathogen shedding depends on many host and pathogen-specific factors. Though infectivity can in some cases range up to many months after resolution of clinically apparent disease, in healthy adults most pathogens of concern here (e.g., respiratory viruses) are unlikely to be transmissible for more than a few days after recovering from illness.

B virus- PREDICT staff are most likely to be exposed by handling live macaques, which should **always** be assumed to be infected with B virus, with or without any clinical signs. *Macaques with oral lesions (right) should be handled with **extreme** caution and only by highly trained staff, if they are handled at all.* Macaques shed the virus in their oral, gingival, and genital mucosa and transmission can occur via bites, scratches, percutaneous inoculation with infected materials (e.g., accidental needlestick), and mucosal splash exposure. There is risk of B virus exposure from macaque CNS (central nervous system) tissues and CSF (cerebrospinal fluid), but peripheral blood from macaques has not been known to cause infection in humans. To prevent exposure to B virus, workers must always follow all PPE procedures and the precautions outlined below. In the event of accidental exposure, workers must stop **IMMEDIATELY** and trigger the **B Virus Emergency Exposure Protocol** detailed in **Appendix II. TIMING IS CRITICAL** and an immediate action can be the difference between life and death. Additional information on B virus can be found here:



<http://www.cdc.gov/herpesvirus/index.html>
<http://www.cdc.gov/mmwr/preview/mmwrhtml/00015936.htm>
<http://www2.gsu.edu/~wwwvir/index.html>

Ebola virus (and related *Filoviruses*) - PREDICT staff are most likely to be exposed by handling dead African ape carcasses, including bushmeat. Transmission can occur through contact with infected tissues, secretions, and body fluids and can be prevented through proper use of PPE and related barrier techniques (see www.cdc.gov/ncidod/dvrd/spb/mnpages/dispages/ebola.htm or <http://emedicine.medscape.com/article/216288-treatment> for more detailed information). Extreme caution must be taken by anyone sampling cases where Ebola infection is suspected. In the event of accidental exposure to Ebola virus (e.g., needlestick injury, any direct contact of eyes, skin or mucous membranes with infected fluids) workers must stop immediately and follow the details in **Appendix III. Ebola Virus Exposure Emergency Protocol**. Symptoms of Ebola and complications are treated as they appear. The following basic interventions, when used early, can significantly improve the chances of survival:

- Providing intravenous fluids (IV) and balancing electrolytes (body salts).
- Maintaining oxygen status and blood pressure.
- Treating other infections if they occur.

Recovery from Ebola depends on good supportive care and the patient's immune response (<http://www.cdc.gov/vhf/ebola/treatment/>).

Section 4. Primate Sampling

Note: *Capturing, trapping, darting, and immobilizing NHP should only be performed by experienced and skilled staff and are not entirely covered in this document (Hughes, T. 2010).*

PREDICT partners are expected to have detailed capture/immobilization protocols (and recording sheets, monitoring sheets, etc.) for any target primate species. This sampling protocol assumes a starting point of either a safely immobilized or an already dead primate. A vervet monkey capture and anesthesia guide is provided in ***PREDICT Vervet Monkey Capture and Anesthesia Guide***.

For the PREDICT project, post-capture processing will entail a number of sometimes concurrent activities. The main objectives during processing are:

1. Safeguard the health of all handlers and any live animals being processed.
2. Collect required sample data.
3. Collect required biological samples.
4. Collect supplemental data and samples.
5. Await animal recovery or dispose of carcass.
6. After recovery, release animals as close to their site of capture as possible and follow all other guidelines for release as stated in the PREDICT IACUC protocol.

In some cases, time constraints, anesthetic risk, inability to prolong immobilization, or other factors may necessitate prioritizing biological sample collection at the expense of collecting any physical measurements. At a minimum:

1. Obtain and record the animal's weight (kg) as this can be important for proper drug dosing or emergency interventions and to estimate the age category.
2. Conduct a cursory physical exam before sampling in order to note any lesions or major abnormalities.
3. If capture wounds are observed, treat as needed.

Sample Data Collection

Please refer to the required data collection templates for data to collect:

1. P2 Animal Data Collection Form
2. P2 Site Characterization Data Collection Form
3. P2 Specimen Data Collection Form

Additional (Optimum) Data to Collect from NHP

Note: *The P2 data templates mentioned above are required to be filled in. Additional data and biometric measurements may be collected at the discretion of the sampling party.*

Ideally, the following additional data should be collected from any NHP that are processed:

- body mass (kg)
- age class (see below)
- sex (and possibly reproductive status if adult female)
- whole body photograph(s)
- identifying characteristic photographs
- morphometric measurements

Body mass: Body mass may be one of the first measurements taken in order to ensure proper drug dosages, etc. Being careful to monitor breathing, and depending on size, NHP should be weighed (kg) in bags, slings, or a suitable container using a calibrated hanging spring scale or, if they are small enough, a tabletop scale with or without a tray or other container. If large NHP exceed the limit of spring scales two or more scales can be linked (one hanging from the other) to distribute the weight. The total weight is the measure of both scales added together. Scales should be zeroed (checked to make sure they measure '0.0' units when empty) and any containers (bags, slings, trays, boxes) should be weighed beforehand and then both primate and container should be weighed together. Once the primate is removed from the container for sampling, the container should be re-weighed and subtracted from previous total. Alternatively, the weighing container can be tared so that the scale reads '0.0' units with the container, and then checked to verify it still measures exactly zero after the primate is removed. If scales are not available or accurate weights cannot be measured for any reason, a weight should still be estimated but the recording sheet *MUST note that it is an estimated and not a measured weight.*

Age class: If exact age is known (e.g., for habituated NHP) that should be recorded. Otherwise, for most primate species it will be possible to classify into one of the age classes in the table:

Age Class	Description
Neonate	Animal shows signs of being born within a few days.
Infant	Animal is unweaned and usually still clinging to mother and suckling.
Juvenile	Animal is mostly independent from mother, not yet adult-sized, and sexually immature.
Immature	Any individual not evidently sexually mature.
Subadult	Animal is fully independent, appears to be sexually mature, but not fully physically mature (e.g., less than full adult size).
Adult	Animal has secondary sexual characteristics, adult size, sexually mature.
Old Adult	Adult showing signs of age degeneration

Sex determination (species identification/examination): Based on morphology and unique characteristics, identify NHP to genus, and species (where possible) and sex. Sex determination for young individuals of many primate species is not always simple and photographs of genitalia should be taken, especially if there is any doubt. For female NHPs, note parity (e.g., presence offspring, evidence of previous lactation), also determine pregnancy status by gently palpating the abdomen (at least for small NHP), and determine lactation status by gently attempting to express milk from the teats (for larger NHP, milk samples can be collected in an empty cryovial and stored frozen). If dependent offspring are captured along with their mothers, they should not be removed from their mothers unless absolutely necessary (e.g., to prevent injury or if they are nearly independent/weaned) and then only for the minimal time required for sampling.

Photographs: At a minimum, the following digital photographs should be taken:

- Anterior/ventral view of full body with arms at sides, preferably with identification card or sheet displaying unique identifying number.
- Full anterior facial view.
- Full lateral facial/head view.
- Views of full upper and lower dentition (to help determine/verify age and sex).
- Frontal/ventral view of fully exposed genitalia.
- Views of any lesions (e.g., cuts, scratches), physical abnormalities (e.g., missing toes), or individually identifying marks or characteristics (e.g., healed scars, abnormal coloration, facial spots or wrinkles, etc.)

Body measurements: Time permitting, the biometrics (in cm or mm) should be recorded with the *minimum standard mammal measurements (all linear)*:

- Head and body length (measured dorsally and linearly from tip of nose to base of tail when head is stretched and aligned with back). Note: For many NHP (e.g., apes) this measure is adjusted to what is called “crown-rump” length that starts at the top of the head in order to produce the longest linear measurement (without wrapping over the head).
- Tail length (from base to tip).
- Hind foot length (heel to tip of longest toe- exclude nail and note which toe).
- Tibia length (‘knee to ankle’).
- Ear length: base of the notch below the ear opening (lower rim of external auditory canal) to the most distant point of the margin of the pinna.

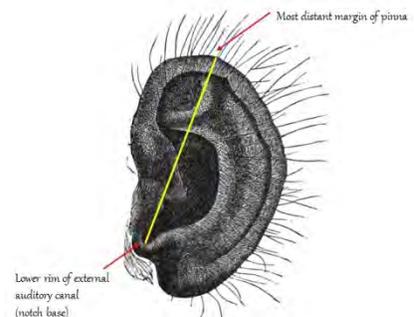
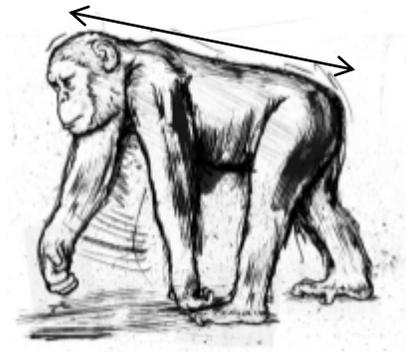


Fig. 5.—Ear of chimpanzee. Anthropoid Apes, 1885

Biological Sample Collection

In addition to the standard PREDICT sampling and analyses, PREDICT partners are encouraged to collect additional samples and pursue routine diagnostics (e.g., blood counts and chemistries, urinalysis, etc.) where resources allow. Sample collecting for archival is also strongly recommended. Opportunities to collect biological samples and related health data from wild NHP are relatively uncommon and maximizing these opportunities can further advance wildlife health.

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two oral swabs** - one in 500 μ L VTM and one in 500 μ L Trizol
2. **Two rectal swabs/ fecal samples** - one swab in 500 μ L VTM and one in 500 μ L Trizol OR 0.5cc (pea size) feces in 500 μ L VTM and 0.5cc (pea size) feces in 1 mL Trizol
3. **Two whole blood samples** - one with max of 500 μ L of whole blood in 500 μ L VTM and one with max of 500 μ L of whole blood in 500 μ L Trizol
4. **Two serum samples** - 2 x 500 μ L aliquots, frozen without media
5. **Urogenital swab/urine samples** – one swab each in 500 μ L VTM and 500 μ L Trizol OR one 500 μ L urine sample each in 500 μ L VTM and in 500 μ L Trizol

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

If there is no **short-term** access (i.e., within 24 hours) to cold chain such as in an emergency situation then samples can be collected in 500 μ L of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

- 1 day at 37°C (i.e., ambient temp)
- 1 week in the refrigerator
- Within one week freeze at -80°C for storage until analysis

Sample Collection from Live NHP

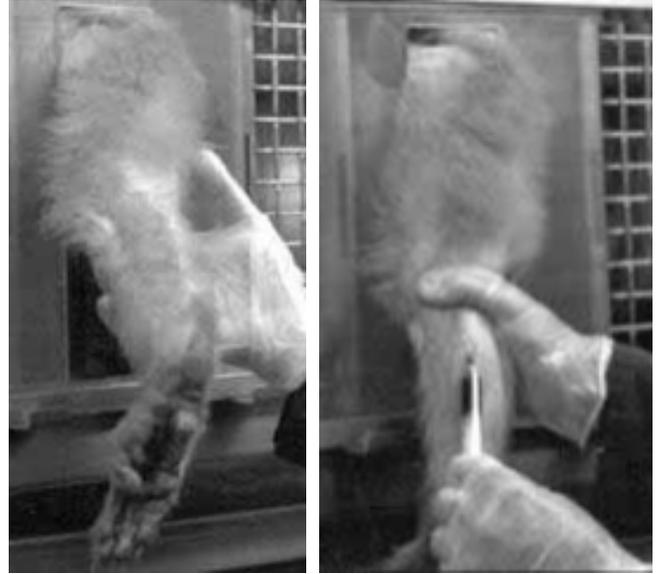
Live NHP should be chemically restrained during any invasive sample collection (e.g., blood collection). Two, preferably three, people are required for these manipulations: one person to safely restrain or position the primate, one to take samples, and a third to manage the tubes (e.g., unscrew the lids, hold them up to the sample taker, make sure the lids are replaced tightly and kept in order) and record sample data.

Blood Collection

Note: *At least one person present should have previous experience in primate venipuncture to avoid injury to the animal.* No more than 1 ml of blood per 100 g (= 10 ml/kg or 1%) of primate body weight should be collected at any one time.

Collection procedure

1. Select appropriate venipuncture site:
 - **Forearm veins**- In larger species (e.g., apes), the cephalic, radial, median, and ulnar veins might be large enough for safe blood collection.
 - **Femoral vein**- Best for small NHP and for large sample volumes. If the femoral artery (just lateral/anterior to the vein) is inadvertently pierced sampling can continue but extra effort must be made to apply post-collection pressure for at least 1 full minute to minimize hematoma formation.
 - **Jugular vein**-This may be the only option in very small NHP and must be accessed carefully.
 - **Caudal saphenous vein** (*Figure right with laboratory macaque*)-With compression of the upper thigh or knee, this vein can be prominent and superficial, but often collapses during collection.
2. Select appropriate size needle and syringe (or vacutainer) for the size of the primate.
3. Disinfect the site with iodine solution or alcohol.
4. Collect sample.
5. **Do not recap needle.**
6. Apply pressure to site of bleeding using a cotton ball or gauze pad until bleeding ceases (approximately 1 minute).
7. Process blood (see below).
8. Properly dispose of sharps and other biohazard materials immediately upon transfer of sample to collection vials and slides.



Blood processing

Place whole blood or blood clots in VTM and Trizol: If animals are large enough, collect whole blood into 1 lavender top tube containing EDTA and in 1 serum separator/serum-clotting factor tube (red top or tiger top) tube. From the EDTA tube, store 500 μ l whole blood in a cryovial with 500 μ l VTM and a second sample of 500 μ l whole blood in a cryovial with 500 μ l Trizol.

For serum, from the red top/tiger top tube, allow blood to clot and/or centrifuge. Use a plastic pipette to take 1 ml of serum and transfer into 2 cryovial tubes, 0.5 ml each. You can harvest additional serum for serum bank as appropriate. Transfer the remaining blood clots to separate cryovials. If the animal is not large enough to collect two blood tubes (for whole blood and serum), save the blood clot after serum separation. The blood clot should be placed in a cryovial with 500 μ l VTM. Freeze all samples in liquid nitrogen in dry shipper or dewar and transfer to -

80°C freezer when possible.

(Optional) **Whole blood in EDTA:** If facilities are available to perform complete blood counts (CBCs) within 5 days, remaining whole blood in lavender top tubes can be refrigerated for analysis. Blood smears can also be prepared in the field.

Oral Swabs

Swabs in VTM and Trizol: Using two sterile, polyester-tipped swabs with a plastic shaft, rub the swab tip gently but thoroughly against the back of the primate's throat, saturating the swab with saliva. Place 1 swab in a cryovial filled with 500 µl VTM and use flame-sterilized scissors to cut the shaft of the swab above the tip. If using plastic shaft swabs when scissors aren't available, insert the swab to the bottom of the vial and then lift the tip and snap the plastic shaft of the swab on the edge of the cryovial. If the plastic shaft is snapped when the swab tip is resting on the bottom, the swab will be too long and the cryovial won't close. Place the other swab into 500 µl of Trizol in a cryovial, following the same procedures. After inserting the swab and closing the vial lid, shake each tube to mix the sample well. Store both cryovials in a liquid nitrogen dry shipper or dewar & transfer to -80°C freezer when possible.

Feces

200 mg in VTM and Trizol: Collect either excreted feces or if primate is large enough (> 1 kg) use a gloved, lubricated (saline or medical lubricant) finger to collect feces directly from rectum. Place a ~200 mg (pea sized) sample of fresh feces in a cryovial with 500 µl VTM and another ~200 mg sample in a cryovial with 1 mL Trizol. Homogenize by shaking. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

If feces are not available, collect **2 rectal swabs- 1 in VTM and 1 in Trizol.** Gently insert one sterile swab tip at a time into the animal's rectum. [Note: DO NOT USE TRIZOL AS A LUBRICANT – IT IS HIGHLY IRRITATING TO TISSUE.] Place 1 swab in a cryovial filled with 500 µl of VTM using a flame-sterilized scissors to cut the shaft of the swab above the tip. Place the other swab into a cryovial with 500 µl Trizol. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80°C freezer when possible.

Urine

Most NHP will urinate as a fear reaction prior to handling, but urine can sometimes be collected free catch or by bladder expression by trained personnel. Place 500 µl of urine in a cryovial with **500 µl VTM**, and another 500 µl of urine in a cryovial with **500 µl Trizol**. Store in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

(Optional) Ectoparasites (e.g., mites, lice, nits, fleas)

Collect any obvious ectoparasites (and hairs if necessary, e.g., for louse nits- pictured right) using forceps and place in labeled, appropriate sized container of **95% ethanol** and store at *room temperature*.

(Optional) Milk

If lactating females are handled, milk may be collected into cryovial(s) and stored frozen at -80°C. For basic analysis 0.5-2.0 ml is adequate and even small NHP (less than 500 g) can be milked to full evacuation one time and provide ~1 ml of milk without risking the health of their infants. Dependent offspring are typically best left with the nursing mothers and separation of nursing young prior to sampling should never be done strictly for the purpose of collecting milk.

Non-Invasive Primate Saliva Sampling (Rope method)

Field Collection Supplies:

- Six inch nylon oral swab ropes (Salimetrics, Inc)
- Nylon swab retrieval strings (if necessary)
- 5 ml compartmental swab storage tubes (Salimetrics, Inc)
- Cryovials
- Backpack/bag for concealing collection supplies
- Attractant (jam, bananas, juice, honey, etc.)
- Viral transport media
- Pipettor and tips (or disposable pipettors)
- Cooler bags and ice packs
- Field centrifuge
- Trash bags
- Spray bottle of disinfectant
- PPE (N95 masks, goggles, gloves, dedicated clothing*)

***Dedicated clothing: long-sleeves and pants**

Tyvek-like suits are not required for non-invasive primate saliva sampling

Primate Groups for Sampling

Saliva sampling using distributed ropes is intended for use with semi-habituated primate species that will allow researchers to approach within a reasonable distance for sample collection.

Precautions should be taken to avoid baiting primates into closer contact with local people or further habituating primate groups by limiting the number of times a single group is sampled and avoiding primates associating humans with distributing food.

Collecting Non-invasive Saliva Samples

1. While wearing gloves, dip 6-inch nylon swab ropes (Salimetrics, Inc) into an appropriate attractant (juice, jam, crushed banana, etc.). For some species (i.e. baboons), disguising the ropes completely inside a banana is more effective.
2. Walk around and look for isolated individual primates that are out of sight of the rest of the primate group. Try to identify individuals that are in the lower canopy or on the ground. When deciding on an area to sample, make sure there are no more than three primates in your perspective sampling area. Distributing ropes where large numbers of primates are present can initiate aggression between individuals.

3. Observe the social structure of the primates present carefully. Throw the rope to the most dominant primate (either adult male if present, or the largest adult female present). Juveniles may get the treat after the adult has discarded it. If you want to sample a juvenile primate make sure they are out of sight of other individuals.
4. Throw the rope when no primates are watching you and continue walking so the primate does not associate you with the treat.
5. Watch the primate as they chew on the rope. Keep a reasonable distance to avoid disturbing them. Follow the primate until it discards the rope. Do not approach a dropped rope until the primate has left the area and is no longer watching you.
6. When collecting the sample, have a designated person wearing PPE (N95 mask, eye goggles, gloves, dedicated clothing) approach the sample. Compress the chewed nylon swab rope with a gloved hand into a swab collection tube (Salimetrics, Inc.). Pipette 1ml of viral transport media (VTM) over the compressed rope in the swab collection tube. Store tubes on ice packs.
7. Move to a different location within the site to collect the next sample so no primates begin to associate you with distributing food. If it is difficult to retrieve dropped ropes because they are lost in tree branches, a thin nylon string can be sewn onto distributed 6-inch ropes to aid with retrieval.
8. In the laboratory or field processing station, centrifuge ropes for 15 minutes at 3,000rpm to elute saliva/VTM into the bottom collection compartment. Pour or pipette the saliva/VTM into labeled cryovial tubes and store in freezer at -80 degrees C.

Sample Collection from Dead NHP

If animals are found dead or must be euthanized by trained personnel per acceptable guidelines due to health or welfare reasons, necropsy samples may be taken. When full necropsies are performed, following the American Association of Zoo Veterinarians (AAZV) great ape necropsy protocol is recommended and can be adapted for all primate species.

***Note:** properly following this extensive necropsy procedure and collecting and measuring all samples can require 4-6 hours per animal.*

If carcasses are not whole or are fairly decayed, see ***Bushmeat Sampling Methods***. If bodies are relatively whole and fairly fresh then blood, organ tissues, urine and (optionally) external parasites may be collected as described below.

Post-Mortem Blood Collection

From recently dead animals, it may be possible to collect whole blood (often clotted) from the right side of the heart where the largest volume of blood is available. Collect all available blood into an appropriate size container (typically one or more blood tubes). Allow the tubes to sit undisturbed for at least 30 minutes, and then centrifuge at high speed (2000 x G for 20 minutes). Use a plastic pipette to take 1 ml of serum and transfer into 2 cryovial tubes, 0.5 ml each. Transfer the remaining blood clots to separate cryovials. Freeze all samples in liquid nitrogen

immediately in the field and transfer to -80°C freezer once back in the lab. If a centrifuge is not available, allow the clots and cells to settle as much as possible and collect serum as above.

Tissue Collection

Collect three, adjacent, approximately 200 mg (pea-sized) samples of the following tissues:

- Adrenal
- Cecum
- Colon
- Duodenum
- Heart
- Kidney
- Liver
- Lung
- Lymph node
- Spleen
- Ovary
- Pancreas
- Testes
- Other, if required

One specimen should be frozen in 500 µL VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

Urogenital Swab/Urine

A urine sample should be collected if the carcass contains an intact bladder holding uncontaminated urine. Ideally, the sample should be collected with a 3 ml syringe attached to a 25 to 22 gauge needle. Insert the needle through the bladder wall and use the syringe to withdraw a maximum of 1 ml of urine. Do NOT stabilize the bladder by placing your hand beneath it, as this will put you at risk for needle injury. If the bladder is contracted (appears grossly empty of urine), use a sterile blade to make a small incision in the bladder wall. Small amounts of urine might be present and possible to suction up with a needleless 1 ml syringe inserted through the open incision. Place 500 µl of urine in a cryovial with **500 µl VTM**, and another 500 µl of urine in a cryovial with **500 µl Trizol**. Alternatively, if urine is not available, two urogenital swabs can be taken, with one placed in **500 µl VTM** and one in **500 µl Trizol**. Store samples in a dry shipper or dewar with liquid nitrogen and transfer later to -80°C freezer.

Section 5. References

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Appendix I. Field Inventory Checklist

General Field Supplies:

- Headlamps
- AAA batteries and AA batteries
- Leatherman/Pocket knife
- GPS unit to mark site coordinates (in decimal degrees)
- Binoculars
- Brightly colored flagging tape

Workstation materials:

- Drapes, sheets, blankets, tarps, towels, plastic sheeting, etc.
- Scale and sacks, harnesses, ropes for weighing
- Disinfectants and clean-up supplies
- Biohazard bags (or plain bags and biohazard stickers) and sealing tape
- Hard, coverable container for transporting biohazard bags (if necessary)
- Anesthetic or immobilization drugs, medications, vaccinations
- Anesthesia monitoring equipment (pulse oximeter, stethoscopes, thermometer, etc.)

Data Collection Supplies:

- Field Data Collection sheets (Site/Event, Animal, and Specimen sheets)
- Clipboard or other weather resistant writing surface
- Pens and permanent markers
- Digital camera and charger
- Blank index cards or paper to label animals in photos
- Printed labels
- Waterproof paper (for use in formalin specimen jars)

Personal Protective Equipment (PPE)

- N95 (or better) respirators and a small stapler* (enough for all team members plus extras)
- Safety goggles or face shields (for every person handling monkeys). Use face shields if handling suspect B virus or Ebolavirus positive NHP.**
- Long clothing/Tyvek-like suits
- Disposable nitrile gloves
- First Aid Kit (with soap and betadine for cleaning wounds)
- Thicker gloves for primate handlers (i.e., leather to be worn over the nitrile gloves) that can be disinfected and re-used (e.g., Hexarmor Hercules 400R6E)
- Emergency exposure kits for B virus or Ebola (if applicable)
- Working communications equipment (cell phone, satellite phone, etc.)
- Emergency response plan (see *Emergency Preparedness Guide*)**

* A small stapler can be used to staple the elastic straps back onto the N95 mask if they snap off

Biological data and sample collection supplies:

- Ruler, tape measure, or calipers appropriate for the size of the animal
- Serum separator blood tubes
- EDTA blood tubes
- Appropriate gauge needles or butterfly needles for smaller primates
- Syringes: 1mL, 3, 6 or 12mL
- Gauze or cotton to apply iodine to blood collection site
- Iodine for preparing blood collection site
- Sterile polyester swabs with plastic shaft for oropharyngeal, nasal, and fecal swabs

- Cryovials for storing serum, blood clots, swabs, feces, etc.
 - “Cryovials” refers to plastic, internally threaded screw-top vials with a silicon O-ring to prevent leakage. NUNC or Corning brand are recommended
- Viral Transport Media (VTM) and Trizol for storing specimens
- 95% Ethanol for storing ectoparasites
- 1.5mL microcentrifuge tubes for storing ectoparasites
- Necropsy kit for post-mortem exam in case of accidental death
 - 21 gauge needles (for cardiocentesis)
 - 22 to 25 gauge needles (for urine collection)
 - 1 and 6 mL syringes (for cardiocentesis and urine collection)
 - Scalpel and surgical blades
 - Forceps
 - Sharp and blunt tip scissors
 - 75%-80% Ethanol in small screw-capped vials for storing forensic entomological specimens
- Small jars containing 10% buffered formalin for histopathology specimens

Sample processing and storage supplies:

- Tube racks or cryovial boxes for storing tubes
- Field centrifuge to spin blood to separate serum
- Sterile plastic pipettes (for aliquoting serum)
- Surgical blades for dividing blood clot
- Cold Storage Container - Cooler and ice packs for specimens during collection
- Charged dry shipper for specimen field storage
- Cryoboxes, canes, or nylon stockings for organizing specimens in dry shipper

Waste Disposal Supplies:

- Sharps container (for needles and pipette tips)
- Trash bags or containers that can be disinfected
- Virkon or similar disinfectant that kills viruses
- Spray bottle for disinfectant
- Portable waste incinerator or other biohazard disposal plan

Appendix II. B Virus Exposure Emergency Protocol

(Adapted from Cohen et al., 2002. Recommendations for Prevention of and Therapy for Exposure to B Virus (*Cercopithecine Herpesvirus 1*). *Clinical Infectious Diseases*, 35: 1191-203.)

FIRST AID *MOST IMPORTANT STEP*****

Mucous membrane exposure: flush eye or mucous membranes with sterile saline solution or water for 15 min (or 1 liter).

Skin exposure: Wash skin thoroughly with a solution containing detergent soap (e.g., chlorhexidine or povidone iodine) for 15 min. Consider washing skin with freshly prepared 0.25% hypochlorite solution, followed by detergent solution, for 10–15 min.

INITIAL EVALUATION

Exposed worker

- Assess the adequacy of cleansing; the health care provider should repeat cleansing.
- Determine and document the date, time, location, and description of the injury, and the type of fluid or tissue contacted.
- Evaluate general health (including medications) and determine when the last tetanus booster was received.
- Determine the need for post-exposure prophylaxis with antibiotics or rabies vaccine and immunoglobulin.

NHP (partly intended for laboratory NHP)

- Identify the monkey associated with the exposure, the species of that monkey, and the responsible veterinarian.
- Assess general health (including medications and involvement in past and present research studies).
- Evaluate prior serologic history (including infection with B virus or simian immunodeficiency virus).
- Consider confining monkey for further evaluation and testing.

EXAMINATION AND LABORATORY TESTING

Exposed worker

- Physical examination, especially evaluation of the site of the exposure and neurologic examination.
- Consider obtaining serum samples at baseline for serologic analysis (pair at 2-3 weeks).
- Consider culturing specimens from the wound site or exposed mucosa after cleaning.

NHP

- Examine the animal for mucosal lesions (e.g., vesicles, ulcers), conjunctivitis, etc.
- Consider culturing specimens from the lesions, conjunctiva, and buccal mucosa.
- Consider serologic testing for B virus (if the animal is not known to be seropositive) and follow-up paired sample at 2-3 weeks.

EDUCATION AND TREATMENT

- Counsel the patient regarding the significance of the injury.
- Provide the patient with information on the signs and symptoms of B virus infection.
- Ensure that the patient has a card (to carry in his or her wallet) that includes information on B virus and a phone number to call for advice in an emergency.
- Ensure that the patient's occupational health care provider and supervisor are notified.

- Review with the patient and his or her work supervisor the safety precautions in place at the time of injury.
- Schedule a follow-up appointment.

CONSIDER POST-EXPOSURE PROPHYLAXIS

Pros and cons of post-exposure prophylaxis for persons exposed to B virus:

Pros

- Initiation of acyclovir therapy within 24 h after exposure to B virus prevents death among animals.
- Initiation of acyclovir therapy within hours of exposure may prevent or modify symptomatic B virus disease.

Cons

- Infection with B virus is very rare relative to the number of possible exposures.
- There are no controlled studies that document the ability of immediate empirical therapy to prevent infection or symptomatic B virus infection in humans.
- Acyclovir therapy can suppress virus shedding and seroconversion, which may make diagnosis more difficult.

Recommendations for post-exposure prophylaxis for persons exposed to B virus.

Prophylaxis recommended:

- Skin exposure (with loss of skin integrity) or mucosal exposure (with or without injury) to a high-risk source (e.g., a macaque that is ill, immunocompromised, or known to be shedding virus or that has lesions compatible with B virus disease).
- Inadequately cleaned skin exposure (with loss of skin integrity) or mucosal exposure (with or without injury).
- Laceration of the head, neck, or torso.
- Deep puncture bite.
- Needlestick associated with tissue or fluid from the nervous system, lesions suspicious for B virus, eyelids, or mucosa.
- Puncture or laceration after exposure to objects (a) contaminated either with fluid from monkey oral or genital lesions or with nervous system tissues, or (b) known to contain B virus.
- A post-cleansing culture is positive for B virus.

Prophylaxis considered:

- Mucosal splash that has been adequately cleaned.
- Laceration (with loss of skin integrity) that has been adequately cleaned.
- Needlestick involving blood from an ill or immunocompromised macaque.
- Puncture or laceration occurring after exposure to (a) objects contaminated with body fluid (other than that from a lesion), or (b) potentially infected cell culture.

Prophylaxis not recommended:

- Skin exposure in which the skin remains intact.
- Exposure associated with non-macaque species of NHP.

Appendix III. Ebola Virus Exposure Emergency Protocol

(adapted from <http://www.cdc.gov/ncidod/dvrd/spb/mnpages/vhfmanual/section5.htm>)

Accidental needlestick injury: Assume any needlestick injury is a suspected contact for viral hemorrhagic fever (VHF) whether or not a break in the skin can be seen. If an accidental needlestick injury occurs, contact the health care provider and treat the exposure site.

1. Immerse the exposed site in 70% alcohol for 20 to 30 seconds, and wash with soap and clean water.
2. Flush the site in running water for 20 to 30 seconds.
3. If needed, cover with a dressing.
4. Report the incident to a supervisor or the physician-in-charge.

The purpose of notifying the physician-in-charge is:

- To identify what caused the problem.
- To take corrective action to solve the problem and prevent accidental transmission.
- To provide appropriate care for the possible case of VHF.

Remind the exposed worker that accidents do happen even when every precaution to prevent them has been taken. Reassure worker that reporting the accidental exposure will have no negative consequences. Explain that reporting the accidental exposure is essential for protecting themselves, their families, other health workers and patients.

Accidental contact with infectious body fluids: An accidental contact can occur if there is unprotected contact between infectious body fluids and broken skin or the mouth, nose or eye. For example, vomit may run under a glove, a primate might cough blood which runs into the health care worker's eye, or splashed blood may run underneath a health care worker's mask and get into the mouth. Treat any accidental contact as a suspected contact with VHF. As soon as the contact occurs:

1. Flush the area in the most appropriate manner with soap and clean water. If a splash occurs in the eye, flush it with clean water.
2. Leave the isolation area and remove the protective clothing as recommended.
3. Take a shower and put on street clothes.
4. Report the exposure to a supervisor or the physician-in-charge. Complete the necessary forms.

Follow up accidental exposures:

1. Monitor the condition of the exposed worker. Take a measured temperature two times per day.

If a fever occurs -- temperature is 38.5°C (101°F) or higher -- the worker should not do any work activities and should seek immediate medical attention. Treat as a suspected case of VHF if the worker's signs and symptoms meet the case definition.

Appendix IV. PREDICT Vervet Monkey Capture and Anesthesia Guide

Monkey Capture

Information in this guide is based on field training with Mountain Gorilla Veterinary Project/Rwanda PREDICT staff and the PREDICT Primate Capture Training Protocol by Chris Whittier.

1. Assess monkey sleeping, foraging, and movement patterns in a given location. Working with local scouts to monitor monkey troop movements can make trapping efforts much more efficient.
2. After assessing movement patterns, identify a trapping location. During initial capture efforts, morning and late afternoon (as monkeys were leaving and returning to sleeping areas) were effective trapping times.
3. Prepare the Tomahawk non-collapsible metal traps by securely baiting them with a fresh banana before placing them near the target monkey troop. A long piece of duct tape folded in half lengthwise makes an effective strip for tying the banana to the bottom of the cage. The banana should be positioned just behind the treadle, so that it doesn't interfere with the ability of the treadle to trigger the trap to close (Figure 1). Tying the banana to the cage with at least 2 loops of duct tape ensures that the monkey can't steal the banana without triggering the trap (Figure 2).



Figure 1: Tying a banana to the trap. Photo credit: PREDICT Tanzania Team



Figure 2: Vervet Monkey triggering the trap while trying to remove a banana. Photo credit: PREDICT Tanzania Team

4. Weigh the baited Tomahawk traps (in kg) using the appropriate spring scale. Set Tomahawk non-collapsible metal traps near the monkey troop. Place the traps on level ground, in a shaded location when possible. Capture staff should monitor the set traps from a distance to avoid disturbing the monkeys.
5. Once a monkey has been captured, weigh the trap with the monkey (in kg), and subtract the trap weight from the total weight to calculate the weight of the monkey. This weight is necessary for calculating doses of anesthetic drugs. Male vervet monkeys typically weigh 3-6.5kg. Females are usually 1.5-5 kg.
6. Following capture and weighing, traps containing monkeys should be removed to a sampling site and placed in a quiet, shaded location away from humans or other disturbances. Continue monitoring open traps to capture additional individuals. Monkeys in troops that have been captured previously may be extremely hesitant to enter traps.

Monkey Processing

Note: *Members of the country/field team should determine the specimens to be collected based on the interface sampled, the species of primates captured, feasibility, safety, and diagnostic requirements.*

1. Fill in site/event information on the data sheet and prepare animal and specimen data sheets for recording individual animal data. Use the GPS unit to collect site latitude/longitude in decimal degrees.
2. Set up a monkey processing station. Place leaves or other vegetation on the ground to insulate the anesthetized monkey from a cold or hot surface. Place a disposable plastic sheet or apron over the vegetation to create a sampling area that can be easily disinfected (Figure 3). Organize supplies in advance for easy access during sampling.



Figure 3: An anesthetized vervet monkey at the processing station with a PREDICT field team member identifying the femoral vein. *Photo credit: PREDICT Tanzania Team*

- All individuals handling monkeys and samples should wear appropriate PPE: N95 or better respirators, nitrile gloves, long clothing/tyvek suits, and safety glasses or face shields. Duct tape can be wrapped around the overlapping tyvek suit and gloves at the wrist to avoid skin exposure.
- Using the weight of the captured monkey, calculate the appropriate anesthetic drug volumes. Vervet monkeys can be safely anesthetized using a combination of ketamine and medetomidine (Dormitor®) with the following doses:

→ 3.5 mg/kg of ketamine

→ 0.035 mg/kg of medetomidine‡

‡Dexmedetomidine may also be used at a dose of 0.0175 mg/kg

Volume of drug needed (mL) = animal weight (kg) * safe and effective dose (mg/kg) ÷ drug concentration (mg/mL).

Ketamine is commonly available in a concentration of 100mg/mL or 200mg/mL, and medetomidine is commonly 1mg/mL. Using these concentrations (100mg/mL for ketamine), a 5 kg monkey would require:

$$5 \text{ kg} * 3.5 \text{ mg/kg Ketamine} \div 100\text{mg/mL} = 0.175 \text{ mL}$$

$$5 \text{ kg} * 0.035 \text{ mg/kg Medetomidine} \div 1 \text{ mg/mL} = 0.175 \text{ mL}$$

The total volume of anesthetics (0.175 + 0.175 = 0.35 mL) can be drawn up in a single syringe for hand-injection of the monkey. A 22 or 25 gauge needle can be used to deliver the drugs.

- Prepare the Atipamezole reversal (dose = 0.175 mg/kg) in a syringe and set aside in a cool place until sampling is complete. Note: when using 5mg/mL concentration of Atipamezole, the reversal volume is the same as the Medetomidine volume with 1mg/mL concentration of Medetomidine.
- Once the drugs have been drawn up and combined in a single syringe, the monkey can be hand-injected through the bars of the trap. To reduce stress and to ensure that the full anesthetic dose is administered, the monkey must be confined to a small area of the trap where its movements are restricted. As a pilot method, the PREDICT Tanzania and Rwanda teams used 2 tools in combination to restrain the monkey in the cage. First, the comb (Figure 4, left) is inserted under the entry door of the trap to block the monkey from leaving. The entry door is then opened, and the plunger (Figure 4, right) is inserted. The comb is removed and then the plunger is pushed **gently** downward to confine the monkey to the bottom portion of the trap.



Figure 4: PREDICT team inserting the comb just under the trap door so that the door can be opened without the monkey escaping (left). Anesthetic drugs are hand-injected through the bars at the bottom of the trap where the monkey is confined by the plunger tool (right). Photos courtesy of Mike Cranfield, Mountain Gorilla Veterinary Project.

7. Inject the anesthetic drugs intra-muscularly (target muscles of the thigh, shoulder or upper arm) and observe the monkey for initial signs of anesthesia: eyelids drooping, decreased movement, leaning against walls of trap, lying on floor of trap, unresponsive to stimuli. Signs of anesthesia should be visible within 5-10 minutes post-injection.
8. Once the monkey is anesthetized, carefully remove it from the trap. Open the sliding rear door of the trap a few inches to avoid escape by a partially anesthetized monkey. Quickly pull the monkey's arms behind its back (Figure 5). This safe strategy for holding small primates prevents a partially anesthetized or even fully aware monkey from biting the handler.



Figure 5: Dr. Mike Cranfield, from the Mountain Gorilla Veterinary Project, demonstrates the proper technique to restrain a vervet monkey. *Photo credit: PREDICT Tanzania Team*

9. Place the anesthetized monkey on its back on the plastic sheet of the sampling station (see Figure 3). Ensure that the monkey has a clear airway, with unimpeded chest movements and unrestricted airflow in and out of the lungs. Assess and continually reassess the monkey's plane of anesthesia. During anesthesia, team members should monitor the monkey's breathing, heart rate, mucous membrane color, and capillary refill time. Rectal temperature and blood oxygen saturation (using a Pulse-oximeter) can also be assessed. Grip reflexes are among the first to return when a monkey is coming out of anesthesia.
10. Collect a **blood sample**. The femoral vein is the most reliable blood collection site in small primates. The femoral vein is found lateral and parallel to the femoral artery, which can be easily palpated in the inguinal region. Prepare the blood collection site by swabbing the area with iodine soaked gauze. Use a 25 gauge or smaller butterfly needle inserted at a roughly 45 degree angle to the skin to locate the femoral vein (Figure 6). Once blood is visible in the tubing of the butterfly needle, attach a syringe and apply gentle suction. Avoid pulling the syringe too quickly, as too much vacuum pressure can collapse the vein.



Figure 6: Blood collection from the femoral vein of a vervet monkey. *Photo credit: PREDICT Tanzania Team*

11. Once the appropriate amount of blood has been collected, remove the needle and use gauze to apply pressure to the collection site for 1 minute to avoid hematoma formation. Store the collected blood in a serum separator tube or EDTA collection tube if whole blood is needed. Place the blood sample tubes in the cooler immediately after collection. **Do not recap the needle! Dispose of needle in the sharps container and the syringe in a biohazard or designated bag.**
12. Collect additional biological samples as described in the PREDICT biological sample collection protocol.
13. Before recovery, perform a physical exam of the captured monkey, noting any evidence of disease or injury. Capture related injuries can occur, and severity should be assessed prior to release. Small wounds and lacerations can be cleaned with iodine or betadine. More serious wounds may require veterinary intervention.
14. Mark the monkey with a temporary marker in a visible location such as the upper thigh (Figure 6) to avoid resampling the same animal when a troop is targeted for multiple days of capture efforts. The mark should wear off within one week.
15. Once sampling and physical examination are complete and the monkey has been anesthetized for a MINIMUM of 45 minutes, administer the Atipamezole reversal by intra-muscular injection. Earlier administration (<45 minutes post-induction) may result in ketamine-induced seizures. Place the monkey in a large dog kennel (or locally constructed container of similar size) in a quiet, shaded location away from humans to allow it to recover from anesthesia. It is extremely important to inspect and choose a safe release area away from hazards such as roads, people, or cliffs that could endanger the recently anesthetized monkey. Monitor the monkey regularly, and when it is fully aware and moving in a coordinated manner, open the door to allow it to return to its troop.



Figure 7: A vervet monkey with a temporary mark recovers from anesthesia. *Photo by PREDICT Tanzania team*

Appendix V. Occupational Primate Disease Safety Guidelines for Zoological Institutions

(Appendix 3 – Standard Necropsy Report for Non-Human Primates Work Sheet; From <http://www.aazv.org>)

Appendix 3: Standard Necropsy Report for Non-Human Primates Work Sheet

Pathology #: _____ Species: _____ Date: _____

Animal #/Name: _____ Sex: _____ Age (DOB): _____

Date of death/euthanasia: _____ Time: _____ (am/pm)

Method of euthanasia: _____

Time and date of necropsy: _____ Duration of necropsy: _____

Post mortem state: _____ Nutritional state: _____

Pathologist or prosector and institution: _____

Gross diagnosis:

Abstract of clinical history:

Please check tissues submitted for histopathology.

External Examination (note evidence of trauma, exudates, diarrhea etc):

- _____ Hair coat:
- _____ Skin:
- _____ Scent glands:
- _____ Mammary glands and nipples:
- _____ Umbilicus (see neonatal/fetal protocol):
- _____ Subcutis (note: fat, edema, hemorrhage, parasites):
- _____ Mucous membranes (note: color, exudates):

Ocular or nasal exudate?:

- _____ Eyes and ears:
- _____ External genitalia:
- _____ Oral cavity, cheek pouches and pharynx: Dentition (see attached dental form):
- _____ Tongue:

Musculoskeletal System (Note fractures, dislocations, malformations?):

- _____ Bone growth plate (rib, distal femur, sternabra)
- _____ Muscles:
- _____ Bone marrow (femur):
- _____ Joints (note any exudates or arthritis):
- _____ Spinal column (examine ventral aspect when viscera removed)

Examination of the neck region:

- _____ Larynx:
- _____ Laryngeal air sac (see protocol for great apes):
- _____ Mandibular and parotid salivary glands:
- _____ Thyroids and parathyroids:
- _____ Cervical/cranial lymph nodes:
- _____ Esophagus:

Thoracic Cavity (Note any effusions, adhesions, or hemorrhage):

Note amount, color and any lesions in mediastinal and coronary fat:

- _____ Thymus:
- _____ Heart (see attached protocol):
- _____ Great vessels:
- _____ Trachea and bronchi:
- _____ Lungs:
- _____ Esophagus:
- _____ Lymph nodes:

Abdominal Cavity (Note any effusions, adhesions, or hemorrhage?):

Note amount, color or lesions in omental, mesenteric and perirenal fat:

- _____ Liver and gall bladder:
- _____ Stomach:
- _____ Pancreas:
- _____ Duodenum:
- _____ Jejunum:
- _____ Ileum:
- _____ Cecum and (in apes) appendix:
- _____ Colon and rectum:
- _____ Lymph nodes:
- _____ Kidneys and ureters:
- _____ Adrenals:
- _____ Gonads:
- _____ Uterus:
- _____ Bladder and urethra:
- _____ Male accessory sex glands (prostate and seminal vesicles):
- _____ Umbilical vessels, round ligaments of bladder in neonates:
- _____ Abdominal aorta and caudal vena cava:

Nervous System:

- _____ Meninges:
- _____ Brain:
- _____ Pituitary:
- _____ Trigeminal (gasserian) ganglia:
- _____ Spinal cord (please note to which lumbar segment the cord extends):
- _____ Brachial plexus and sciatic nerves:

Is there an identifiable pineal gland?

WEIGHTS AND MEASUREMENTS (in grams, kilograms, and cm, please)

Body weight : _____

Lymphoid tissue:

R. axillary LN: _____ L. axillary LN: _____

R. inguinal LN: _____ L. inguinal LN: _____

Jejunal LN: _____

Spleen: _____ Thymus: _____

Abdominal Organs:

Liver: _____
R. Kidney: _____ L. Kidney: _____
R. Adrenal: _____ L. Adrenal: _____
R. Ovary: _____ L. Ovary: _____
Uterus: _____
Placenta (weight and measure disc(s)): _____

Thoracic Organs:

Heart wt: _____ Thymus (above): _____
Height: _____ Circumference at coronary groove: _____
Left Vent. Thickness: _____ Rt. Vent. Thickness: _____
Septum: _____
Lt. AV valve circ. _____ Rt. AV valve circ. _____
Aortic valve circ. _____ Pulmonary v. circ. _____
Rt. Lung: _____ L. Lung: _____

Other:

Brain: _____ Tumors? _____
R. Testes (wt.): _____ L. Testes: _____
Length x dia: _____
Penis (length x diameter): _____

STANDARDIZED BODY MEASUREMENTS FOR NONHUMAN PRIMATES INCLUDING APES:

Crown rump length (linear) _____
Crown rump length (curvilinear) _____
Cranial circumference (above brow ridge) _____
Length of head (tip of jaw to top of crest) _____
Width of brow ridge _____
Chest circumference (at nipples) _____
Abdominal circumference (at umbilicus) _____
Left arm: Shoulder-elbow: _____ elbow-wrist: _____
wrist-tip of middle finger: _____ pollex: _____
Right arm: Shoulder elbow: _____ elbow- wrist: _____
wrist-tip of middle finger: _____ pollex: _____
Left leg: hip-knee: _____ knee-ankle: _____
ankle-tip of big toe: _____ heel-tip of big toe: _____
hallux: _____
Right leg: hip-knee: _____ knee-ankle: _____
ankle-tip of big toe: _____ heel-tip of big toe: _____
hallux: _____

ANCILLARY DIAGNOSTICS (CHECK IF PERFORMED, GIVE RESULTS IF AVAILABLE, NOTE LOCATION IF STORED, OR TO WHOM SENT):

Cultures:

bacterial: fungal:

viral:

Heart blood:

serum:

filter paper blot:

Parasitology:

feces:

direct smears:

parasites:

Tissues fixed in 10% formalin (list tissues or specific lesions other than those checked above):

Tissue fixed for EM: _____ Tissue frozen: _____

Impression smears: _____

Comments: _____

NONHUMAN PRIMATE POST MORTEM EXAMINATION

Collection of tissues

Tissues to be fixed in 10% neutral buffered formalin should be less than 0.5 cm thick to ensure penetration of formalin for fixation.

Initial fixation should be in a volume of fixative 10 times the volume of the tissues. Agitation of the tissues during the first 24 hrs is helpful to prevent pieces from sticking together and inhibiting fixation. Once fixed tissues may be transferred to a smaller volume for shipment.

Labeling of specimens

If pieces are small or not readily recognizable (e.x. individual lymph nodes) they can be fixed in cassettes or embedding bags or wrapped in tissue paper labeled with pencil or indelible ink. Another alternative is to submit lymph nodes with attached identifiable tissue, e.x. axillary with brachial plexus, inguinal with skin, bronchial with bronchus, etc.

Sections from hollow viscera or skin can be stretched flat on paper (serosal side down) and allowed to adhere momentarily before being placed in formalin with the piece of paper. The paper can be labeled with the location from which the tissue came.

The formalin container should be labeled with the animals name or number, the age and sex, the date and location, and the name of the prosector.

Tissues to be preserved

From the skin submit at least one piece without lesions, a nipple and mammary gland tissue, scent gland, any lesions and subcutaneous or ectoparasites.

Axillary and or inguinal lymph nodes may be submitted whole from small animals and should be sectioned transversely through the hilus in large primates.

Mandibular, and/or parotid salivary glands should be sectioned to include lymph node with the former and ear canal with the latter. Thyroids, if it is a small primate, may be left attached to the larynx and submitted with the base of tongue, pharynx, esophagus as a block. In larger primates, take sections transversely through the thyroids trying to incorporate the parathyroids in the section.

Trachea and esophagus and laryngeal air sac sections may be submitted as a block.

Cervical lymph nodes may be submitted whole if small or sectioned transversely.

A single sternebra should be preserved as a source of bone marrow. A marrow touch imprint may be made from the cut sternebra and air dried for marrow cytology.

Section of thymus or anterior pericardium should be taken perpendicular to the front of the heart.

Heart: weigh and measure heart after opening but before sectioning. Please fix longitudinal sections of left and right ventricles with attached valves and atria in large animals and the whole heart opened and cleaned of blood clots in smaller animals. In tiny animals the heart may be fixed whole after cutting the tip off the apex.

Lungs: if possible inflate at least one lobe by instilling clean buffered formalin into the bronchus under slight pressure. Fix at least one lobe from each side and preferably samples from all lobes. In little animals the entire "pluck" may be fixed after perfusion and sampling for etiologic agents.

Gastrointestinal Track: Take sections of all levels of the GI track including: gastric cardia, fundus and pylorus (or presaccus, saccus, tubular stomach and pylorus in colobines); duodenum at the level of the bile duct with pancreas attached; anterior, middle and distal jejunum; ileum; ileocecolic junction with attached nodes; cecum and (in apes) appendix; ascending, transverse and descending colon. Open loops of bowel to allow exposure of the mucosa and allow serosa to adhere momentarily to a piece of paper before placing both bowel section and paper in formalin; or gently inject formalin into closed loops.

Liver: Take sections from at least two lobes, one of which should include bile ducts and gall bladder.

Spleen: Make sure sections of spleen are very thin if the spleen is congested; formalin does not penetrate as far in very bloody tissues.

Mesenteric (jejunal) nodes: section transversely; colonic nodes may be left with colon sections.

Kidneys: Take sections from each kidney: Cut the left one longitudinally and the right one transversely so they will be identifiable (or label). Please make sure the sections extend from the capsule to the renal pelvis. Adrenals: small adrenals may be fixed whole but larger ones should be sectioned (left - longitudinal and right transversely) making sure to use a very sharp knife or new scalpel blade so as not to squash these very soft glands.

Bladder: sections should include fundus and trigone. Please make sure to include round ligaments (umbilical arteries) in neonates.

Male gonads and accessory sex glands: Section the prostate with the urethra and seminal vesicles transversely. Section testes transversely. If testes are being collected perimortem for sperm retrieval, try to arrange to take small sections before the gonads are manipulated.

Female reproductive organs: Fix the vulva, vagina, cervix, uterus and ovaries from small and medium sized primates as a block (after making a longitudinal slit to allow penetration of formalin). Rectum and bladder (opened) can also be included in this block. In somewhat larger animals make a longitudinal section through the entire track.

In great apes make transverse sections of each part of the track and the ovaries. (See reproductive track protocols from the contraception advisory group if animals are to be included in their database.)

CARDIAC EXAMINATION FOR GREAT APES (AND OTHER PRIMATES IN WHICH CARDIAC DISEASE IS PRESENT)

Examine heart in situ. Check for position, pericardial effusions or adhesions. Collect for culture or fluid analysis if present.

Remove heart and entire thoracic aorta with "pluck".

Examine heart again. Check the ligamentum (ductus) arteriosus for patency. Check position of great vessels. Open pulmonary arteries to check for thrombi.

Remove heart and thoracic aorta from the rest of the "pluck". Examine for presence of coronary fat. Examine external surfaces especially coronary vessels. Note relative filling of atria and state of contracture (diastole or systole at death) and general morphology. (The apex should be fairly pointed.)

Measure length from apex to top of atria. Measure circumference at base of atria (around coronary groove).

Open the heart:

Begin at the tip of the right auricle and open the atrium parallel to the coronary groove continuing into the vena cava. Remove blood clot and examine the AV valves. Cut into the right ventricle following the caudal aspect of the septum and continuing around the apex to the anterior side and out the pulmonary artery. Remove postmortem clots and examine inner surface. Open left atrium beginning at the auricle and continuing out the pulmonary vein. Remove any clots and examine valves. Open the left ventricle starting on the caudal aspect and following the septum as for the right ventricle. When you reach the anterior aspect, clear the lumen of blood and identify the aortic outflow. Continue the incision around the front of the heart and into the aorta, taking care to cut between the pulmonary artery and the auricle. Open the entire length of the thoracic aorta. Remove all postmortem clots. You may gently wash the heart in cool water or dilute formalin to better visualize the internal structures and valves. Sever the thoracic aorta from the heart just behind the brachiocephalic arteries. Examine intima and adventitia and section aorta for formalin. Sever the pulmonary vessel and vena cava close to the heart. Weigh and measure the heart and record.

Measure thickness of right and left ventricular free walls and the septum. (On the left side, do not measure directly through a papillary muscle.)

Measure the circumference of the right and left AV valves and the aortic and pulmonary valves using a pliable measuring tape (or use a piece of string and measure the string on a straight ruler).

Take sections for histopathology:

Sections should include:

longitudinal sections of left and right ventricles AV valves and atria.

Sections of myocardium from left and right ventricles including coronary vessels. Sections of papillary muscles. Sections from the septum at the base of the AV valves (area of conduction system).

In small animal like callitichids, you may fix the heart whole.

Fix the entire heart, if possible by immersion in 10% buffered formalin for more detailed examination by a cardiac pathologist.

Other vessels:

Make sure to examine the abdominal aorta, iliac arteries and popliteal arteries (frequent sites of aneurysms in humans).

Note the location and severity of fibrous plaques, fatty streaks and atherosclerotic plaques and presence of mineralization or thrombosis.

POSTMORTEM EXAMINATION OF NONHUMAN PRIMATE FETUSES AND NEONATES

External examination of the fetus:

Weigh the fetus and make body measurements.

Measure the placental disc(s) and weigh the placenta. Note umbilical length and vascular patterns on the placenta.

Note presence of hair, freshness of the carcass (if dam is dead, is the decomposition of the fetus consistent with that of the dam) and any evidence of meconium staining.

Internal examination of the fetus:

Follow the general nonhuman primate necropsy protocol.

Make sure to note whether ductus arteriosus and foramen ovale are patent. Note also whether the lungs are aerated and to what extent.

Note dentition / erupted teeth.

Identify umbilical vein and arteries and check for inflammation. Make sure to save umbilicus and round ligaments of the bladder (umbilical arteries) for histology.

Make sure to save a growth plate (e.g. costochondral junction or distal femur) in formalin. Cultures: Take as many of the following as possible: Stomach content or swab of the mucosa; lung; spleen or liver; placental disc and extra-placental membranes. Do both aerobic and anaerobic cultures if possible.

POST MORTEM EXAMINATION OF THE AIR SACS OF ORANGUTANS AND OTHER NONHUMAN PRIMATES

Examine the skin over the air sac for signs of fistulae or scars. Note thickness of the skin and presence of fat or muscle overlying the air sac.

Incise the air sac through the skin on the anterior aspect. Note color and texture of air sac lining. Note presence or absence of exudate.

Note presence or absence of compartmentalization by connective tissue and presence of diverticulae.

Note extent of air sacs (e.g., under clavicle, into axilla, etc.)

Identify and describe the opening(s) from the larynx into the air sac (e.g. single slit-like opening, paired oval openings etc.). Note any exudate.

Note the location, size and shape of the opening in the larynx (e.g. from lateral saccules or centrally at the base of the epiglottis). Note length of any connecting channel between larynx and air sac and direction a probe must take to go from inside the larynx to the air sac.

Cultures:

Please culture several different sites within the air sacs (we need data to determine normal flora and if infections are "homogeneous" or compartmentalized).

Pangolin Sampling Methods

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Objectives: To safely collection biological samples from live and dead pangolins.

Section 1. Confirmation of Knowledge

When you are familiar with the information in this Guide, take the PREDICT quiz.

Section 2. Brief Overview of PPE and Safety

MINIMUM PPE REQUIRED FOR HANDLING PANGOLINS

The minimum PPE for sampling pangolins includes:

- Designated clothing (consider long-sleeves if handling awake animals)
- Nitrile gloves*
- N95 facemask
- Protective eye wear
- Closed-toed shoes

*Pangolin scales are sharp and frequently cut through gloves; double-gloving is recommended.

(See the Biosafety and PPE Guide (Section 4) for detailed instruction on PPE use).

Note: *Although pangolins are not known to be carriers of rabies, as mammals they remain susceptible to the virus. As such, all personnel handling pangolins should be vaccinated against rabies beforehand as described below.*

OTHER SAFETY CONSIDERATIONS

Zoonotic Diseases

There are few publications identifying the potential risks pangolins pose as sources of disease for humans. Due to this lack of knowledge, a wide array of diseases affecting mammals should be considered when handling pangolins to reduce infection risk. It is recommended that immunocompromised personnel not work directly with live or dead animals.

Rabies is a virus that is endemic in many mammal populations, especially carnivores and bats. While pangolins are not known as a reservoir, rabies can affect any mammal and it is important to take appropriate precautions when handling the species. Clinical signs of rabies are variable and typically include non-specific neurologic abnormalities such as depressed mental activity, a general sign displayed by nearly all sick or injured animals. Most zoonotic exposure to rabies is via bite wounds from live animals, which is highly unlikely with pangolins given their lack of teeth and inability to bite. However, exposure to infected bodily fluids (particularly saliva, blood and cerebrospinal fluid) must also be considered when collecting samples from pangolins (even if deceased).

Coronaviridae is a viral family that includes Severe Acute Respiratory Syndrome coronavirus (SARS CoV) and Middle East Respiratory Syndrome coronavirus (MERS CoV). Clinical signs vary

with the type of coronavirus but typically cause moderate to severe respiratory disease in humans. Coronaviruses have also been shown to cause enteritis and peritonitis in non-human animal species. SARS CoV was detected in at least six pangolin specimens sampled during the SARS CoV outbreak in 2003. While extensive disease surveillance of pangolins has yet to be performed, consideration for zoonotic coronaviruses should be considered when handling the species. Transmission methods for coronavirus are not always clear, but both direct and indirect contact have been indicated. Given their relatively unknown status as disease reservoirs, there is a potential for pangolins to carry other zoonotic viruses that may cause serious human illness. As such, gloves and respirator masks are recommended for minimizing risk.

Enteric bacteria and parasites (both external and internal) have not been extensively researched in the eight pangolin species. While current literature has reported the presence of internal and external parasites in multiple pangolin species, the parasites have not yet been evaluated as potential zoonotic threats. A novel *Anaplasma* species was identified in Malaysian pangolins in 2016, but zoonotic potential was not determined. The natural bacterial flora of pangolins not been evaluated. Due to the unknown zoonotic disease risks of parasites and bacterial flora in pangolins, general precautions are recommended (e.g. gloves and N95 facemasks).

Wearing gloves and practicing proper hand hygiene will reduce the transmission risk of most pathogens. Personnel working with animals should always wash their hands thoroughly before eating, drinking, using tobacco products, or performing any other activity that involves touching the face or mucus membranes.

Staff vaccinations and medical concerns

All personnel working with mammals, including pangolins, must be vaccinated against rabies, ensure they have a recently confirmed protective titer, and be aware of appropriate exposure prophylaxis in case of salivary transmission into an open wound.

Bites & Scratches

While pangolins neither have teeth nor the ability to open their mouths wide enough to cause any significant damage to a handler, they do have sharp claws and scales that have the ability to inflict mild to moderate cuts and scratches leaving a handler with open wounds and potentially exposed to zoonotic diseases. Furthermore, the pangolin defense mechanism to curl is very strong and has the potential to injure handlers. Even debilitated pangolins can curl tightly around handlers' limbs, and are very difficult to remove. This generally results in considerable scratching and bruising, and it can be incredibly painful.

Section 3. Special Considerations for Capture and Restraint of Pangolins

This section supplements [Section 5.2.5: Safe Animal Capture and Sampling](#), with which anyone handling animals is expected to already be thoroughly familiar. Note also that sampling from deceased animals destined for food is covered in [Section 5.2.12: Bushmeat Sampling Methods](#).

STRESS

Pangolins are especially susceptible to stress and have been known to develop gastrointestinal ulceration and other debilitating health effects due to stress from capture and handling. As such, extreme care should be taken to decrease the stress experienced by animals prior to and during immobilization. This includes keeping your distance from the captured animal, speaking in a soft voice and minimizing noise, and covering box traps with a towel or tarp to decrease visual stimuli. Similarly, anesthetized animals should have their eyes covered with a towel or cloth. It is especially critical that the animal is free (to the extent possible) of visual and auditory stimuli immediately following initial drug administration, as the quality of the induction often impacts the whole anesthetic event. During recovery, the caged animal should be kept in view but at a great enough distance that the animal does not feel threatened by your presence. As always, noise should be kept to a minimum. If possible, keep the pangolin in a dark environment during recovery to reduce stress.

HANDLING

Prior to handling, all animals should be visually inspected for any injuries or illness that might place them at risk during sampling. Pangolins typically roll into a defensive ball when handled, and if they feel threatened may emit a noxious smelling acid (similar to a skunk) from glands close to their anus. In healthy pangolins, this defensive posture is very difficult to undo without injuring the animal and specimens selected as the result of capture by local hunters often suffer from significant traumatic injury as a result of forced manual unrolling. Although some samples may feasibly be collected in a tightly balled, awake specimen, healthy adult or sub-adult pangolins will be anesthetized for sampling to avoid traumatic injury while unrolling. Rarely, pangolins may be manually restrained by grasping the tail and holding in the midair (if calm) or by supporting the underside of the body with the other hand. Prior to anesthetization, a brief physical exam should be performed to identify injuries/abnormalities and a weight should be recorded for drug calculations. Pangolins identified by veterinarians or trained personnel as having a high risk for anesthetic complication (i.e. neonates, sick, or injured) should be sampled under manual restraint without anesthesia.

CAPTURE, RESTRAINT & ANESTHESIA

To minimize stress, all pangolins should be anesthetized prior to sampling unless there is a high risk of anesthetic complication as determined by a veterinarian or trained researcher. Pangolins are easily captured and handled manually as described in the handling section. If present and

available, box traps may also be set in anticipation of eventual capture. All traps, regardless of padding or safety features, pose some degree of hazard to the animal, especially when there are active attempts to escape. Pangolins are capable of self-injury in any type of trap. For example: they might tear off their claws, damage their scales, or damage the ventral skin not protected by scales – this is particularly apparent with wire traps, and their use is not recommended. The safest box traps to use are those with sturdy, smooth wooden sides and hinged lids. Traps should be prepared carefully, be in good working order, and be checked frequently (at least every 12 hours). Veterinary staff should be prepared to deal with a variety of trap injuries, including lacerations.

Anesthetic restraint is accomplished using isoflurane anesthesia administered at 2-5% via chamber/ box induction, and maintained at 0.5-1.5% thereafter via face mask delivery. The animal should be maintained on the gas anesthetic for the duration of the procedure unless respirations are reduced or unapparent.

If gas anesthesia is unavailable, injectable anesthetics may be used with a combination of ketamine (9 mg/kg) and xylazine (1 mg/kg) administered intramuscularly (IM) for short sampling procedures. An IM injection (at a 45-degree angle) may be accomplished in the thigh with the needle entering the skin between scales.



Box chamber for induction (right), wooden box for post anesthesia recovery; Photo Credit: WCS/ Viet Nam



Anesthetized specimen, maintained on isoflurane via facemask; Photo Credit: WCS/ Viet Nam

Pangolin skin is thick and resistance to plunging the syringe may indicate that the needle is intradermal and needs to be advanced a few millimeters to reach the muscle. Ketamine is a non-reversible drug and recovery is contingent upon the animal metabolizing the drug. Xylazine will be reversed with the reversal agent Atipamezole hydrochloride at a dose of 1-2 mg/kg. NOTE: ketamine has been documented to cause excessive salivation in pangolins, which may cause respiratory complications during and after anesthesia. Pangolins generally uncoil within 5-10 minutes following injectable anesthesia administration and recover after about 20 minutes following administration of the reversal drug.

Animals recovering from anesthesia should be housed in a closed, wooden box in a quiet, dark environment until they are fully recovered to prevent injury or death from drowning, falls, or predation. Animals are typically ready for release when they can hold their head steady and follow movements with their eyes, but pangolins specifically may roll up into a ball instead. If the animals become more tightly rolled when handling, this is a good indicator of recovery.

Anesthetized animals should be monitored regularly during recovery until they can no longer be safely handled, at which point they should be confined in a trap or cage. Essential monitoring includes measuring and recording heart rate, respiratory rate, body temperature, and pulse quality every 5-10 minutes throughout the procedure. The eyes should be lubricated with ophthalmic ointment and protected from debris. Normal vital parameters have not been determined for most of the eight species, though a single study on Chinese pangolins (*Manis pentadactyla*) recorded rectal temperatures of healthy specimens to be about 34.4 to 35.5 °C (94 to 96 °F). Resting awake Chinese pangolins in the study showed a respiratory rate of 14-53 breaths per minute and a heart rate of 86-88 beats per minute, while those under ketamine anesthesia (16-25 mg/kg) exhibited a respiratory rate of 33-38 breaths per minute and a heart rate of 86-100 beats per minute. Animals should be kept out of direct sunlight. Overheated animals should be cooled by placing rubbing alcohol on their paws, administering cool subcutaneous fluids, or wetting their ventrum. Hypothermic animals should be kept warm using tarps, blankets, or warmed SQ fluids.

Should any life-threatening complications occur, anesthesia should be discontinued and the reversal drug administered, if appropriate. While non-invasive (orotracheal) intubation is not possible in pangolins, emergency tracheotomy may be performed if respiration arrests. The latter procedure should only be performed by a veterinarian or well-trained individual.

Section 4. Pangolin Sampling

Please refer to the three required data collection templates for data to collect. These include:

1. P2 Animal Data Collection Form
2. P2 Site Characterization Data Collection Form
3. P2 Specimen Data Collection Form

For more information on downloading templates from EIDITH see [Section 5.2.3. General Data Collection Templates and Applications](#).

In some cases, time constraints, anesthetic risk, inability to prolong immobilization, or other factors may necessitate prioritizing biological sample collection at the expense of collecting any physical measurements. At a minimum:

1. Measure and record the animal's mass (kg) immediately as this can be important for proper drug dosing or emergency interventions. (Weight can be collected through measuring box and animal and then subtracting box weight, to avoid handling an animal).
2. Note any physical abnormalities on visual exam that may indicate injury or illness.
3. Measure and record the animal's tail and nose-to-tail lengths (cm) as these can assist with species identification and age classification.

SAMPLE COLLECTION

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two oral swabs** - one in 500 μ L VTM and one in 500 μ L Trizol
2. **Two fecal samples** - one with max of 500 μ L/0.5cc feces in 500 μ L VTM and one with max of 500 μ L/0.5cc feces in 1 mL Trizol
OR
Two rectal swabs - one in 500 μ L VTM and one in 500 μ L Trizol
3. **Two blood samples** - 2 x 500 μ L aliquots, one in 500 μ L VTM and one in 500 μ L Trizol
4. **Two serum samples** - 2 x 500 μ L aliquots (only if more than 2ml of blood available), frozen without media.
Note: If animals are too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in VTM after serum separation
5. **Two urogenital swabs/urine samples** - one with max of 500 μ L of urine in 500 μ L VTM and one with max of 500 μ L of urine in 500 μ L Trizol

NOTE: Store all cryovials in a liquid nitrogen dry shipper or dewar & transfer to -80°C freezer when possible.

Oropharyngeal Swabs

Using a sterile, polyester tip swab, rub gently (but thoroughly) at the back of the animal's throat, taking note not to push too far into oral cavity if resistance is met. Because the pangolin mouth does not open like a typical mammal, blindly pushing a swab deep into the oral cavity may cause significant soft tissue/ mucosal trauma. Collect two samples and place 1 swab into a cryovial containing 500 μ L VTM and 1 swab in 500 μ L Trizol.



Oropharyngeal swab collection; Photo Credit: WCS/ Viet Nam

Fecal samples/ Rectal swabs

500 μ L or pea-sized piece of feces (200 mg) in VTM and Trizol: Collect either excreted feces, or if animal is large enough (> 1 kg) use a gloved, lubricated (saline or medical lubricant) finger to collect feces directly from rectum. Place two ~200 mg (pea size) samples of fresh feces into 2 vials, one containing 1 mL Trizol (= maximum final ratio of 1:2) and one containing 500 μ L VTM (= maximum final ratio of 1:1). Homogenize by shaking. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

If feces are not available, collect 2 rectal swabs, one in VTM and one in Trizol: Gently insert one sterile swab tip at a time into the animal's rectum. [Note: DO NOT USE TRIZOL AS A LUBRICANT – IT IS HIGHLY IRRITATING TO TISSUE.] Place one swab in a cryovial filled with 500 μ L of VTM using a flame-sterilized scissors to cut the shaft of the swab above the tip. Place the other swab into a tube with 500 μ L of Trizol. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80°C freezer when possible.

Blood

Blood may be collected from the ventral coccygeal (tail) vein in pangolins of all ages. Needle size will vary with animal size, but consider the use of sterile 23 to 21 gauge 1 inch needles as a frame of reference for venipuncture. Volume of collection should be no more than 1% of bodyweight. If animals are severely dehydrated, perceivably unwell or neonatal, venipuncture should be abstained from depending on animal condition. NOTE: Most confiscated specimens are likely to be severely dehydrated.

Coccygeal venipuncture

1. If manually restrained (for young, sick, or injured specimens – note: for *M. javanica* and *M. pentadactyla* this will only be possible in profoundly debilitated specimens) and animal is naturally unrolled: may rest body of animal on a table and restrain behind the forearms to stabilize. The tail may be held off the edge of the table and accessed ventrally for the ventral coccygeal vein. If anesthetized: may place animal in dorsal recumbency (on its back) for venous access.
2. Clean and sterilize a ventral area about 5 to 10 scales caudal to tail base with an antiseptic/ alcohol.
3. Place needle between scales on the ventral midline of the tail to a depth of about 2 cm, at a 45-60 degree angle from skin surface.
4. Withdraw sample.
5. Apply pressure to the venipuncture site after withdrawal of needle, until bleeding stops.



Coccygeal venipuncture on anesthetized specimen; Photo Credit: WCS/ Viet Nam

Jugular venipuncture

1. The right jugular vein may be accessible in pangolins that are not obese, though can be difficult to locate due to their enlarged salivary tissue.
2. Jugular vein venipuncture should only be performed in anesthetized animals. Place the animal in dorsal recumbency and extend neck to access the jugular vein on the right side.
3. Clean and sterilize the neck region with an antiseptic/ alcohol.
4. Apply slight pressure at the base of the neck to distend the vein and manually palpate to locate it.
5. Insert the needle into the vein at a 30 to 45-degree angle to skin surface.
6. Withdraw sample.
7. Apply pressure to the venipuncture site after withdrawal of needle, until bleeding stops.

Femoral venipuncture

1. The femoral vein of Asian pangolins may be obstructed by the testes in males and significant fat pads in females making it difficult and inappropriate to access. Femoral access should only be considered if the person performing venipuncture has significant experience in all species.
2. Femoral vein venipuncture should only be performed in anesthetized animals. Place the animal in dorsal recumbency and stabilize behind forearms and outstretched tail. Stretch out pelvic limb of vein to access.
3. Clean and sterilize the inguinal region with an antiseptic/ alcohol.
4. Palpate femoral arterial pulse in area of the femoral groove/ femoral triangle.
5. Insert needle medially (1-2 mm) to the pulse to access the vein at a 30 to 60 degree angle to skin surface.
6. Withdraw sample.
7. Apply pressure to the venipuncture site after withdrawal of needle until bleeding stops.

Blood should be collected into red/tiger top (serum) and purple top (EDTA) vacutainers. Gently invert purple-top tube several times to mix. Whole blood can be aliquoted from the purple top tube into cryovials with VTM and Trizol using a pipette gun. Whole blood collected in a red/tiger top tube can either be centrifuged (if available) or placed vertically in a cooler with ice bricks and allowed to stand undisturbed overnight (~ 12 hrs) for clean serum separation. Serum can then be aliquoted into cryovials.

Urine/ Urogenital Swabs

Using a sterile, polyester tip swab dipped in VTM, rub the external urethra of the penis or inside the vagina of animal. Collect two samples and place 1 swab into a cryovial containing 500 μ L VTM and 1 swab in 500 μ L Trizol.

Urine may also be collected if excreted and actively collected as a “free-catch” sample into plastic vials. Add up to 500 μ L of urine directly into 2 vials, one containing 500 μ L VTM and one containing 500 μ L Trizol (maximum ratio 1:1).

ADDITIONAL OPTIONAL SAMPLES

These are not a part of current PREDICT protocols but may be useful samples to collect for future disease surveillance and genetic analysis.

Ectoparasites

Parasites (e.g. ticks) can be collected and transferred into an appropriately sized cryotube filled with molecular grade (95-99%) ethanol and stored at room temperature.

Hair Samples

Place in separate paper envelopes and store dry at room temperature. Collect 5-10 strands.

Biometrics

Additional biometric information such as sex, age, body length, and tail length can be recorded while the animal is anesthetized.

DECEASED SPECIMENS

If a fresh pangolin carcass is to be sampled (e.g. deceased animal in a wet market, or after confiscation from the illegal trade), then the standard PREDICT necropsy protocol may be used.

Approximate Body Measurement Ranges for the 8 species of pangolin:

	Nose to Tail (cm)	Tail (cm)	Weight (kg)
Asian Species			
Chinese pangolin (<i>Manis pentadactyla</i>)	40 - 58	25 - 38	2 - 7
Sunda pangolin (<i>Manis javanica</i>)	40 - 65	35 - 56	7 - 10
Indian pangolin (<i>Manis crassicaudata</i>)	51 - 75	33 - 47	10 - 16
Philippine pangolin (<i>Manis culionensis</i>)*	40 - 65	35 - 56	7 - 10
African Species			
Tree pangolin (<i>Phataginus tricuspis</i>)	25 - 43	35 - 62	1.6 - 3
Long-tailed pangolin (<i>Phataginus tetradactyla</i>)	28 - 34	55 - 80	2.2 - 3.6
Giant pangolin (<i>Smutsia gigantea</i>)	125 - 140**		18 - 30
Ground pangolin (<i>Smutsia temminckii</i>)	50 - 60	40 - 50	15 - 18

**M. culionensis* is very similar in appearance to *M. javanica*, usually distinguishable by shorter head and a smaller tail to body length ratio.

** *S. gigantea* is readily distinguishable by total body length and weight; only total length measurement available.

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Section 6. Appendix I. Supply and Equipment List

Note: Supply details, availability, and vendor sources may vary.

PPE

- Designated clothing (e.g. overalls or other clothes which can be put on before sampling and removed following sampling; long-sleeved shirts should be considered if handling awake animals)
- Flexible face shield or other eye protection
- N95 or P100 respirator
- Nitrile examination gloves (double-gloving recommended)
- Closed-toed, washable shoes

First Aid

- Betadine or (or benzalkonium chloride)
- First aid kit (with post-exposure prophylactic vaccine if working in remote areas where vaccine is not rapidly accessible)

Data Collection

- Datasheets (or EIDITH tablet for direct data entry)
- Pencils
- GPS

Capture and Handling

- Leather gloves
- Smooth sided box traps (w/ bait)
- Towels/tarps for covering box traps
- Clear plastic induction box (for gas anesthesia)
- Spring/electronic scale
- Dial/digital caliper
- Tape measure
- Chemical restraint requirements (drugs, portable anesthesia machine, etc.)
- Camera
- Identification guides

Sampling

- Towels/tarps for processing
- Permanent lab markers for tube numbering
- Cryotubes
- Needles 20G, 22G
- Syringes for blood draws
- Sterile swabs (dacron/polyester)
- Cryo resistant tube labels
- Cryovial rack
- Cryoboxes and dividers
- Plastic vacutainers (EDTA and dry)
- Pipettors and disposable tips
- Portable centrifuge for vacutainers
- Cryo gloves
- Forceps
- Scissors
- Dissection kit
- Trizol reagent
- Viral Transport Medium (VTM)
- RNAlater reagent
- Buffered formalin
- 95% ethanol
- Lighter
- Liquid nitrogen shipper/liquid nitrogen

Waste Disposal and Decontamination

- Paper towels
- Sharps containers
- Bleach
- 95% ethanol
- Biohazard bags
- Sprayers

Rodent Sampling Methods

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Objectives: To safely collect biological samples from live and dead rodents.

Section 1. Brief Overview of PPE

Minimum PPE Required for Handling Rodents

The minimum PPE for handling of rodents during capture and sampling includes:

1. Eye protection
2. N95 or P100 respirator
3. Nitrile gloves¹
4. Tyvek-type suits ***
5. Washable shoes or shoe covers

***Tyvek-type suits are required for ALL sampling activities that involve direct contact with live wild rodents and/or situations where contact with any fluids or excreta from live wild rodents could soil clothing. If there is risk of being bitten or scratched by a live wild rodent, other protective gear should be employed as appropriate, such as **heavy gloves (e.g. leather, sterilizable), long-sleeve clothing and pants should be worn underneath Tyvek-type suits**. As always, field teams should take necessary precautions to avoid additional routes of exposure by wearing eye protection, fitted respirator (N95 or P100), and nitrile gloves.

(See the *Biosafety and PPE Guide* for detailed instructions regarding PPE Use)

Section 2. Data Collection

Please refer to the **required data collection templates** for data to collect:

1. P2 Animal Data Collection Form
2. P2 Site Characterization Data Collection Form
3. P2 Specimen Data Collection Form

Section 3. Rodent Capture, Handling, and Sampling

Capture techniques will vary based on the species being targeted and the location where the samples are being collected. Details of the main techniques, including information on Sherman traps, are available in other documents such as the CDC guide *Methods for Trapping and Sampling Small Mammals for Virologic Testing* (pp 15-18)

(http://www.cdc.gov/hantavirus/pdf/rodent_manual.pdf).

Note: Field staff must use this PREDICT guide for specimen collection techniques.

The PPE requirements for handling animals during capture or during processing are the same. All animal capture, handling, and sampling must also be done in accordance with your current in-country IACUC approval, which should be amended accordingly and be consistent across the pertinent approvals.

¹ Nitrile gloves are recommended for handling rodents, in the absence of nitrile gloves and allergies to latex, double latex gloves could be considered.

Age Classification -Updated March 2018

Age classes: For some rodent species it will be possible to classify rodents into one of four age classes. Most rodent biologists will be familiar with the rodent age classifications given below. However, for the PREDICT-2 project, we had to create generalized age classifications that would be appropriate across all taxa that are included in the eBook. For data that will be entered into EIDITH specifically, follow the EIDITH Age Classification rather than the Rodent Age Classification. For clarity, both are listed in the table below.

Note: Follow this equivalency table to correctly classify rodents using the same EIDITH definition. *For teams using this protocol for sampling of shrews, the rodent age classification may not fit all situations, however the EIDITH age classifications should be workable.*

PREDICT SOP Rodent Sampling

RODENT Age Classification	Cross-taxa EIDITH Age Classification
Fetus (in utero)	fetus (in utero)
Neonate (i.e. female gave birth to litter in trap)	neonate (newborn)
Does not apply Note: In the rare case a mother is captured with a pre-weaned young – other than a neonate – it can be marked in EIDITH as juvenile.	juvenile (dependent on dam)
Juvenile/Subadult (presumed independent from parental care and presumably weaned. Not yet adult-sized, not yet adult weight and not in full adult pelage. Absence of secondary sexual characteristics such as testes descended, lactating, vaginal plug)	subadult (immature, independent)
Adult (adult size and weight, full adult pelage, reproductively active: (e.g. testes descended, enlarged nipples/lactating, vaginal/copulatory plug, palpating fetuses); OR reproductively inactive – Note 1: There are rodent species where males have inguinal testes; others have scrotal testes solely during breeding season. Note 2: Shrews have internal testes and some species have cloaca).	adult (reproductive age)

Rodent Capture and Handling

Note: Country Coordinators are responsible for following all local laws and regulations regarding veterinary anesthetic drug use.

1. Free-ranging rodents will be captured using metal box traps (Sherman/Tomahawk traps). Traps will be set open at sunset and checked at sunrise. If adverse weather is expected, such as abrupt changes in precipitation intensity that could cause flash flooding events/animals getting wet, or if researchers are working in areas where predation is common, traps will be checked more frequently, and those traps will be closed during the adverse weather event. Traps will be located in areas protected from direct sunshine (e.g. ground cover vegetation or artificial reflective foil insulation) to prevent consequent heat stress. Otherwise, traps will be closed between sunrise and sunset.

2. To avoid hypothermia, allow thermoregulation, and reduce capture stress, always provide nesting material (e.g., pulped cotton fiber, such as Nestlets by Ancare) to each trap. If the animal is in hypothermic shock, wrap the animal in a dry drape, place a hand warmer (e.g., reusable exothermic pad or air-activated disposable pad) in the outer layer of the wrap, place the animal in a dry recovery chamber (i.e. clear plastic box), monitor recovery and then release at the capture location.
3. Remove animals from traps by placing a clear plastic handling bag or large Ziploc-style bag over the trap, opening the door into the bag and coaxing animal out by gently shaking the trap. DO NOT BLOW INTO THE TRAP.
4. Weigh the animal in the handling bag using a Pesola scale or a flat digital scale prior to inserting the anesthetic ball (see below). Temporarily record the animal plus bag weight and after the animal is anesthetized and removed, reweigh the empty bag and subtract the bag weight from the total to obtain the animal weight.
5. Scruffing is the best way to handle smaller rodents for sampling. Hold the anesthetized animal by pinching the skin between the thumb and forefinger at the point where the rodent's spine meets the head (Figure 2). For a more secure hold and/or access to the ventral surface, position the rodent's body firmly across your hand by extending your forefinger and thumb back as far as possible while maintaining a firm grip on the scruff. Place the tail between the fingers of this same hand (Figure 3).

Note: If you grasp too much skin, the airway will become restricted and the rodent will become cyanotic. Monitor the condition of the animal the entire time it is restrained, being careful to observe the breathing rate and color of the ears, nose, and oral cavity. The animal should be released immediately if there are any signs of gasping or change in coloring from pink to blue.



Figure 1 (left): scruff restraint technique. Figure 2 (right): positioning the animal in the hand while scruffing, in order to collect samples. Both: © Leticia Gutiérrez Jiménez.

Rodent Anesthesia

Anesthetize rodents with isoflurane.

Note: *Pregnant women should avoid or minimize exposure to isoflurane.*



Figure 3: Nose cone for anesthesia. Figure: PREDICT

1. Keep in mind species-specific Isoflurane hypersensitivity (e.g. voles) and that age, sex, and reproductive stage can affect drug potency, which in turn may cause abrupt respiratory depression - especially in older individuals.
2. Apply 0.4 ml of isoflurane to a cotton ball and put the cotton ball into a metal tea ball or plastic tube (nose cone; 0.4 ml is the appropriate dose for a ca. 20g mouse, adjust the dose as needed for larger rodents). If tea ball is used, place the metal ball into the clear plastic holding bag or container with the rodent; if nose cone is used, place the rodent's nose into the tube.
3. Watch the animal closely until anesthetized--Breathing rate will increase then slow as the animal progresses further under anesthesia.
4. When withdrawal reflex is suppressed (use toe pinch to measure the withdrawal reflex), remove rodent from bag for processing.
5. Place an additional cotton ball soaked with 0.2 ml isoflurane into a 50 ml tube and screw on the cap. If the animal begins to wake up during blood collection, unscrew the cap of the nose cone and position over the animal's nose for as brief a time as possible to re-anesthetize the animal (see Figure 1 below). A nose cone for supplementary anesthesia should be used with care as different animals respond differently to isoflurane inhalation.
6. Collect samples immediately after the rodent is anesthetized.

Note: For larger rodents, chemical restraint and anesthesia (ketamine alone 25-50mg/kg, or ketamine combined with xylazine 80-100 mg/kg ketamine + 10-12.5 mg/kg xylazine) can be applied. Drugs can be administered to manually-restrained or squeeze-caged animals by syringe or by blow dart, depending on size and situation.

Basic Sample Set for Rodent Sampling

The following basic set of samples should be collected from each animal where possible. *If only one sample can be collected, then place into VTM.*

1. **Two oral swabs** - one in 500 μ L VTM and one in 500 μ L Trizol
2. **Two rectal swabs** - one in 500 μ L VTM and one in 500 μ L Trizol
or
Two fecal samples - one with max of 500 μ L/0.5cc feces in 500 μ L VTM and one with max of 500 μ L/0.5cc feces in 1 mL Trizol
3. **Two urogenital swabs/urine samples** – one in 500 μ L VTM and one in 500 μ L Trizol
4. **Two whole blood samples** - one with max of 500 μ L of whole blood in 500 μ L VTM and one with max of 500 μ L of whole blood in 500 μ L Trizol

Note: *If animals are too small to collect two blood tubes (for whole blood and plasma/serum), collect plasma/serum and save remaining clot or red cell fraction in VTM after plasma/serum separation.*

5. **Two serum samples** - 2 x 0.5ml aliquots frozen without media

Swab Sample Collection

1. Collect **2 oral swab** samples using sterile, polyester-tipped swabs with plastic or aluminum shafts. A sterilized handle of a pair of forceps can be used to safely open the animal's mouth. Rub the polyester tip of the swab gently, but thoroughly, against the back of the animal's throat. Place one swab into a cryovial containing 500 μ L of VTM and one in a cryovial with 500 μ L of Trizol. Cut the shaft of the swab with alcohol-wiped (or ethanol-wiped), flame-sterilized scissors. To cut the swab shaft, lift the swab a little above the bottom of the vial and then cut it. If the shaft is cut when the swab tip is resting on the bottom, the swab will be too long and the cryovial won't close.
2. Collect **2 rectal swabs** using appropriately-sized, sterile polyester swabs. For smaller animals, use urethral or pediatric swabs. Rectal swabs can be moistened with sterile saline prior to animal sampling.

DO NOT USE VTM TO MOISTEN SWABS BEFORE SAMPLING

DO NOT USE TRIZOL AS A LUBRICANT – IT IS HIGHLY IRRITATING TO TISSUE.

DO NOT FORCE TIP OF SWAB INTO RECTUM. IF IT WON'T ENTER EASILY, DO NOT COLLECT THIS SAMPLE.

Gently insert one sterile swab tip at a time into the animal's rectum. Place 1 swab in a cryovial filled with 500 μ L of VTM, and using isopropyl alcohol-wiped (or ethanol-wiped), flame-sterilized scissors, cut the shaft of the swab. Place the other swab into a cryovial with 500 μ L of Trizol. Store in a dewar or dry shipper with liquid nitrogen and transfer to -80°C freezer when possible.

Alternatively, collect fresh feces: Add 500 μ L (pea-sized) pieces of feces directly into two vials, one containing 500 μ L VTM (maximum final ratio of 1:1) and one containing 1 mL Trizol (maximum final ratio of 1:2) and mix each tube well. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

3. Collect **2 urogenital swabs** and place one in 500 μ L of VTM and one in 500 μ L of Trizol. Swabs can be moistened with sterile saline prior to animal sampling. If the animal urinates, collect up to 500 μ L of **urine** using pipettor and place into one cryotube with 500 μ L of VTM and mix well. Pipette up to another 500 μ L of urine into a cryotube with 500 μ L of Trizol and mix well.

Blood Sample Collection

Make sure the total blood collected from the animal does not exceed 1% of weight of animal (i.e., a maximum of 500 μ L should be collected from a 50g rodent).

If animal size allows, collect blood and place in a red-top vacutainer tube with clotting factor and in a lavender-top tube (e.g., EDTA). Allow blood in red-top tube to clot, then centrifuge and pipette two 500 μ L serum samples and freeze without media. From lavender top tubes, add up to 500 μ L of whole blood to a cryotube with 500 μ L of VTM and 500 μ L of blood to a second cryotube with 500 μ L of Trizol (if blood volume is <500 μ L then only collect into VTM).

If animals are too small to collect vacutainer blood tubes, blood collection can be done with Sarstedt Inc Microvette Capillary Blood Collection Tubes (100ul to 600ul capacity), Becton Dickenson BD Microtainer™ Capillary Blood Collector and BD Microgard™ Closure tubes (250ul-500ul capacity), Terumo™ Capiject™ capillary blood collection tubes (500ul capacity), or heparinized 75 μ L glass hematocrit tubes.

For blood collected into hematocrit tubes, use a bulb to expel the whole blood in a cryotube with 500 μ L of VTM. Hematocrit tubes can be centrifuged using portable hematocrit or standard vacutainer centrifuges to separate plasma. Score glass tube using a razor blade or X-acto knife where the serum meets the red cell fraction and snap the tube. Use a bulb to expel plasma into a micro-cryovial and freeze. If two or more capillary tubes are filled, collect two aliquots of plasma. Preserve the remaining red cell fractions in a separate cryovial with VTM and freeze.

Guidelines for Selecting Bleeding Method

Rodent Taxa	Mice, field mice, jumping mice	Voles, gerbils, and hamsters	Squirrels and chipmunks	Rats and rat-like species
Bleeding Method	1) Facial vein 2) Saphenous vein	1) Facial vein 2) Saphenous vein	1) Saphenous vein	1) Saphenous vein 2) Lateral tail vein Larger animals: Ventral tail vein, jugular vein

Table 1: Recommended Bleeding Technique(s) by Rodent Taxa

Local personnel should be acquainted with the local fauna and their anatomy and apply this knowledge to determine the most appropriate sampling methods to use in the field. Do not attempt venipuncture without at least one field person present with prior experience, especially with the facial vein and jugular vein bleeding approaches.

General Techniques for Bleeding Rodents

NOTE: Bleeding by any method should only be performed on anesthetized animals.

SUBMANDIBULAR OR FACIAL VEIN BLOOD COLLECTION

Adapted from the MEDipoint, International Inc. Goldenrod lancet ‘For use on mice’ website.

For videos of the technique, please visit

http://www.medipoint.com/html/pc_video_instructions.html (for PC)

http://www.medipoint.com/html/mac_video_instructions.html (for Mac)

For this technique you will need to purchase lancets to prick the skin on the rodent’s cheeks.² You can use any lancet, but as guidance, Goldenrod lancets are recommended. They come in four point lengths: 3, 4, 5 and 5.5mm point. You should use the best option depending on the age/size of the rodents you will sample. If you only need one or two drops, use 3mm point (keep in mind that a standard blood drop is equal to ~50ul). Always be careful that the volume of blood harvested does not exceed the total volume recommended for that size animal’s safety and health (≤1% body weight).

Step-by-step procedure:

1. Choose the proper lancet point length (see above), corresponding to the size of rodent.
2. Open the lancet/needle wrapper and prepare a collection vial.

² Lancets provide the greatest control of puncture depth and are the recommended device for facial bleeding; however, sterile 20G-22G hypodermic needles may also be used **after** the facial bleeding technique has been completely mastered.

3. Hold the anesthetized animal by pinching the skin at the back of the head (scruff restraint technique – see Figure 2) and taking the tail between the fingers of the same hand (Figure 3).
4. Locate the back of the jaw bone, the submandibular area. There is a vascular bundle located at the rear of the jaw bone providing a convenient and consistent source of blood (Figures 4, 5, and 6).

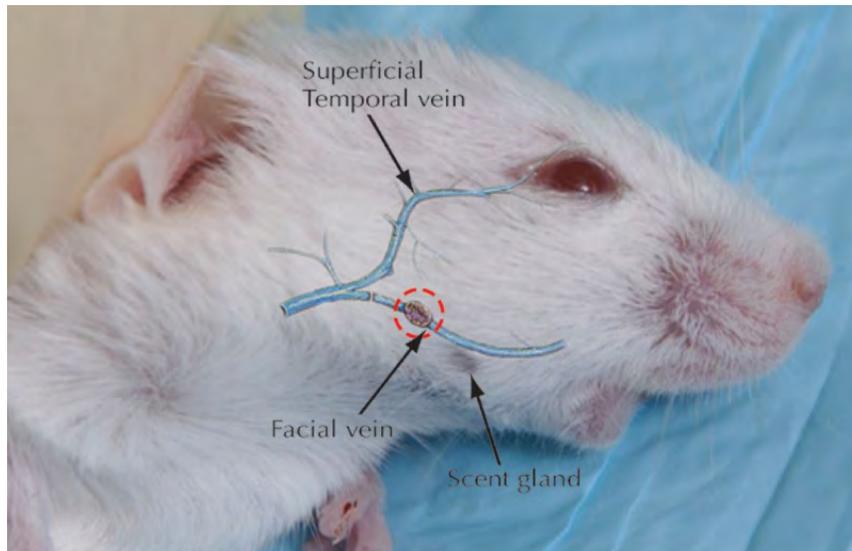


Figure 4: The vascular bundle targeted during blood collection from the facial vein. The dashed circle indicates the puncture site. Figure: Golde, Gollobin, and Rodriguez, 2005.

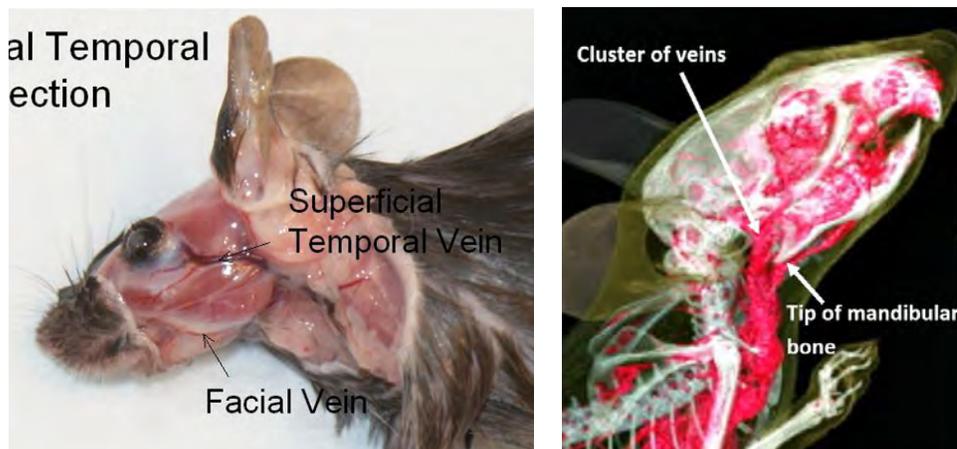


Figure 5 (left): Superficial temporal vein and facial vein in the mouse. Figure 6 (right): The targeted cluster of veins is just behind and above the tip of the mandibular bone. Both: MEDipoint International, Inc.

5. Once the site is located, properly align the tip of the lancet: The facial muscles of a mouse or rat run fairly parallel to the bottom of the jaw line, that is, along the face from the nose/whiskers towards the ear. Align the lancet with the striations of the muscles in

the animal's face so that when the tip enters the facial muscles, it will go between the striations instead of across them. This will cause less damage to the muscle tissue and therefore cause less scarring, and there will be less chance of hematoma formation.

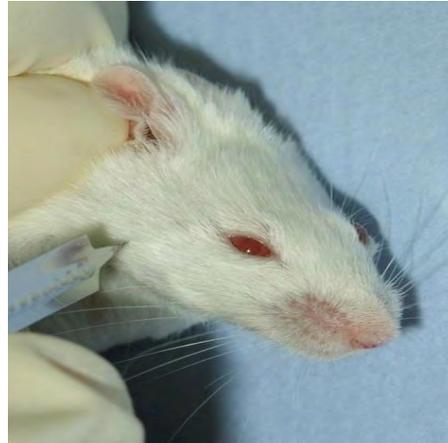


Figure 7 (left): Facial vein puncture site in *P. maniculatus*. Image: © Leticia Gutiérrez Jiménez. Figure 8 (right): After locating the puncture site, apply enough pressure to insert only the tip of the lancet into the cheek.

6. Quickly poke the cheek of the animal, applying enough pressure to insert only the tip the lancet, and withdraw immediately. A blood drop will form instantly. Approach the tube to collect the blood as it flows freely (Figures 7 and 8).

Note: To avoid sample contamination, do not rub or press the collection tube against the puncture site.



Figure 9: Collect blood into the vial as it flows freely, avoiding rubbing or pressing the tube against the puncture site. Both: MEDpoint International, Inc.

If you are not getting enough blood, try the following technique: When scruffing the animal, hold the skin on the back of its neck between your thumb and middle finger. When more blood is desired, put your index finger on the top of the rodent's head and gently move its head up and down. This will keep the wound open a little longer and pump more blood into the submandibular area and out the puncture site.

Blood may also be drawn from both cheeks at one sampling event if the amount of blood to be collected is large.

7. Stop the blood flow at any time by releasing the head/neck scruffing pressure point and applying a compress (gauze, cotton) to the puncture site for a few seconds (or 1-3 minutes to prevent hematoma formation), as needed.

Note: Always ensure complete hemostasis before returning the animal to its individual trap.

8. To avoid hypovolemic shock in small animals ($\leq 200\text{g}$), fluid volume replacement should be administered subcutaneously after collecting all biological samples and immediately before placing the individual into its trap/recovery chamber. (See Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines)

Note: Recumbent or dehydrated individuals must be treated immediately and should **NOT be sampled**. Those animals should be released at the trap capture location as soon as they recover. To avoid dehydration and hypoglycemia, always provide a small apple or other fruit slice (1 apple = ~16 slices) as part of the bait mix and to each trapped individual while it is waiting to be processed.

LATERAL SAPHENOUS VEIN BLOOD COLLECTION

Adapted from Guide to the Care and Use of Experimental Animals. Volume 1, Second Edition. University of British Columbia.

For videos of this technique, please visit
<https://norecopa.no/films-and-slide-shows/mouse>
[Blood sampling of the rat from the saphenous vein](#)

A minimum of two people are required for this type of blood collection in rodents: one for anesthetizing, handling and bleeding, and one for collecting the blood sample and data recording.

A volume of 100 μ -300 μ l can readily be collected using the lateral saphenous vein bleeding technique; however, the number of attempts to take a blood sample should be minimized. Staff should make no more than three needle sticks in any one attempt. Warming the animal immediately prior to blood collection will increase blood flow considerably.

Step-by-step procedure:

1. Choose the appropriate anesthesia protocol and secondary restraining technique for the species. Mechanic and manual restraint causes stress, and therefore the duration of restraint should be minimized. Where a restraint tube is used, it should be appropriate to the size of the rodent. All forms of restraining equipment should be frequently washed to prevent pheromonally-induced stress or cross-infection.
2. The animal is held head-first in a restrainer so that only the rear legs and tail are free. The rear leg can be stretched out to its natural position. The head, nose, and ear color should be monitored closely. Apply the isoflurane nose cone for a couple of seconds if needed. For larger – and feisty – rodents, it is recommendable to wrap the animal in a drape or towel in case of premature awakening.



Figure 10 (left): Mouse or vole restraint. Figure 11 (right): Rat or squirrel restraint. Both: Guide to the Care and Use of Experimental Animals.

3. To secure the animal and elevate the vein, the skin on the upper thigh is gently but firmly squeezed, using the same hand holding the restrainer.
4. The vein is easier to see when the fur is shaved either by clippers or by using scissors. A thin film of bland ointment, such as petroleum jelly or Glycerin, can be applied to prevent blood from seeping and allow for blood drop formation. If you are not shaving the area, find the vein by “combing” or clearing away the fur using a cotton-tipped swab has been dipped in jelly.



Figures 12-14 (left to right): Clearing the fur on the mouse, the rat or squirrel and the vole. Figures 12 and 13: Guide to the Care and Use of Experimental Animals. Figure 14: © Leticia Gutiérrez Jiménez

5. Locate the vein.

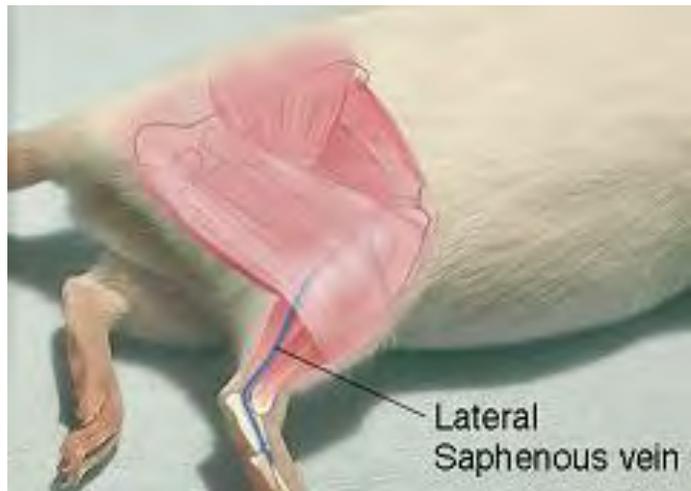
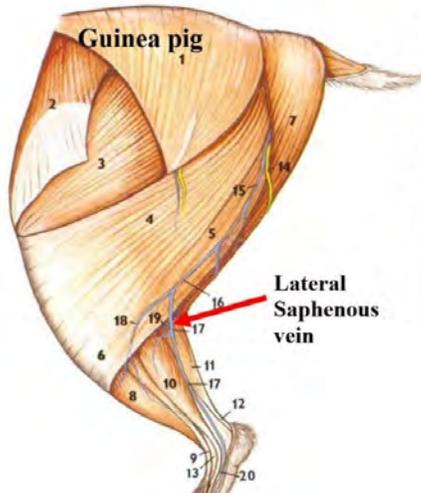


Figure 15 (left): The lateral saphenous vein in the guinea pig. Figure: Guide to the Care and Use of Experimental Animals. Figure 16 (right): The lateral saphenous vein in the mouse. Figure: Theodora.com



Figure 17: Lateral saphenous vein in the Least chipmunk (*Tamias minimus*). Figure: © Leticia Gutiérrez Jiménez

- Using a **23-27 gauge needle or an appropriate depth size animal lancet**, puncture the vessel at a 90° angle at the most proximal visible aspect. Collect blood as it drips from the vein.



Figures 18-20 (left to right): Puncturing the lateral saphenous vein of the mouse, rat or squirrel, and vole. Figures 18 and 19: Guide to the Care and Use of Experimental Animals. Figure 20: © Leticia Gutiérrez Jiménez

Rodent taxa	Mouse (25g)	Hamster/Vole	Rat (250g)
Max. sample volume	200ul	0.5% bodyweight	1600ul
Needle size	27G-25G needle or lancet	25G needle or lancet	23G needle or lancet

- Collect your sample.



Figures 21(left) and 22 (right): Collecting blood from the rat/squirrel and vole via the lateral saphenous vein. Figure 21: Guide to the Care and Use of Experimental Animals. Figure 22: © Leticia Gutiérrez Jiménez

- Use a dry gauze pad and/or povidone-iodine prep pad to apply pressure to the puncture site as the pressure on the upper thigh is released. If bleeding does not stop, use a silver nitrate styptic pencil (moisten the pen slightly with sterile saline solution and then carefully place onto the puncture site). Silver nitrate will stop vigorous hemorrhage, but it stings sharply on the application site and should not be used habitually to stop minor bleeding.

9. In small animals ($\leq 200\text{g}$), fluid volume replacement should be administered subcutaneously after collecting all biological samples, as described in Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines.

Note: Recumbent or dehydrated individuals must be treated immediately and should **NOT be sampled**. Those animals should be released at the trap capture location as soon as they recover. To avoid dehydration and hypoglycemia, always provide a small apple or other fruit slice (1 apple = ~16 slices) as part of the bait mix and to each trapped individual while it is waiting to be processed.

10. Remove the rodent from the restrainer and place it in its individual trap.

11. Monitor the animal for 5-10 min to ensure hemostasis (bleeding has stopped).

Techniques for Bleeding Rats and Larger Rodents

LATERAL TAIL VEIN BLOOD COLLECTION

1. Use a tourniquet. The veins are located on either side of the tail and are quite superficial.
2. Use a needle with the appropriate gauge (usually 27G or 25G), entering the skin at a shallow angle about one third down the length of the tail. Be careful not to collapse the vein.
3. Consider using a small syringe or hematocrit tubes to collect the blood.
4. In small animals ($\leq 200\text{g}$) administer fluids after sampling as described above and in *Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines*.
5. Remove the rodent from the restrainer and place it in its individual trap.
6. Monitor the animal for 5-10 min to ensure hemostasis (bleeding has stopped).

VENTRAL TAIL VEIN BLOOD COLLECTION

1. Use a tourniquet, as above (lateral tail vein bleeding).
2. The vein is located both centrally and ventrally in the tail. The ventral tail vein is deeper than the lateral tail vein used in mice and it is not readily visualized. Take care not to bleed from the ventral caudal artery, which is sheathed by a thick fascia.
3. As with lateral tail vein bleeding, start one third of the way down the tail.
4. After bleeding, ensure hemostasis by applying pressure to site of bleeding using a cotton ball or gauze until bleeding ceases (approximately 1 minute).
5. Administer fluids as described above and in *Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines*.
6. Monitor the animal for recovery.

JUGULAR VEIN BLOOD COLLECTION

Jugular vein bleeding is recommended only for larger rodents (e.g., guinea pigs, chinchillas, etc.), and even then should be considered only if appropriate alternatives are impossible or have been unsuccessful. The technique requires a potentially challenging degree of animal restraint and a strenuous physical positioning of the animal to be sampled, which can be difficult with alert and resistant animals. A high level of competence is required in the animal sampling staff to avoid permanent harm to the animal.

1. On the anesthetized animal, clean and apply pressure to the jugular vein on one side.
2. Direct the needle into the vein and collect the sample.
3. After bleeding, ensure hemostasis by applying pressure to site of bleeding using a cotton ball or gauze until bleeding ceases (approximately 1 minute).
4. In the case of small animals or visibly dehydrated animals, administer fluids as described above and in *Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines*.
5. Monitor the animal for recovery.

Necropsy Sample Collections

In case of accidental death before or during animal sampling, or where dead animals are available opportunistically for sampling, perform a necropsy examination and collect the samples described above. If euthanasia is required see the AAZV and **AVMA guidelines (Section 8.5.2)**.

In all cases, samples going into VTM and Trizol should be collected sterilely, limiting cross-contamination between tissues (see below). Collect as much blood as possible via cardiac puncture. In addition, collect **three** adjacent, approximately 200 mg (pea-sized) samples of the following tissues:

- Adrenal
- Colon
- Heart
- Liver
- Lymph node
- Ovary
- Testes
- Cecum
- Duodenum
- Kidney
- Lung
- Spleen
- Pancreas
- Other, if required

One specimen should be frozen in 500 μ L VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment).

To avoid cross contamination among individual specimens, follow sterile tissue collection technique.

1. Use clean scalpel handle, scissors and tweezers (Clean instrument = wiped with 70% EtOH and flamed). Use a new, sterile scalpel blade to cut skin and to open body cavities. Discard the used scalpel blade.
2. Open the abdominal and thoracic cavities carefully to prevent contamination between cavities and among organs.
3. Observe organs, noting abnormalities.
4. Use a new scalpel blade for specimen collection. Secure aseptic specimen collection by wiping (EtOH 70%) and flaming the instruments between every tissue sample.

Section 4. References

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Section 5. Appendix I. Supply and Equipment List

Note: Supply details, availability, and vendor sources may vary.

PPE

- Tyvek-like suits
- Flexible face shield or other eye protection
- N95 or P100 respirator
- Nitrile examination gloves
- Washable shoes

First Aid

- Betadine or (or benzalkonium chloride)
- First aid kit

Data Collection

- Datasheets (or EIDITH tablet for direct data entry)
- Pencils
- GPS

Capture and Handling

- Sherman traps
- Flagging tape
- Leather gloves
- Holding bags
- Manual restrainer
- Spring/electronic balance
- Dial/digital caliper
- Nestlets or similar bedding material for traps
- Hand warmers or similar hypothermia treatment method
- Large/gallon-sized Ziploc-style (resealable) plastic bags
- Chemical restraint / anesthesia requirements
- Metal tea ball and/or nose cone for anesthesia maintenance, and/or isoflurane vaporizer
- 20ml plastic tubes
- Cotton balls
- Camera
- Identification guides
- Apples or other fruit (food with high water content) to help prevent animal dehydration

Sampling

- Clippers or scissors for hair / fur
- Glycerin or petroleum jelly
- Cotton-tipped swabs (for “combing” or clearing fur only, **NOT** for sampling)
- Processing trays
- Permanent lab markers for tube numbering

- Cryotubes
- Lancets
- Needles: 23G, 25G, 27G (Note: 20-22G needles may be used for facial bleeding after the technique has been mastered)
- Needles and syringes for blood draws
- Insulin syringe (27G-29G, x ½ inch, 1.0 ml) for fluid replacement
- Lactated Ringer's or 0.9% NaCl solution for fluid replacement
- Sterile swabs (dacron/polyester-tipped), appropriate sizes for sampling needs
- Sterile saline
- 2inch x 2inch gauze pads
- Povidone-iodine, 70% Isopropyl Alcohol, or Benzalkonium Chloride prep pads
- Silver nitrate styptic pencil
- Cryo resistant tube labels
- Cryovial rack
- Cryoboxes and dividers
- Plastic hematorit tubes
- Plastic vacutainers (EDTA and dry)
- Small vials or jars for tissue samples (necropsy sampling only)
- Pipetters and disposable tips
- Portable centrifuge for vacutainers
- Portable centrifuge for hematocrit tubes
- Cryo Gloves
- Fine Point Forceps
- Scissors
- Dissection kit
- 70% EtOH for sterilizing necropsy instruments
- Alcohol burner for sterilizing necropsy instruments and/or sterilizing scissors to cut swabs
- Trizol Reagent
- Viral Transport Medium (VTM)
- RNA Later
- Buffered formalin
- 95% ethanol
- Lighter
- Liquid nitrogen shipper/liquid nitrogen

Waste Disposal and Decontamination

- Paper towels
- Sharps containers
- Bleach
- 95% ethanol
- Biohazard bags
- Sprayers

Supply References

Goldenrod lancets: http://www.medipoint.com/html/for_use_on_mice.html

Microcapillary tubes with caps:

<https://www.fishersci.com/us/en/catalog/search/products?storeId=10652&nav=122540&sortBy=default&keyword=Microvette+Capillary&searchType=PROD&SWKeyList=%5B%5D&typeAheadCat=>

Microtainers with microgard closures: <https://www.fishersci.com/shop/products/bd-microtainer-capillary-blood-collector-bd-microgard-closure-7/p-178764#tab2>

Capillary blood collection tubes: <https://www.fishersci.com/shop/products/terumo-capiject-capillary-blood-collection-tubes-8/p-178577>

Nestlet bedding material: <http://www.ancare.com/our-products/nestlets-ancare>

Appendix II. Aseptic Subcutaneous Fluid Administration Guidelines

- a) To minimize contamination, choose the smallest fluid vial volume (i.e. 10mL) of Lactated Ringer's (preferred) or 0.9% NaCl solution. Warm the solution up to ~37°C/100°F.
- b) Check the expiration date of fluid vial/bag. Visually inspect the vial integrity and fluid aspect (discoloration, haziness, crystallization, or particulate matters). Discard any contaminated, punctured or cracked vials/bags.
- c) Select an injection site in the loose skin over the animal's neck or flank that has no evident skin nor tissue damage.
- d) Wipe the chosen area with a 70% Isopropyl Alcohol or Benzalkonium Chloride prep pad.
- e) Using – for small rodents – an insulin syringe (27G-29G, x ½ inch, 1.0 ml), insert the needle 5-10 mm through the skin before making the injection. Lack of resistance to the injection is indicative that you are in the right location.
- f) Depending on individual size, inject subcutaneously 0.5-1ml (respectively for a juvenile and adult mouse) sterile fluid (see Figure below). Small rodents (~25gr) can safely receive up to 2-3ml subcutaneously and larger rodents (~200gr) up to 3-5ml.
- g) Check for leaking from the injection site, especially if a larger volume is injected.
- h) Discard syringe in sharps container.
- i) You can draw multiple fluid doses from the same vial/bag used during the same sampling day. Discard any opened fluid vial at the end of the sampling day, even if it is not empty.



Replace fluids after collecting blood from rodents. Image © Leticia Gutiérrez Jiménez

Safe Animal Capture and Sampling

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Last updated: 28 November 2016

Objective: To provide principles and general considerations for the safe capture of wild animals and safety of personnel during these captures.

Section 1. Principles & Guidelines for Personnel Safety & Health During Wild Animal Capture

General Principles on Personal Safety

Capture, handling, and anesthesia of wildlife during field projects are often carried out in remote areas away from medical assistance. Therefore, every possible effort must be made to prevent injuries to personnel. The following precautions should be considered for fieldwork that involves handling animals for diagnostic sample collection:

Field teams, particularly animal capture teams, must be prepared to deal with potentially hazardous situations and have contingency plans in place to respond to accidents, injuries or other unexpected circumstances.

Investigators implement measures, in accordance with protocols or established guidelines, to protect their staff, co-workers and themselves against possible injury or exposure to potentially dangerous procedures, drugs, chemicals, animals, or animal fluids and waste.

Investigators must clearly identify and discuss with project personnel the hazards to human health and safety and the appropriate safety precautions to be taken when working with wild animals.

Investigators should ensure that all project personnel are properly trained, have written procedures, and have the appropriate protective clothing and personal protective equipment (PPE) for their safety.

Investigators should familiarize themselves with known biohazards specific to the species under study and with the procedures to avoid exposure to these agents.

Personnel should work in teams of at least two people in the field, especially when involved in physical or chemical restraint and handling of animals or other high-risk situations.

If an animal becomes difficult to handle safely, the handler should release the animal if it is safe to do so. Additional restraint, chemical or physical, may be needed to adequately control the animal safely.

Keep an open route of escape when working with animals.

Personnel may need to wear protective clothing including protective footwear with non-slip soles, sturdy clothing (e.g., long-sleeve shirts, long trousers, plastic aprons, etc.), gloves, and face masks. The appropriate protective clothing depends on site and species-specific field conditions.

Field workers should be trained in the tasks and safety procedures relevant to the animal capture and handling activities, including how to avoid injury from equipment or animals and how to avoid exposure to potential pathogens. They should also be trained to avoid transmission of human pathogens to captured animals.

Special considerations:

Individuals with known allergies associated with animals, with immune deficiency diseases, or on immunosuppressant therapy, should not engage in studies involving the handling of wild animals.

Certain animals, including but not limited to bats, dogs, and non-human primates, are known to harbor disease agents considered to be deadly to humans such as rabies. Any capture and manipulation of these animals warrant that the handlers wear double gloves, catching gloves (if necessary), and immediately report any bites or exchange of blood/fluids to supervisors, field coordinator, or medical professionals if present in the area. Preventative vaccination is recommended.

General Guidelines on Safe Handling of Animals

Supervising veterinarians and other trained PREDICT staff will handle animals as part of surveillance activities and sampling fieldwork. Staff must be trained on the potential hazards and safe handling techniques for the specific types of animals they are likely to handle. Animal hazards may include injuries due to sudden animal movements, bites and scratches, and exposure to zoonotic pathogens. The following precautions should be considered for the safe handling of animals:

Handlers should have a basic understanding of the animal's typical behavior.

All animal handlers should be trained in basic animal handling techniques and those techniques should be used consistently. Improvements to techniques should be tested and implemented when available.

Generally, slow and deliberate movements should be used around animals.

Animal behavior can be unpredictable. Therefore, personnel should remain constantly alert when handling wild animals. Personnel should watch for warning signs of animal aggressiveness and fear. These signs vary with animal species and may include vocalizations, raised fur, flattened ears, twitching tails, or bared teeth. An animal that feels threatened or cornered may be more aggressive than under normal circumstances.

Extra caution should be used when handling animals that are sick, hurt, or are new mothers or highly territorial species.

If capture and sampling procedures may cause pain, animals should be handled safely and humanely under the supervision of a veterinarian trained in wild animal chemical immobilization and animal restraint devices.

Workers should use extreme caution when giving injections and handling sharps around animals; sudden animal movements could cause a needle stick injury to the personnel or injury to the animal's vital organs.

Investigators should be aware of the potential for human pathogen transmission to wildlife and ensure adequate use of protective equipment to avoid exposing animals. In addition, sick workers should not be allowed to participate in animal handling.

Safe Operation of Equipment

All personnel involved in wildlife capture should have current training in the use of pertinent equipment, including but not limited to different kind of traps, nets, and snare poles (rabies-poles used for capture and restraint. Specific on appropriate capture and handling of PREDICT target species are described in the taxa-specific PREDICT protocols. Overall field team knowledge on the correct use of equipment will help to minimize injury due to accident or the misuse of equipment during animal capture and handling. Use of drug delivery equipment, such as dart rifles, dart pistols and darts, jab-sticks (pole-syringes) must be done under the supervision of a veterinarian trained in wild animal physical and chemical restraint.

Safe Use of Anesthetic Drugs and Other Chemicals

Capture of free-ranging wildlife may place personnel at risk of injury and anesthesia or other forms of chemical immobilization may be necessary. All use of drugs or chemicals must be done other the supervision of a veterinarian trained in wild animal physical and chemical restraint. Injury can occur, not only from animal attacks, but also capture equipment, or exposure to potent drugs. Every possible effort must be made to minimize the probability of human injury when undertaking chemical restraint and/or anesthesia of wildlife. The following precautions should be followed when using anesthetic drugs or other chemicals:

The risks involved in using drugs for the capture and immobilization of wildlife must be identified and communicated to all project personnel.

At least two people on the team should be trained in first aid and cardio-pulmonary resuscitation (CPR) (one of the two should be a person NOT in charge of handling anesthetics). First aid or CPR may be required in an accidental drug exposure emergency. A well-stocked first aid kit customized for each project should be kept within easy reach during fieldwork activities.

An evacuation plan for an anesthetic drug related accident should be developed and communicated to all field personnel. Local medical authorities should be informed of the

potential hazards of the field work and an evacuation plan to medical facilities should be discussed prior to beginning fieldwork.

All drugs and chemicals used in field research should be handled in such a way as to prevent human exposure. Researchers or personnel authorized to use immobilization drugs should protect themselves against eye, respiratory and cutaneous exposure to drugs and chemicals and accidental injection. The use of gloves, long-sleeve clothing, and protective goggles and/or face-shields may be indicated in some cases.

Those utilizing immobilization drugs for restraint of wild animals should have the appropriate training and information available to aid in their medical care should accidental contamination occur. It is advisable to always have drug manufacturer information for all medications in use as some human emergency facilities will not be familiar with drugs used for the immobilization of wildlife particularly in countries where such drugs are not available commercially.

It is recommended to work in pairs when utilizing highly potent drugs, so that there is constant monitoring of the person handling anesthetics. When anesthesia drugs being used have an appropriate reversal agent, both people should carry a full dose of reversal drugs (for human treatment) at all times. There should be adequate quantities (for human treatment) of reversal drugs on hand in the field if these exist. Potent anesthetic drugs should be handled only by trained veterinarians.

When darts are used to restrain animals, every reasonable attempt should be made to recover all darts that miss the target animal as they contain chemicals that could pose a public or animal health risk. As with syringes used to draw up medications, darts should be placed in a special container to avoid accidental exposure to personnel.

National and local regulations with regard to drugs, specialized equipment (rifles, pistols), and liability issues concerning medical treatment of humans and/or human well-being should be clearly understood and followed before fieldwork begins.

Biohazards and Zoonotic Diseases

Investigators and field workers are at risk of exposure to zoonotic diseases (diseases transmitted from animals to humans). Pathogen transmission may occur through direct contact with contaminated dirty hands and equipment, bites and other direct exposure to animal fluids (blood, urine, saliva) or inhalation of contaminated dusts. Investigators and supervisors should familiarize themselves with known biohazards specific to the species under study and with the procedures to avoid exposure to these agents as detailed in the taxa-specific protocols.

Prior to fieldwork, the Country Coordinator or field supervisors should provide training and information regarding all potentially hazardous biological or zoonotic agents that may be encountered in the field situation or that are relevant to the species under study.

Personnel must wear the specified PPE, as indicated for each taxonomic group. Additional PPE could be warranted given certain field situations, and or as determined by the PREDICT field supervisor. PPE is required to prevent bites or scratches. Protective eyewear and respirators is also needed to prevent exposure to pathogens transmitted by splashing of body fluids or secretions, or inhalation of contaminated aerosols.

The Country Coordinator or field supervisor must ensure that safety procedures are established for the conduct of postmortem examination in the field and that appropriate protective equipment (e.g. aprons, gloves, face-masks, eye protection, etc.) is available and used correctly. The Country Coordinator is responsible for ensuring that all personnel be trained in the postmortem techniques appropriate for the species.

Field workers should wash hands or use disinfecting hand wipes frequently before and especially after animal capture and field activities. Frequent handwashing is the best defense against diseases transmitted through contact with contaminated animal saliva, other body fluids and wastes.

If injured by an animal or potentially exposed to a diseased animal, workers should immediately report to their supervisor and/or coordinator and seek the appropriate medical attention and follow-up. See ***Emergency Preparedness*** for detailed instructions and accident reporting forms.

Field personnel should also take precautions (i.e. long sleeve shirts, insect spray, etc.) to avoid exposure to external animal parasites such as ticks, fleas, as well as to animal feces that may contain internal animal parasites (ova or larvae) infective to humans.

If personnel become sick or show unusual symptoms they should immediately report this to the supervisor and should contact medical authorities knowledgeable about the diseases and parasites in the region. Field staff should discuss animal and field exposures and potential zoonotic hazards with their medical care staff.

Immunizations and Pre-Exposure Screenings

The Country Coordinator or field supervisor should ensure that personnel have consulted with a human health worker with regard to the immunizations required prior to participating in fieldwork that involves handling animals. See ***Emergency Preparedness*** for details and immunization forms.

Vaccines and immunizations will vary depending on the geographical area, animal species to be handled, and personal medical history. Only a human health professional can provide vaccination and immunizations to the staff.

Due to the significant risks of working with wild mammals (bats, rodents, etc.), field personnel should be required to receive pre-exposure rabies vaccination (before starting any field project)

for themselves. Tetanus immunization should also be required for all staff that will have any contact with wild animals.

Pre-exposure screening for tuberculosis is required for personnel that will be handling non-human primates. Tuberculosis screening and interpretation of results should only be conducted by a human health professional.

Training Records

A record must be kept of all training given to PREDICT personnel and reported into the EIDITH system for tracking training activities. For each training activity (on-the-job, self-study, small group training, workshop) the following information should be reported into the EIDITH system for tracking training activities for training forms and directions on how to enter data into EIDITH):

- Date of training
- Type of training (workshop, on-the-job, simulation, field or lab training)
- Location of training (town and country)
- Topics covered
- Instructor(s)
- Trainees' names, gender, phone, email, title, organization, and sector

Country Coordinators and field supervisors should maintain a record (on the PREDICT report form) of any injuries or illnesses incurred while handling wildlife (whether in the field or laboratory). Such information should accompany the individual when examination or treatment by a medical practitioner is needed. See ***Emergency Preparedness*** for accident reporting forms.

Applicable local regulations regarding the documentation and reporting of workplace injuries should be consulted and followed.

Field supervisors should also maintain a record and pertinent product information of all immobilization drugs in their possession and as well as their usage.

Section 2. Principles and Guidelines for Animal Care and Safety during Capture

All appropriate measures should be taken to minimize injury or harm to animals during capture and handling. Animals can sustain injuries or develop pathologic conditions during capture that could put their life in danger or decrease their chances of survival in the wild. Appropriate handling and restraint techniques should be used, and training in how to apply them should be provided to avoid injury to animals.

The investigator also must ensure that all workers fully understand and are trained in the techniques to be used for restraint and handling of wild animals. Improperly trained individuals or improperly applied techniques could harm the animal during capture and handling. Capture and handling can be accomplished using physical or chemical restraint or a combination of both.

Several factors must be considered to determine what type of restraint will be used on a specific project:

- Animal species and condition (sick, stressed, nursing, etc.)
- Safety for the staff to carry out the capture
- Animal safety
- Feasibility of accomplishing the capture's objective with the type of restraint
- Availability of drugs and specialized equipment to carry out the capture procedure
- Ability to protect, observe, and assist an animal until it has fully recovered after the procedure

Physical Restraint

Physical restraint may be most appropriate for some species and/or for short procedures. Animal handlers must ensure that physical restraint is performed in such a way that the animal will not suffer excessive stress or any injury during the process.

Physical restraint should be accomplished with necessary PPE, including latex or nitrile gloves and specialized equipment such as leather gloves, nets, rabies-poles, as needed for the species being handled. The capture team must be capable of correctly using and operating such equipment to avoid animal and human injuries during capture.

At a minimum, disposable gloves must be worn during handling and during the operation of specialized equipment. Researchers must be trained and capable of using and operating all equipment used for capture to avoid injuring the animal. When using leather gloves to restrain an animal, the operator must ensure that excessive pressure is not applied to avoid suffocating the animal. When using nets to capture wild animals, removal from the net should occur immediately to avoid further entanglement and possible fatal and non-fatal injuries to the animal.

The risk of causing trauma to an animal when using a snare or rabies-pole is high, thus the benefits should outweigh the risks when considering their use. If using a snare-pole, it is important that the snare be placed around the neck AND one of the front legs of the animal to prevent the risk of asphyxiation.

In certain cases and with species that are vulnerable to stress, chemical restraint may be more appropriate.

Chemical Restraint

Chemical restraint should be considered when physical restraint is not safe for either the personnel or the animal being captured. Chemical restraint should be performed by veterinary professional with previous experience and specialized training in the use of anesthetic drugs and field anesthesia procedures.

The following considerations should be taken into account when deciding to chemically restrain an animal:

Considerations in Drug Selection

- Investigators should be familiar with the different drugs and drug combinations that can be used to safely capture a particular wild animal species.
- All drugs have intrinsic cardiovascular effects. The supervising veterinarian must be familiar with these effects and how to respond to any complications caused by these effects.
- Supervising veterinarians must be aware of any contraindication for the use of particular drugs on target species under the existing conditions.
- Investigators and supervising veterinarians should be aware of the availability (or lack of) of certain anesthetic drugs (and their reversal agents) and the regulations in place to import them into the country.

Considerations in Drug Administration

- During field captures, drugs that are often administered by injection can be administered via hand injection, pole-syringe (manual, spring-loaded) or darts (dartgun or blow darts).
- Staff should be familiar with the use of equipment (darts, pole-syringes) to avoid harming the animal during drug administration and immobilization.
- When hand injection is elected, the correct size needle should be used to avoid excessive trauma to the muscle or a penetrating wound in vital organs.
- Staff using darts to administer drugs should be trained in the use of the equipment required for the species and field setting. In general, only animals >15kg should be darted with powered dart-delivery equipment (i.e. pistols, guns).
- Darts have the potential to cause trauma if projected with excessive force or injected into a non-targeted area. Practice is the best way to assure that appropriate force is used when darting an animal.

Sites of Injection

- Anesthetic drugs should be injected into large muscle masses.
- Excessive force should be avoided when firing a dart as this could result in broken bones or perforation.
- Darts placed into the abdominal or thoracic areas are potentially fatal for the animal. Personnel should take great care to avoid placing a dart into one of these body areas.

Monitoring Anesthesia

- Anesthesia should be kept as brief as possible to minimize risks.
- When an animal is under chemical restraint, it should be monitored constantly to detect possible complications during anesthesia.
- An anesthetized animal should never be left unattended.
- Anesthetized animals cannot regulate their body temperature. Measures should be taken to prevent hyper- or hypothermia.
- Monitoring should include at least temperature, heart rate, respiratory rate and partial saturation of oxygen using a portable pulse-oximeter.
- The staff should be trained to respond properly to any emergency or complication occurring during anesthesia (e.g., how to treat hyperthermia), and appropriate reversal/termination of anesthesia to avoid complications.

Recovery

- Animals must be released only once fully recovered from anesthesia .
- Animals should be allowed to recover in safe areas, away from hazards and areas where potential predators or aggressive con-specifics may be present.
- Once released, the animal must be observed for as long as is required to ensure it is awake, alert, and active
- If the animal suffers an injury during capture, the injuries should be treated appropriately before releasing the animal.
- If the injury sustained is life-threatening or will render the animal incapable of surviving in the wild, humane euthanasia should be considered. In some cases placing injured animals in rehabilitation facilities might be an option.

Note: A wealth of knowledge and expertise, as well as additional training materials are available within the PREDICT consortium. Staff should raise any concerns or questions regarding procedures for safe and ethical animal capture and handling to their partner surveillance leads. Regular surveillance team operational meetings are held to address questions or concerns encountered during surveillance activities and facilitate cross-partner distribution of knowledge and best practices in safe animal capture and handling.

Section 3. Safe Animal Capture Guide Checklist

Procedures Checklist: Personnel working in the field with wild animals should follow these basic personal procedures:

- Coordinators should provide all personnel a “Useful Contacts” list with address and numbers of local medical and emergency response services.
- Researchers working with wild mammals should consider pre-exposure rabies vaccination.
- Rabies vaccination should be given to personnel who routinely handle high-risk species in the wild (bats, raccoons, etc).
- Researchers and their assistants should also consider vaccination against tetanus in those situations where exposure to this pathogen is possible.
- Individuals who are exposed to potential vectors of rabies (e.g. animals with neurological signs) should immediately report the exposure to medical authorities and the supervisor.
- All animal tissues, fluids, and excrement should be handled so that the potential for human contact is minimized.
- Staff should thoroughly wash and/or sanitize hands and any other contaminated skin surfaces with a germicidal skin cleanser immediately after handling wild animals or their samples.
- All personnel handling wild animals should practice good hygiene and avoid rubbing their eyes after animal handling.
- Appropriate planning and specific precautions (trained staff, equipment and tools in good working condition, PPE, etc.) should be taken in order to prevent injuries from bites, scratches and skin punctures from wild animals. Even minor wounds or scrapes may become infected and can potentially result in disease transmission.
- If an injury occurs, clean the wound with a disinfectant and immediately contact a coordinator/supervisor.
- Where there is a risk from aerosolized pathogens from saliva, feces or urine, protective gear such as gloves, eye protection, respiratory protection (masks, face-shields or respirators), foot protection and protective clothing should be used as necessary.
- Researchers should always wear gloves and facemask when handling sick or dead animals.
- Personnel performing post-mortem examinations in the field should wear at least a plastic apron, gloves and facemask or goggles.
- After any post-mortem examination is performed, staff should wash and disinfect hands and any other contaminated skin surface.
- All contaminated equipment should be cleaned and disinfected immediately after use while still wearing the appropriate PPE. Disposable used equipment must be adequately disposed of on site (i.e. buried, burnt, etc).
- All drug containers, needles, scalpel blades, suture needles and other sharp instruments should be used and disposed of in a manner that prevents accidental human injury.
- Physical restraint of wild animals should be kept as brief as possible.
- Care should be exercised when using equipment such as nets, gloves, rabies-pole, etc to capture wild animals.
- Staff should be familiar with dart equipment, sites of injection and drugs when chemical restraint is elected.
- Anesthesia monitoring equipment and emergency drugs must be available and staff should be familiar with their use.
- Staff will make sure each animal is fully recovered from anesthesia prior to release.
- A list of the equipment and supplies needed to correctly implement the recommendations of this Safety Guide for Animal Capture for Sampling is available and checked prior to departing for the field.

Checklist for Supplies for Animal Capture Activities

Check as appropriate:

PPE

- Nitrile (recommended) gloves
- Leather or kevlar gloves
- Face-mask
- Respirator
- Goggles
- Face-shield
- Disposable (Tyvek) suit
- Sharp-container
- Closed-toed shoes

Monitoring

- Thermometer
- Stethoscope
- Stopwatch or other timing device
- Pulse oxymeter with probes
- Penlight
- Warm-water bottles (to prevent hypothermia)
- Buckets or water bottles (to prevent hyperthermia)

Immobilization equipment

- Dart equipment (rifle/pistol/blowpipe, CO₂ or powder cartridges, and dart protectors)
- Drugs (sedatives, tranquilizers, anesthetic drugs, reversals or antagonists)
- Calculator to use for calculating drug dosages
- Darts and dart needles
- Ropes/hobbles
- Nets
- Pole-syringe
- Cargo-net
- Blindfold
- Ear-plugs
- Carrying bags
- Syringes and needles
- Towels
- Snare-pole

Emergency

- Emergency medications (doxapram/ atropine/epinephrine/diazepam)
- IV catheters
- Fluids (NaCl, lactated Ringer's, Dextrose)
- IV administration set
- Antibiotics, disinfectants
- Tongue swabs
- Vet wrap and tape
- Flashlight
- Minor surgery (and suture) pack
- Euthanasia solution
- Alcohol (to treat hyperthermia)
- Tissue glue or super-glue

- Blanket/towel (to help treat hypothermia)
- Cold pack/hot pack
- Laryngoscope, tracheal tubes, ambu bag

Recovery and release

- Crates/containers in which to place animal during recovery
- Binoculars

Section 4. References

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Small Carnivore Sampling Methods

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Objective: To safely collect biological samples from live and dead small carnivores.

Section 1. Brief Overview of PPE

Minimum PPE Required for Handling Small Carnivores

The minimum PPE for sampling small carnivores includes:

- Designated clothing
- Nitrile gloves
- Protective glasses
- N95 facemask for self-protection and to avoid contaminating samples

(See the ***Biosafety and PPE Guide*** for detailed instructions regarding PPE Use)

Note: *In order to protect both human handlers and sampled small carnivores, all personnel handling small carnivores should be vaccinated against rabies beforehand as described in the following section.*

Section 2. Special Considerations for Handling Small Carnivores

This section supplements ***Safe Animal Capture and Sampling***, with which anyone handling animals is expected to already be thoroughly familiar. Note also that sampling from dead animals, whether destined for bushmeat or not, is also covered in ***Bushmeat Sampling Methods***. However, for completeness, much of that protocol is repeated here.

Handling small carnivores involves a number of special considerations:

1. Zoonotic diseases
2. Staff vaccinations and medical concerns
3. Other hazards (bites and scratches)
4. Capture and handling

1. Zoonotic Diseases

There are numerous zoonotic diseases that may be transmitted from small carnivores to humans. Here we highlight the most important diseases that are a risk to human handlers. Research teams should be familiar with additional zoonoses that may be present in their target and bycatch species and geographic areas. It is recommended that immunocompromised people not work directly with live or dead animals.

Rabies is endemic in many carnivore populations, and may show up sporadically in any carnivore. Therefore, anyone who expects to handle live or dead carnivores should be appropriately vaccinated. Clinical signs of rabies are quite variable and can include any neurologic abnormality; this includes depressed mentation, which is a general sign displayed by nearly all sick or injured animals. When handling a carnivore, the potential for rabies should be

assumed and appropriate precautions taken. Most zoonotic exposure is via bite wounds from live carnivores. However, exposure to infected bodily fluids (particularly saliva, blood and cerebrospinal fluid) must be considered when collecting samples from dead carnivores. Rabies is not transmitted by casual contact. Many other viruses can also be zoonotic.

Leptospirosis is a bacterial disease that affects a wide variety of mammals. It causes septicemia, kidney, liver, pulmonary, and/or reproductive dysfunction and is spread by contaminated water and urine. Gloves and good hygienic practices (i.e., thorough hand-washing) should provide protection.

Salmonella spp. are bacteria that can cause severe disease in humans and carnivores, but can also be carried by these species asymptotically. *Salmonella* can cause septicemia as well as diarrhea, and all carnivores are potential carriers. Special care should be taken when working with animals, live or dead, that have evidence of diarrhea. *Salmonella* spp. are most likely to be shed in the feces, but fur, traps, and vegetation where animals are held can all become contaminated. For that reason, gloves should be worn while handling animals and while cleaning/handling traps and cages in which animals have been captured or housed.

Mycobacteria bovis, the cause of bovine tuberculosis, is a bacterium sometimes found in carnivores that prey on species in which *M. bovis* is endemic. The most likely exposure for staff would be during necropsy, so gloves and mask should be worn during necropsies of carnivores in endemic areas (i.e., East Africa). This disease is of special concern for those who are immunosuppressed, but it can cause disease in immunocompetent individuals as well.

Echinococcus spp., a cestode parasite of canids and their mammalian prey, causes disease in humans when the eggs are ingested. Infective eggs are shed in the feces by the carnivore definitive hosts, which includes domestic dogs as well as wolves, foxes, jackals, lions, and sometimes other canids and felids. The disease is caused by large cysts formed by the larvae; cysts are most commonly found in the liver and lungs. For prevention, gloves should be worn and hands should be thoroughly washed after handling carnivores or items that could be contaminated with carnivore feces.

Parasites, both external and internal, may be transmitted from carnivores to humans. Internal parasites are most commonly transmitted via the feces, so precautions taken for salmonellosis should also protect against these organisms. External parasites, such as *Sarcoptes* mites (the cause of mange) and fleas, causing dermatitis, may also transmit pathogens (e.g., fleas may carry *Yersinia pestis*, the causative agent of plague). Wearing gloves, long sleeves and pants while handling carnivores can help prevent transmission of parasites. When performing necropsies on heavily infested carcasses, the carcass may be dusted with an acaricide prior to handling.

A variety of other pathogens may be transmitted from wild carnivores to humans, and risk of exposure varies by geography and other factors. Wearing gloves, good hand washing protocols, and common sense hygienic practices will protect against transmission of most pathogens.

Personnel working with animals should always wash their hands thoroughly before eating, drinking, using tobacco products, or any other activity that involves touching the face or mucus membranes.

2. Staff vaccinations and medical concerns

All those working with carnivores should be vaccinated against rabies, ensure that they have a protective titer, and be aware of appropriate post exposure prophylaxis in the case of bites.

3. Bites and scratches

With few exceptions, carnivores are predators and have formidable weapons. Small carnivores can inflict powerful bites that cause massive tissue damage and inoculate bacteria deep into tissue. Most carnivores have claws on all four feet and felids, especially, can do severe damage with their claws. Small carnivores such as civets, otters and mongooses should not be underestimated; they are remarkably agile and cannot be safely sampled without chemical restraint. No one should attempt to work with larger carnivores without proper training and a healthy respect for the risk in working with these species.

4. Capture and handling

Most carnivores are captured with traps, such as box traps, foothold traps, or snares. All traps, regardless of padding or safety features, pose some degree of hazard to the animal. Carnivores are capable of severe self-injury in any type of trap; they can break canine teeth or tear off claws in box traps, or chew off limbs caught in foothold traps. Snares set for large animals have obvious risks for smaller carnivores. For these reasons, traps should be prepared carefully, in good working order, and checked frequently, at least every 12 hours. Veterinary staff should be prepared to deal with a variety of trap injuries, including lacerations, broken teeth, and crush injuries.

Many carnivores captured in footholds or snares can be dangerous to personnel. Trapped animals should be approached carefully. Most species will need to be chemically immobilized by blowdart or pole syringe, but some smaller animals (such as some jackal species) can be handled with manual restraint and tools such as gloves and nets. Animals in box traps can be hand-injected or pole-syringed. Ketamine hydrochloride combined with a tranquilizer (such as a benzodiazepine or an alpha-2 agonist), or Tiletamine-Zolazepam (Telazol, Zoletil) are the mainstay of carnivore immobilization protocols, but many protocols have been used and protocol success varies by species. Appropriate protocols for individual species can be found in various publications such as Kreeger, Fowler, Fowler and Miller, Nielsen, and West, Heard, and Caulkett (See References Section). Chemically immobilized animals should be housed in a trap or cage until they are fully recovered from anesthesia to prevent injury or death from drowning, falls, predation, or intraspecific aggression. While many protocols include one or more reversible drugs, neither ketamine nor the tiletamine component of Telazol are reversible, so protocols containing either of these drugs are **not** fully reversible. Therefore, giving a reversal agent is not a guarantee of an immediately awake and aware animal. Animals should be observed carefully to ensure that they are fully recovered before they are released, keeping in mind that a reactive

animal is not necessarily a completely awake animal. Animals are typically ready for release when they can hold their head steady and follow movements with their eyes.

Care should be taken to decrease the stress experienced by animals prior to and during immobilization. This includes keeping your distance from the captured animal, speaking in soft voices and minimizing noise, and covering box traps with a towel or tarp to decrease visual stimuli. Similarly, anesthetized animals should have their eyes covered with a towel or cloth. It is especially critical that immediately after initial drug administration, the animal is free (to the extent possible) of visual and auditory stimuli; the quality of the induction often impacts the whole anesthetic event. During recovery, keep the caged animal within view, but at a great enough distance that the animal does not feel threatened by your presence. As always, noise should be kept to a minimum.

Anesthetized animals should be monitored regularly during recovery until they can no longer be safely handled, at which point they should be confined in a trap or cage. Essential monitoring includes measuring and recording heart rate, respiratory rate, body temperature, mucous membrane color, and pulse quality every 5-10 minutes throughout the procedure. The eyes should be lubricated with a bland ophthalmic ointment and protected from debris. Animals should be kept out of direct sunlight and overheated animals ($>105^{\circ}\text{F}/40.6^{\circ}\text{C}$) should be cooled by placing rubbing alcohol on their paws, administering cool subcutaneous fluids, or wetting their fur. Cold animals ($<100^{\circ}\text{F}/37.8^{\circ}\text{C}$) should be kept warm using tarps, blankets, or warm SQ fluids. Emergency drugs, appropriate-sized endotracheal tubes, and a mechanism for providing positive pressure ventilation (i.e., Ambu bag) should be available whenever animals are immobilized.

Section 3. Small Carnivore Data Collection

Please refer to the three required data collection templates for data to collect. These include:

1. P2 Animal Data Collection Form
2. P2 Site Characterization Data Collection Form
3. P2 Specimen Data Collection Form

In some cases time constraints, anesthetic risk, inability to prolong immobilization, or other factors may necessitate prioritizing biological sample collection at the expense of collecting any physical measurements. At a minimum:

1. Measure and record the animal's mass (kg) initially as this can be important for proper drug dosing or emergency interventions.
2. Conduct a cursory physical exam before sampling in order to note any wounds or major abnormalities and to protect the health of both handler and animal.
3. Ensure the animal's parameters (heart and respiration rates, body temperature, etc.) are adequate for continuation of procedures. If they are not, attempts to correct them should be made and reversal of anesthesia considered if the animal's life is at risk.

Additional (Optimal) Data to Collect from Small Carnivores

The P2 data templates mentioned above are required to be filled in. Additional data and biometric measurements may be collected at the discretion of the sampling party.

Ideally, the following additional data should be collected from any small carnivores that are processed for PREDICT:

1. Body mass (weight)

Body weight: Although in an ideal world the body weight of an animal would be measured prior to drug dose calculation, because of the dangerous nature of carnivores this is rarely possible in field conditions. Weight ranges of the target (and likely non-target) species should be known before capture is attempted, and the veterinarian or biologist should have experience estimating weights. Animals should be weighed (g or kg) in bags, slings, or a suitable container using a calibrated hanging spring. (Note: If an animal exceeds the limit of spring scales, two or more scales can be linked (one hanging from the other) to distribute the weight. The total weight is the measure of both scales added together). Scales should be zeroed (checked to make sure they measure '0.0' units when empty). If scales are not available or accurate weights cannot be measured for any other reason, a weight should still be estimated but the recording sheet MUST note that it is an estimated and not a measured weight.

2. Age class (see below)

Age class: Usually the exact age will not be known. Individuals should be placed into one of the following age classes:

Age Class	Description
Juvenile	Dependent young, likely unweaned
Subadult	Animal is fully independent, appears to be sexually mature, but not fully physically mature (e.g., less than full adult size).
Adult	Animal has secondary sexual characteristics, adult size, sexually mature.
Old Adult	Adult showing signs of age degeneration (i.e. tooth wear)

3. Species Identification and Sex Determination (and reproductive status if adult female)

Species identification and sex determination: Based on morphology and unique characteristics, identify animal to genus and species (where possible) and sex. If dependent offspring are captured along with their mothers, they should be kept confined while their mother is anesthetized to prevent them from wandering off. Reintroduce them only when the mother is fully recovered.

4. Whole body photograph(s)

Photographs: At a minimum, the following digital photographs should be taken of each individual:

- a. Right and left lateral views while animal is recumbent.
- b. Full anterior facial view.
- c. Full lateral facial/head view.
- d. Views of full upper and lower dentition (which can help determine/verify age and sex).
- e. Views of any lesions (e.g., cuts, scratches), physical abnormalities (e.g., missing toes), or individually identifying marks or characteristics (e.g., healed scars, abnormal coloration, torn ears, etc.)

5. Morphometric measurements

Body measurements: Time required for collecting the biometrics (in cm/mm) should be recorded with the minimum standard mammal measurements (all linear) as follows:

- a. Head and body length (measured dorsally and linearly from tip of nose to base of tail when head is stretched and aligned with back).
- b. Tail length (from base to tip).
- c. Hind foot length (heel to tip of longest toe- exclude nail and note which toe).
- d. Tibia length ('knee to ankle').
- e. Hind foot (tarsal) length
- f. Ear length- base of the notch below the ear opening (lower rim of external auditory canal = meatus) to the most distant point of the margin of the pinna.

Additional Optional Measurements

Head length, trunk height, hip breadth, hand length and breadth, foot breadth, limb segments (thigh, lower leg, upper arm, forearm).

Chest circumference, abdominal circumference, and cranial circumference (at or above brow).

Section 4. Small Carnivore Sampling

Capturing, trapping, darting, and immobilizing small carnivores should only be performed by experienced and skilled staff.

PREDICT personnel are expected to have detailed capture/immobilization protocols (and recording sheets, monitoring sheets, etc.) for any target small carnivore species. This sampling guide assumes a starting point of either a safely immobilized or an already dead small carnivore.

In addition to the standard PREDICT sampling and analyses, PREDICT partners are encouraged to collect additional samples and pursue routine diagnostics (e.g., blood counts and chemistries,

urinalysis, etc.) where resources allow. Opportunities to collect biological samples and related health data from wild animals are uncommon and maximizing these opportunities can further advance wildlife health knowledge.

The following basic set of samples should be collected from each animal where possible (If only one sample can be collected, then place into VTM):

1. **Two oral swabs** - one in 500 μ L VTM and one in 500 μ L Trizol
2. **Two fecal samples** - one with max of 500 μ L/0.5cc feces in 500 μ L VTM and one with max of 500 μ L/0.5cc feces in 1 mL Trizol
Or
Two rectal swabs - one in 500 μ L VTM and one in 500 μ L Trizol
3. **Two whole blood samples** - one with max of 500 μ L of whole blood in 500 μ L VTM and one with max of 500 μ L of whole blood in 500 μ L Trizol
4. **Two serum samples** - 2 x 0.5 ml aliquots frozen without media
5. **Two urogenital swabs/urine samples** – one in 500 μ L VTM and one in 500 μ L Trizol

***Note:** If animals are too small to collect two blood tubes (for whole blood and serum), collect serum and save remaining clot in 500 μ L VTM after serum separation.*

Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab.

If there is no **short-term** access (i.e., within 24 hours) to cold chain such as in an emergency situation then samples can be collected in 500 μ L of RNAlater instead of Trizol and VTM. Storage times and temperatures for samples in RNAlater are as follows:

1. 1 day at 37 °C (i.e., ambient temp)
2. 1 week in the refrigerator
3. Within one week freeze at -80 °C for storage until analysis

Sample Labeling

Tubes must be labeled with a unique specimen ID per Animal/specimen labeling guide.

Sample Collection from Live Small Carnivores

In most cases, live small carnivores should be chemically restrained for handling. At least two, and preferably three people are required for these manipulations: one person to monitor the animal, one to take samples, and a third to manage the tubes and record data.

1. **Two oral swabs in VTM and Trizol** (if only one is collected, place sample in VTM): Using sterile, polyester-tipped swabs with a plastic shaft, rub the swab tip gently but thoroughly against the back of the animal's throat, saturating the swab with saliva.

Place 1 swab in a cryovial filled with 500 μ l of VTM and use a flame-sterilized scissors to cut the shaft of the swab above the tip. Place the other swab into 500 μ L of Trizol in another cryovial and cut shaft as above. [Note: If the plastic shaft can be snapped, then scissors are not necessary and the risk of cross-contamination is reduced. To snap the swab, lift the swab a little above the bottom of the vial then snap it. This will ensure the swab will not block the cap].

Mix each tube well. Store both cryovials in a liquid nitrogen dry shipper or dewar and transfer to -80°C freezer when possible.

2. Fecal samples

500 μ L or pea-sized piece of feces (200 mg) in VTM and Trizol: Collect either excreted feces, or if animal is large enough (> 1 kg) use a gloved, lubricated (saline or medical lubricant) finger to collect feces directly from rectum. Place two ~200 mg (pea size) samples of fresh feces into 2 vials, one containing 1 mL Trizol (= maximum final ratio of 1:2) and one containing 500 μ L VTM (= maximum final ratio of 1:1). Homogenize by shaking. Freeze in dry shipper or dewar with liquid nitrogen and transfer to -80°C freezer when possible.

If feces are not available, collect **2 rectal swabs, one in VTM and one in Trizol:** Gently insert one sterile swab tip at a time into the animal's rectum. [Note: DO NOT USE TRIZOL AS A LUBRICANT – IT IS HIGHLY IRRITATING TO TISSUE.] Place one swab in a cryovial filled with 500 μ L of VTM using a flame-sterilized scissors to cut the shaft of the swab above the tip. Place the other swab into a tube with 500 μ L of Trizol. Store in a dewar or dry shipper with liquid nitrogen dry shipper and transfer to -80°C freezer when possible.

3. Whole blood and serum samples

Precautions

- **At least one person present should have previous experience in small carnivore venipuncture to avoid injury to the animal.**
- Animals should be immobilized using either injectable or gas anesthesia according to appropriate guidelines. On occasion some species may be manually restrained for venipuncture but extra care must be taken to avoid injuries to personnel and animals.
- The person restraining the animal is responsible for monitoring respiration and other vital signs and communicating the status of the animal appropriately.
- No more than 1 ml of blood per 100 g (= 10 ml/kg or 1%) of body weight should be collected at any one time; it is best to limit collection to 0.6 ml blood per 100g.
- Blood should always be considered highly infectious and hazardous.

Collection Procedure

1. Select appropriate venipuncture site:

Animal family	Venipuncture site
----------------------	--------------------------

Felids

Medial saphenous vein: With compression of the inner thigh, this vein can be prominent and superficial, but often collapses during collection.

Use of a butterfly needle and extension set may help avoid this problem, as well as using a smaller syringe and pulling back slowly on the plunger.

Cephalic vein: In larger species the cephalic vein might be large enough for safe blood collection.

Jugular vein: This may be the only option in very small animals and must be accessed carefully.

Lateral tail vein: This may be accessed in larger felids; the same comments as for the medial saphenous vein apply.

Canids and Hyenids

Lateral saphenous vein

Jugular vein

Cephalic vein

Femoral vein: It is easy to hit the femoral artery instead; if this happens, be sure to apply firm, direct pressure for several minutes to effect hemostasis

Medial saphenous vein: May be accessible in some species

Mustelids

Jugular vein: Due to their short muscular necks, this can be a difficult vein to access. Placing pressure in the thoracic inlet bilaterally often helps to occlude this vein and help it pop up.

Femoral/saphenous vein: These tend to be short and difficult to hit, and the femoral artery can be accidentally hit as in canids and hyenids.

Viverrids and Herpestids

Jugular vein: Best option for adequate samples

Cephalic: Small volumes

Tail (ventral midline): Small volumes

Ursids

Cephalic vein

Saphenous vein

Jugular vein

Note: When using an alpha-2 agonist in a chemical immobilization protocol, the peripheral veins often collapse due to vasoconstriction. Using the jugular vein will often be necessary for venipuncture.

2. Select appropriate sized needle and syringe (or vacutainer) for the size of the animal.
3. Disinfect the site with iodine solution or alcohol.
4. Collect sample.
5. **Do not recap needle.**
6. Apply pressure to site of bleeding using a cotton ball or gauze pad until bleeding ceases (approximately 1 minute).
7. Process blood (see below).
8. Properly dispose of sharps and other biohazard materials immediately upon transfer of sample to collection vials.

Blood Processing

- a. **Whole blood in EDTA:** Collect 1 tube of whole blood in EDTA (lavender top vacutainer). Add up to 500 μ L of whole blood into 2 vials, one containing 500 μ L Trizol and one containing 500 μ L VTM (= maximum final ratio of 1:1) and mix each vial well. Place vials into liquid nitrogen in dry shipper or dewar and transfer to -80°C freezer when possible.
- b. **Aliquot serum into cryovials:** Collect blood into a serum tube (red top or tiger top, if >1 mL blood is collected, or into 1.5 mL conical Eppendorf tubes). Place labeled blood tubes in a rack on ice (optimally) for up to 2 hours prior to centrifuging. Centrifuge the blood samples. If a centrifuge is not available, red top tubes with blood can be left standing on ice overnight to allow serum to separate. Use a pipette to draw off serum, aliquot into 0.5 mL volumes per cryovial, and store cryovials in a dry shipper or dewar. As soon as possible, remove samples and place in cryoboxes and store in an -80°C freezer.

4. Urine

Collect 2 urogenital swabs and place one in 500 μ L of VTM and one in 500 μ L of Trizol. If the animal urinates, collect up to 500 μ L of urine using pipettor and place into one cryotube with 500 μ L of VTM, and another up to 500 μ L of urine into a cryotube with 500 μ L of Trizol and mix well.

Sample Collection from Dead or Euthanized Small Carnivores

If carcasses are not whole, then the ***Bushmeat Sampling Methods*** may be more applicable. If bodies are relatively whole and fairly fresh then sample as described above. If an animal must be euthanized due to humane or veterinary care reasons, see ***American Veterinary Medical Association guidelines***.

As discussed throughout this protocol, all wildlife should be considered potentially infectious for a wide variety of dangerous pathogens and dead animals in particular should be sampled only following all safety measures including proper PPE use, proper work station decontamination, and proper carcass disposal as outlined here and in other PREDICT documents.

Though not required for PREDICT sampling, thorough necropsy procedures can be very beneficial and might pertain to some animals (e.g., valuable or known individuals, suspicious deaths, etc.). Necropsy protocols are addressed in separate documents. Time and skill permitting, when full necropsies are performed, following any Association of Zoos and Aquariums/AZA (or similar) necropsy protocol is recommended and most can be adjusted for application to other species. *(Note that properly following extensive necropsy procedures and collecting and measuring all samples can require 4-6 hours for a single animal.)*

Post-Mortem Blood Collection

From recently dead animals, it may be possible to collect whole blood (often clotted) from the right side of the heart where the largest volume of blood is available. Collect all available blood into an appropriate size container (typically one or more blood tubes). Allow the tubes to sit undisturbed for at least 30 minutes, and then centrifuge at high speed (2000 x G for 20 minutes). Transfer the serum (clear, yellow or red-tinged fluid at the top), preferably via pipetting, to appropriately labeled cryovials. Transfer the remaining blood clots to separate cryovials. Refrigerate or freeze both the serum and blood clots.

If a centrifuge is not available, allow the clots and cells to settle as much as possible, and then collect the serum and clots as described above. If the animal's death is recent enough that the blood has not yet clotted and a centrifuge is not available, invert the blood tubes after the blood has been collected to allow the clot to form on the rubber stopper. After the blood has clotted, turn the tube right side up and carefully remove the stopper with the adhered clot, thereby leaving a clean serum sample in the tube.

At a minimum, as many of the following blood samples as possible should be collected:

- 2 samples of 500 μ L (**whole blood**) placed in 2 vials, one containing 500 μ L **Trizol** and one containing 500 μ L **VTM** (= maximum final ratio of 1:1). Mix each vial well.
- 2 or more aliquots (0.5 ml) of **separated serum**, frozen

Tissue Collection

Collect **three**, adjacent, approximately 200mg (pea-sized) samples of the following tissues:

- Adrenal
- Cecum
- Colon
- Duodenum
- Heart
- Kidney
- Liver
- Lung
- Lymph node
- Spleen
- Ovary
- Pancreas
- Testes
- Other, if required*

One specimen should be frozen in 500 μ L VTM in a cryovial, one should be frozen in 1 mL Trizol in a cryovial, and one should be stored at room temperature in a small vial or jar in 10% buffered formalin at a volume of fixative 10 times the volume of the tissue (once fixed, the tissue may be transferred to a smaller volume for shipment). **If only one sample can be collected, then place it into VTM.**

*It will usually require experience to identify abnormal tissues, but potentially recognizable gross lesions include masses, discolored areas, ulcerations, etc. Samples for histopathology (i.e., in formalin) should be collected at the abnormal margins to include both normal and abnormal sections in the same piece of tissue. Collection of any obvious internal parasites in ethanol is also recommended.

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Qualitative Research Guides

Qualitative Research: Introduction and Observational Research Guide

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Objectives: To provide principles and general guidelines for the conduct of targeted qualitative research to understand the context and potential risk practices and behaviors of individuals at high risk of zoonotic disease spillover.

Section 1. Qualitative Research: An Introduction

Qualitative Research is an exploratory type of research that is used to gain insight into people's lives. Qualitative data can be collected at multiple levels within the community using different and complementary methods. Figure 1 shows three different levels: human environment, community life, and individuals and households. These three levels are linked to three different qualitative methods of data collection: observational research, focus groups and ethnographic interviews.

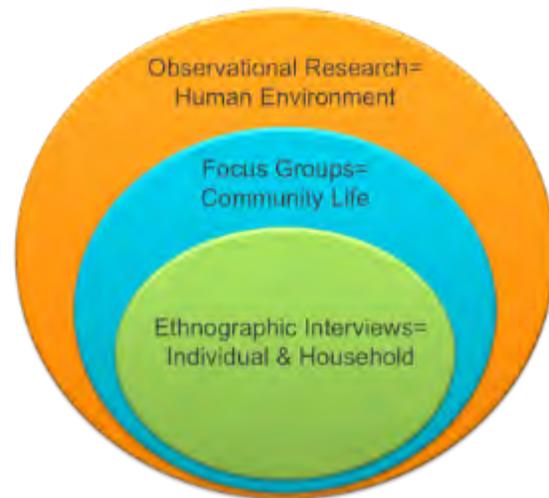


Figure 1: Levels of Qualitative Data

Qualitative research is the best method for understanding the individual motivations that influence behaviors, particularly private, unpopular or taboo behaviors. People are more likely to provide information on such behaviors if they are able to provide the context or a justification. For example, a person who would not admit to hunting in a protected area if asked in a survey may disclose hunting activities in a one-on-one ethnographic interview, offering the justification that hunting was necessary to feed the family.

Qualitative research may be general and implemented over long periods of time. Alternatively, this type of research may be targeted and focused on a set of specific issues, as is the case for PREDICT qualitative research.

The limitation of qualitative research is that findings may only apply to small groups of people who are similar to those participating in the research. While there is great depth and detail to the data collected using these methods, and much important information is learned, it cannot be said with certainty that the behaviors and practices identified in small group settings are the same as those in the larger community. That is why a qualitative approach is often combined with other types of data collection (e.g., large surveys) to address complex issues that require timely intervention.

The PREDICT project strategy is to use the data collected through the qualitative research step of the process to improve on behavioral risk questionnaires that have been conducted with large populations. The qualitative data will be analyzed based on the experiences of people who are at increased risk of zoonotic disease transmission. In addition, after the behavioral survey has been completed with a larger population, the findings from the qualitative analysis can be used to help interpret survey findings, as well as to inform risk mitigation strategies.

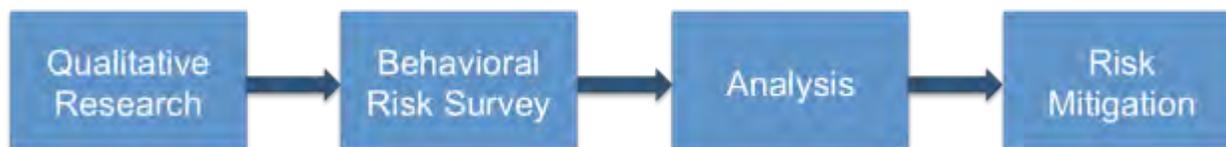


Figure 2: PREDICT project strategy

This protocol reviews the objectives and methods of conducting observational research. Observational research may be conducted immediately at a site, and can be conducted at any time. Focus groups and ethnographic interviews require institutional review board (IRB) or other in-country ethical committee approvals before they may be conducted. All staff conducting ethnographic interviews or focus groups or participating in data analysis must complete human research ethics training (e.g., Collaborative Institutional Training Initiative [CITI] training; National Institutes of Health Protecting Human Research Participants training) before working with research subjects or their identifying information.

Section 2. Targeted Approach

The qualitative methods outlined in this protocol use a targeted qualitative approach. This approach involves assessing current knowledge and perspectives of specific populations, in order to gain insight into a set of core themes.

Section 2a. Core Themes

The **core themes** are the topics that guide the research in this protocol. These are topics about which incomplete or no information is known, particularly in regard to their relationship with zoonotic disease transmission. There are five core themes that interviewers will focus on when guiding the conversation. The five core themes and the research goals for each theme are listed below. Examples of types of questions that can be asked for some of the core theme are included in the [***Ethnographic Interview Guide \(Appendix 5.6.5c.\)***](#) at the end of the protocol.

1. **Human Movement:** To understand how far people travel and why
2. **Socioeconomics and Daily Living:** To understand a typical day and how socioeconomic factors impact animal contact risk
3. **Biosecurity in Human Environments:** To understand how sanitation or hygiene factors could play a role in disease transmission
4. **Illness, Medical Care/Treatment and Death of Humans:** To identify any unusual disease experiences—signs, symptoms and sources—as well as how people respond to illness
5. **Human-Animal Contact:** To understand 1) physical interactions and exposure to animals, 2) the use of animals and animal byproducts, and 3) knowledge and beliefs about animals

Section 2b. Timeline

Table 1: Timeline for Behavioral Research Activities

Method	Timeline
Observational Research	Can occur as soon as PREDICT staff are at a site at any time/place.
Focus Groups	4-8 weeks total (can occur concurrently with Observational Research and Ethnographic Interviews)
Ethnographic Research	4-8 weeks total (can occur concurrently with Observational Research and Ethnographic Interviews)

Section 2c. Target Population

The **target population** is the group of people who are actively exposed to animals along one of the PREDICT project pathways: 1) land use conversion, 2) animal production intensification, or 3) animal value chain.

Different kinds of people will be found in the three PREDICT project pathways. For example, the kinds of people that may be found on the land use change pathway could include extractive industry workers (e.g., the people who cut down and carry logs out of the forest), the foreman at a mine, engineers working at a new port or roadway being built, or the people who sell animals or other food to the workers. Below is a list of some of the kinds of people that may be found on the project pathways. There are others not included on this list.

Table 2: Examples of Members of Targeted Populations for each of the three Pathways

Land Use Conversion	Animal Production Intensification	Animal Value Chain
<ul style="list-style-type: none"> • Laborers • Foremen/headman on site • Family members of laborers • Local food suppliers (e.g. local or informal restaurants for workers) • Transporters • Residents near changing land • Fuel/wood harvesters • Farmers • Pastoralists • Miners/loggers 	<ul style="list-style-type: none"> • Farm or ranch owner • Farm or ranch worker • Backyard animal raiser • Distributors • Transporters • District vets • Feed/supplement sales people • Abattoir workers • Butchers • Traders • Herders 	<ul style="list-style-type: none"> • Wildlife farmers • Market vendors • Wildlife restaurant owners/worker • Transporters • Users of animal based medicine • Healers/traditional medicine • Hunters • Consumers • Marketplace owners/managers

It is important to interview a diverse group of people from the target population; therefore, approximately 35-40% of participants should be women. Efforts should be made to include a large variety of people, including those of different religions or ethnicities, younger people and older people, and people who have more power or influence (e.g., farm owners), as well as those with less (e.g., market cleaners). All of these different groups of people are likely to have different risk behaviors, practices and experiences. An important goal is to be able to understand and report on as wide a range of experiences as possible.

There are no strict rules concerning sample size or how many people need to be interviewed in targeted qualitative research. The most important factor is diversity of the people interviewed. The goal of this type of research is to get many different perspectives on a limited set of core themes. The lists above have approximately 10 different kinds of people that can be found on the project pathways. Each individual type of person may differ by age, gender, ethnicity, social status, or place of birth. These types of differences should be represented in the final sample. Researchers who have considered sample size issues suggest a range of 20-30 participants per site.

Section 3. Observational Research

Purpose: Observational Research is intended to be the first step in the research process and is carried out in order to observe the setting and the people who may meet the targeted population criteria at the sites that are being considered for surveillance and sampling.

Section 3a. Observational Research Overview

Table 1: Observational research key points

What Is It?	Research Goals
<ul style="list-style-type: none"> • A first step in the qualitative research process • Passive observation and field note taking of the structure and characteristics of the site and the people who inhabit it • Informal conversations with ‘key informants’ • Mapping of land and community 	<ul style="list-style-type: none"> • Identify key informants • Establish relationships with individuals from target populations and key informants • Prepare for next stages of qualitative work (i.e. focus groups and ethnographic interviews) • Write up field notes of observed environment and interactions • Map the setting

Section 3b. Who is Involved in Observational Research

The main individuals involved in observational research are the **Observer, Key Informants** and any other individuals interested in speaking with the Observer in an informal way.

Observer: The Observer is the person conducting the observational research (e.g., can be country coordinator, head field worker, or any other PREDICT staff person). The Observer should let people know about the study and the things we would like to learn. This is an excellent opportunity to engage people and to spread the word about the PREDICT project. The Observer should pursue informal and active introductions to people and members of the target communities, especially people of influence. Identification of formal leadership structures will be important in terms of identifying opportunities and challenges for the implementation of the study, as well as any future interventions targeting structural or behavior change.

The Observer is often introduced to people of influence by local contacts that have already been established. This is the easiest way to identify key informants who may then introduce the Observer to others. It is much more challenging to engage in informal conversations without local contacts, but not impossible. Simple observation of the setting should provide clues to identify the people in authority or who have influence. This observed information is just as important and should be collected independently of any informal conversation by the Observer.

Key Informant: To gather information rapidly on a particular topic, such as the locations, practices and activities of the target population, it is necessary to identify people of power in the community (e.g., government officials, business people) or those with influence with the target population (e.g., religious leaders, market managers, community elders). Key informants are often those who are easy to approach. It is important to speak with a range of key informants.

Section 3c. Observational Research Methods

Observational Research methods include making observations, having informal conversations with community members who are willing to speak with the Observer, and mapping the sites being considered for future surveillance and sampling. Informal conversations must be limited to casual or introductory conversations about what PREDICT is doing in the community and cannot involve direct questions about the Informant or community member's work or personal life, as in-depth discussions that reveal dynamics that we are trying to understand about zoonotic disease transmission would be considered 'Human Subjects research' and would require the completion of a Participant Consent form according to PREDICT's human research ethics review board approvals.

Informal conversations often provide a good opportunity to inquire about other key informants: for example, "Is there a market manager whom I might talk to and can you direct me to her?" or

“Is there a site foreman and where is his office?” All observation and informal conversations must be documented as Field Notes.

Field Notes (i.e. the data collected in Observational Research), can help contextualize subsequent qualitative or quantitative findings. Observational research can be conducted independently by the Observer or with the help of key informants, who guide the observational experience through their intimate knowledge of the area and culture. Excerpts from Field Notes are included in [**Section 5.6.4. Appendix I. Observational Field Notes Excerpts.**](#)

The observational process entails looking for specific features of a potential research site, meeting people, talking with anyone who is interested, identifying individuals in positions of authority or influence in the target community or those who interact regularly with the target community, and trying to establish relationships with these individuals. Observation is an active activity, requiring focused attention to one’s surroundings and involving all five of the human senses, including visual, auditory or olfactory information.

In addition, drawing maps of potential surveillance and sampling sites is an important and visual way to document the human environment. For example, an important feature in a market may include the separation of livestock and wildlife in different sections of the market. Hand-drawn maps can serve as reminders of where specific features are located or, over time, if these features change. Examples of maps are included in [**Section 5.6.5. Appendix II. Observational Map Examples.**](#)

Observational research should continue through the life of the project. Observational research does not require IRB approval.

Section 4. Appendix I. Observation Field Notes Excerpts

Brief Summary

Observer: Jim Desmond
Date: Sunday, November 2, 2014
Setting: Guangzhou TaiPing Market (SARS market)
Weather: Overcast and comfortable weather
Time: 10:30am – 12:30pm

Tai Ping market is about 100 km southwest of GaungZhou. I had previously visited this market with GuangJian and Jin Ping in 2011. At that time there were many more animals, both domestic and wild, at this market.

The market is quite large, covering a large area. On this particular many of the stalls were closed and there didn't seem to be a lot of activity, not many buyers. The market is divided into two sections. There is a section that contains, reptiles, amphibians, fish and other aquatic animals. The other section contains birds and mammals. We focused solely on the bird and mammal section.

There were approximately 50 vendors – but that is a very rough estimate and it's also difficult to say if some of the closed shops were only closed that day or if they were closed permanently. Of the vendors that were open they generally seemed to sell either birds or mammals but not both. With birds, there was more mixing with vendors selling a variety of chicken, goose and duck breeds as well as pigeons. Some vendors had pheasant or quail. Some of the duck breeds looked like wild birds, for example there were a lot of mallards and there were other ducks that I could not identify the species but they did not look like domestic ducks. My guess is they are farmed but at some point in the past they had been wild caught. There was a roughly and equal number of bird vendors vs. mammal vendors

We observed a wide variety of mammals, a mixture of wild and domestic. However, there were far fewer mammals present and much less diversity than our previous visit in 2011. GJ said that the market had been shut down several months following our visit due to an article published in the paper regarding the illegal wild animal market. All the vendors are aware of the risk of disease. GJ said he overheard some guys talking when we got out of the car and they assumed we were looking for diseases in the animals. The presence of westerners definitely is a red flag for them and maybe even the presence of non-local Chinese. Unless you speak the local dialect, vendors there will be unwilling to speak with you according GJ.

Here is a list of some of the animals seen: wild boar, bamboo rats, another species of wild rat, nutria, raccoon dogs, another type of wild rodent? That looked a bit like a marmot - need to look it up, domestic cats, domestic dogs, goats, cows (jerseys). I may be missing a few but that covers most of it. The raccoon dogs were sort of hidden so they vendors must be concerned about them being seen. There were a lot more wild boar than the last visit but less animals and less diversity overall.

Observer: Arif
Setting: DLS in Dhaka
Dates: Jan 21-28, 2015

I spoke with some persons of DLS and also discussed with cattle traders in Dhaka city market regarding cattle marketing channel across Bangladesh. I visited three cattle Markets in Dhaka for getting information where the cattle come from.

The vast illegal trade thrives since cows are considered holy in India, and New Delhi is unable to legalize their export. It becomes 'legal' when traders pay up revenue officials in Bangladesh

They told that cattle come through Jessore border. Putkhali Khatal in Benapole border in Jessore district where is most of cattle trading occur.

Bangladesh and India share a 4,096-kilometer (2,545-mile)-long international border consisting 28 districts. Cattle traders say that cattle trading is occur in following districts:
Dinajpur, Kurigram, Lalmonirhat, Panchagarh, Thakurgaon, Meherpur, Kushtia, Chuadanga, Jhenaidah, Rajshahi, Chapainawabganj, Naogaon, Nilphamari and Jessore District

Above mentioned districts, Many cattle come across Meherpur border. Although, it is small district only 716 sq km but most are bordered with India. Cattle trader say that even Beef illegally come through Meherpur border. After slaughtering cattle at night, the beef come across border.

I tend to think that we can choose Meherpur district in Y-1 and Jessore in Y-2.

Near to Nepal border: Thakurgaon & Panchagarh District: there is Banglabandha, a major inland port in northern Bangladesh established to provide a trade link with India, Nepal and Bhutan. The three nations are separated by 52 km only. So either Thakurgaon or Panchagarh District can be choose for Y-3/Y-4 PREDICT-2

Myanmar border: Bangladesh and Myanmar share a 193 kilometer crossing Cox's Bazar (in Teknaf Upazila) and Bandarban District. We can choose some sites with Myanmar border

It seems to me that it will be really good to include Medical doctors of One health scholar for conducting observational research under my supervision.

Finally, the present political situation is not good here. The indefinite transport blockade is still going on.

Observer: Maureen Miller
Date: 1/7/15 Wed morning 8:30 start 11:30a end
Setting: Live animal markets in Queens, New York City
Weather: frigid it snowed last night

Site 1: Almadina Halal poultry shop

Time: 9-9:30

We got lost trying to find the place and got directions from a man coming off the subway. We had to walk through a tunnel and ended up at a cross roads of abandoned looking warehouses. He sent us off in one direction while we walked in another. There were metal shops, glass works and car buyers/repairers/parts shops strewn throughout. There was one section on the opposite side of the street where houses had been converted into 3 or 4 different kinds of church congregations. Nobody was walking on the streets. The sidewalks were unshoveled, some were icy where people had walked.

We started looking for 157th street where the poultry shop we were going to was located. We ended up bumping into the guy who gave us directions at 156th. He was a guard at the blocked off street that led into a factory complex. It turns out that the complex was a distributor of live and butchered animals. We asked another guard for directions. I showed him the address. It was pretty clear that none of these guys knew how to read. I asked about the live poultry shop and he sent us back to exactly where we had come from. One of the abandoned looking buildings was actually Another shop—not the one we had targeted.

There were two delivery trucks out front advertising halal butchered goat and cow. There was also a food cart with a long line of poultry shop workers. The cart looked like regular halal, but most of the workers were buying cup-o-soup by lipton or coffee. As we stepped on the curve, we stepped over a large frozen puddle of blood. There was also quite a bit of feces around.

I went in and asked for Raja—the name of the man I had spoked with. The first guy didn't speak English. The guy behind the clear plastic ribbon protected cutout in the wall directed me to the door next door, which was for employees only. I went in and asked several people for Raja. One finally spoke English and corrected me: Raya. The room was small high ceilinged and dark. There were plastic crates about 8" high filled with chickens that could not stand up: one had 3 chickens but most had 6 or more. There was liquid deep on the floor: a combo of melting snow, urine and feces. The air was fetid, warm and difficult to breathe.

Raya came out. I explained who I was and what we wanted to do. He said he had never spoken to me. I asked if we could observe anyway. He said no, but gave me detailed directions to the shop we were trying to go to. There were many men working there and I saw one woman. I think they were Pakistani.

People were eating and drinking in with the animals and presumably the butchery and slaughter areas too.

Qualitative Research: Focus Groups, Ethnographic Interviews, and Data Analysis Guide

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Objectives: To provide principles and general guidelines for the conduct of targeted qualitative research to understand the context and potential risk practices and behaviors of individuals at high risk of zoonotic disease spillover.

Note: Focus groups and ethnographic interviews require institutional review board (IRB) or other in-country ethical committee approvals before they may be conducted. In addition, all staff conducting ethnographic interviews or focus groups or participating in data analysis must complete human research ethics training (e.g., Collaborative Institutional Training Initiative [CITI] training; National Institutes of Health Protecting Human Research Participants training) before working with research subjects or their identifying information.

Section 1. Focus Groups

Purpose: To assess the distribution and overlap of animals in the community setting; and to discuss 1) animal contact and context, 2) illness in animals and humans, and 3) rules and restrictions surrounding both wildlife and livestock.

Section 1a. Targeted Focus Group Overview

Table 1: Focus group key points

What Is It?	Research Goals
<ul style="list-style-type: none"> • Guided group discussion focused on limited topics • A group of 6-10 people not from the same household/family • Group members share relevant characteristics • Conducted over a short time period (all within 4-8 weeks) 	<ul style="list-style-type: none"> • Identify how groups of people think or feel about behaviors and practices that may be linked to disease transmission • Explore reasons why certain opinions are held • Examine social, cultural and economic factors

Section 1b. Who is Involved in Focus Group Research

The people who conduct a focus group are the **Moderator** and the **Recorder/Observer**.

Moderator: the person who leads the focus group discussion. A moderator should have a charismatic, friendly personality and should not be timid, authoritarian, or judgmental. The moderator introduces each question or activity and encourages all focus group participants to contribute to the discussion. The moderator asks follow up questions, a process also called probing, until a topic is exhausted or no new information is being learned. It is the responsibility of the moderator to make sure that all voices are heard, and that the participants share and discuss a full range of information.

Recorder/Observer: the person who supports the moderator and records the focus group. The support is provided by observing the behaviors and responses of the focus group participants, as

well as documenting highlights of the topics discussed, particularly for any new or unique information. In addition, the recorder/observer may become aware of additional follow up questions that the moderator may wish to probe. It is the responsibility of the recorder/observer to discretely share this information with the moderator and suggest probing questions. The highlight notes that the recorder/observer writes are part of the data that will be analyzed.

Section 1c. Focus Group Methods

A **targeted focus group** is conducted by two people, one who leads the discussion (the **moderator**) and the **recorder/observer** who supports the moderator. Focus groups are generally conducted with a group of 6 to 10 people from the target population who share a relevant characteristic (e.g., wildlife farmers or workers). A focus group generally lasts between 60 and 90 minutes. The setting where the focus groups take place should be selected and prepared ahead of time. It should be a private area where the group will be undisturbed for the length of the Focus Group. Focus groups will be tape recorded, so that they may be transcribed, coded and analyzed.

The discussion is semi-structured and guided. That means that the topics of discussion for the group are well defined before the focus group begins. The questions that are used to guide the discussion are called the **Focus Group Guide** (see [Appendix I. PREDICT Focus Group Guide](#)). The questions and activities included in the Focus Group Guide are meant to engage all members of the focus group and to stimulate the discussion.

The Focus Group Guide for this project includes a ‘community mapping’ component. Community mapping is an activity that immediately engages all group members as they provide information about the location of various animals in the community. Examples of animal maps are included in [Appendix II. Focus Group Animal Mapping Exercise Examples](#). This introductory step also allows the moderator to identify participants who may try to dominate the discussion, as well as those who may be shy. It is important for the moderator to make sure that everyone has a turn to speak. After the community mapping activity, the group focuses on animal contact and context, illness in animals and humans, and rules and restrictions surrounding both wildlife and livestock. The map may be used for reference during the discussion.

Section 2. Ethnographic Interviews

Purpose: To understand the personal context and potential risk practices and behaviors of individuals at high risk of zoonotic disease spillover.

Section 2a. Targeted Ethnographic Interview Overview

Table 1: Targeted ethnographic interview key points.

What Is It?	Research Goals
<ul style="list-style-type: none">• One-on-one semi-structured interviews• Focused on limited topics (core themes)• Conducted over a short time period (all within a total of 4-8 weeks)	<ul style="list-style-type: none">• Identify behaviors and practices that may be linked to zoonotic spillover• Explore reasons underlying behaviors/practices• Examine social, cultural and economic factors

Section 2b. Ethnographic Interview Methods

A **targeted ethnographic interview** consists of the **Interviewer**, the person who conducts the interview, and a **Respondent**, an individual from the target population. Targeted ethnographic interviews are semi-structured and guided discussions. The topics of discussion are well defined before the interview begins and are based on the **core themes** of interest, described in detail on below.

The **core themes** guide the ethnographic interview discussion. The themes are topics for which limited information is known, but which are strongly suspected to play a role in the transmission of diseases from animals to humans. The **Interview Guide** (see [Section 5.7.7. Appendix IV. PREDICT-2 Ethnographic Interview Guide](#)) is a list of core themes that also includes subthemes and suggested questions that may be asked during an ethnographic interview. Not all questions listed will be asked in any one interview. In fact, if the respondent is providing detailed information that is unique (e.g., the person is describing burial methods in a culture where no one is comfortable talking about death), the interviewer should spend time asking additional questions in order to get more detailed information.

One of the biggest challenges to using the Interview Guide is figuring out which of the many questions should be asked during one interview. One way to address this challenge is to imagine different kinds of respondents and think about the kind of information those people could provide. For example, a 13-year-old girl who lives next to a forest with bats may not know the family income, but she could provide insight as to where the bats live during the day, the kinds of bats she sees, how frequently and where she bathes, whether her parents travel for work and how far, what she learns about animals at school, her responsibilities with the family chickens,

how they differ from her brother's responsibilities and from what hers will be when she gets older. Thinking through the kinds of information that a particular respondent could provide helps in selecting appropriate questions from the Interview Guide. The key to a successful ethnographic interview is being as prepared as possible *before* the interview begins, and being flexible during the interview.

Interviews generally last between 60 and 90 minutes, and should not last longer than 120 minutes. The setting where the interviews take place should be selected and prepared ahead of time. Individual interviews are conducted in private, ensuring that others cannot hear the interviews. A barrier should be created so that no other individuals can view the respondents while they are being interviewed. Depending on the location, this could be a private room, behind a building or fence, or behind a line of trees, obstructing view so that confidentiality may be maintained. Interviews will be audio recorded with permission, so that they may be transcribed, coded and analyzed.

The **Interview Checklist** is a document that lists the core themes and subthemes that are included in the interview guide (see Section 8. Appendix V. Interview Checklist). Because it is not expected that all core themes and subthemes will be discussed in every interview, the Interview Checklist allows the interviewer to check off only the themes that were discussed during the interview. This document is important for the coding and analysis of ethnographic interview data. Qualitative data coding and analysis can be time consuming. A completed Interview Checklist ensures that time is not wasted looking for data on a theme that was not discussed in the ethnographic interview. The Interview Checklist should be filled out immediately following the completion of the interview.

Section 3. Analysis

Purpose: The primary goals of the qualitative data analysis are to 1) systematically review and prepare the data for analysis, 2) uncover new information that was shared by individuals during the interviews and focus groups, 3) review information from the observational research that may help contextualize research findings.

Section 3a. Data Analysis Process

There are two steps involved in the analysis of qualitative data: 1) coding and 2) preliminary data analysis in the form of summary notes.

Section 3b. Coding

Data coding is the way that data are defined in qualitative research. Codes can be thought of as “tags” that are applied to discrete sections of narrative text. Codes allow researchers to assemble information into meaningful analytic groupings. Each coded piece of information represents a data point that can then be analyzed or considered in relation to other data points.

Data coding for preliminary data analysis will be focused exclusively on the five core themes and subthemes that guide the PREDICT qualitative research. Coding data requires a close reading of the transcribed document. Reading the document closely for the first time provides an opportunity for the researcher to objectively review the range and type of information that was collected during the interview, as well as to take good notes on the major themes discussed preparatory to coding.

The coding process uses the suggested Coding keywords document (see [Section 5.7.10. Appendix VII. Suggested Coding Key Words](#)) to code the transcribed focus group and ethnographic interview documents. **Coding keywords** are words that are associated with the core themes and subthemes. Coding keywords help the coder search through a document to identify the information to be coded. The list of keywords is meant to be an aide in the coding process. The list of **coding keywords** will be provided to all research staff who will code the data.

In countries where internet is stable and the resources are available, it is ideal to use qualitative software package for data analysis (Dedoose, NVivo, Atlas.ti, etc.), as any of these platforms allow for the data analyst to query data to look for patterns across interviews. In countries without stable internet, data can be coded using the COMMENT function. Examples of coded transcripts are found in [Section 5.7.11. Appendix VIII. Examples of Coded Text](#).

Section 3c. Quote Selection (for Ethnographic Interviews and Focus Groups)

From the transcript of each interview and focus group, the researcher will select a few quotes that are good examples of specific core themes. The close reading that the researcher does in preparation for coding also provides a good opportunity to identify quotes that ‘stand out’ because they clearly express one of the core themes in an interesting way or they provide new information. These ‘stand out’ quotes should be highlighted in the transcribed document and copied to the final summary document, with the transcript page number noted. In addition to selecting the quotes, each researcher will identify which core themes or subthemes the quotes represent, as well as provide an opinion as to why these quotes were selected as good examples.

Section 3d. Brief Summary Notes (for Ethnographic Interviews and Focus Groups)

The researcher codes the document by each core theme, one at a time. For example, for the core theme of **socioeconomics**, throughout the interview a market cleaner may talk about the unpredictability of the schedule, the certainty of blame when inspectors come, unreliability of payment for services and sometimes stealing food from the butcher table when the butcher is not looking. When this same interview is coded for **human movement**, the market cleaner may reveal not having a home and sleeping with animals in the market to stay warm at night, having moved to the area from the countryside for work and finding limited options, and of wanting to return home but the situation is worse there.

The analytic object is to briefly summarize the situations and experiences of the individual as they relate to the core themes. For each of the five core themes, there will be summary notes describing the major ideas or issues discussed by the individual. New information should be emphasized and transcript page numbers for new information should be included in the summary notes. If a core theme was not discussed in the interview, please note that fact in the summary document.

Section 3e. Summary Documents

A summary document will be required for each ethnographic interview and focus group. The summary document will consist of quotes that are good examples of specific core themes, as well as the explanation and transcript page numbers for quote selections. The summary document will also contain brief summary notes by each of the five core themes.

Section 3f. Training

Preliminary data coding and analysis may be conducted by local research staff; however, preliminary analysis of qualitative data is not mandatory. For countries interested in conducting preliminary analyses for the PREDICT project, training will be provided on request.

Section 4. Appendix I. PREDICT Focus Group Guide (Version 2; May 1, 2015)

The focus group discussion is initiated by naming all of the animals that can be found in the community. The goal of this exercise is to explore animal diversity.

The community mapping activity locates where the different kinds of animals can be found relative to the site of the focus group. It should be emphasized that this will not be an 'accurate' map. This exercise is designed to assess the distribution and overlap of animals. Prompts such as 'anywhere else?' should be used. The animal list will contain insects, reptiles and fish. Map only mammalian and avian species.

These two activities together should be limited to 10-15 minutes. The themes to be explored in the discussion are 1) contact and context, 2) illness in animals and humans, and 3) rules and restrictions. Events such as animal die-offs should be added to the map, if they are discussed.

1. Contact and context

- Which of these animals do you see the most often? The least? (Probe: where, why)
- What animals do you come into physical contact with? (Probe: where, why, how often)
- Which of these animals do you eat?
 - Where do you get them? How are they prepared? Which are for special occasions only?
- What are animals good for other than food? (probe: labor, medicinal, magic, pets, by-product uses)
- Which animals come into buildings or places where people are? Is water shared with animals?
- How are unwanted animals kept out? (probe: which animals, all methods used)
- Who takes care of the animals? (Probe: who, specific jobs, animal movements)

2. Illness in animals and humans

- Animals
 - What happens when animals get really sick? How are the animals cared for?
 - Has this happened recently? Do people try to hide animal sickness?
 - Is animal sickness reported to anyone? (probe for differences between wild and domestic animals)
 - Have any animals been destroyed or killed by authorities? Describe.
 - What happens to animals when they die? (probe: eaten, buried, left to rot, depends if wild or not)
- Humans
 - What is the most unusual or memorable sickness anyone has had? What happened?
 - What are the causes of illness or sickness?
 - Do you know anyone who has gotten sick from an animal? What happened?
 - What do you know about animals that can give you infections or diseases?

3. Rules and restrictions

- Are there places in the community where you aren't allowed to go? Why not?
- Are there any rules about hunting or trapping animals? (Probe: cultural, legal)
- Are there any animals that you don't eat or that are avoided? Why?
- Are there official rules or laws about garbage disposal? human waste? Animal waste?

4. Is garbage a problem in this community? What's the problem?

Final question for all: If you could change one thing in your life, what would it be and how would you do it?

Section 5. Appendix II. Focus Group Animal Mapping Exercise Examples

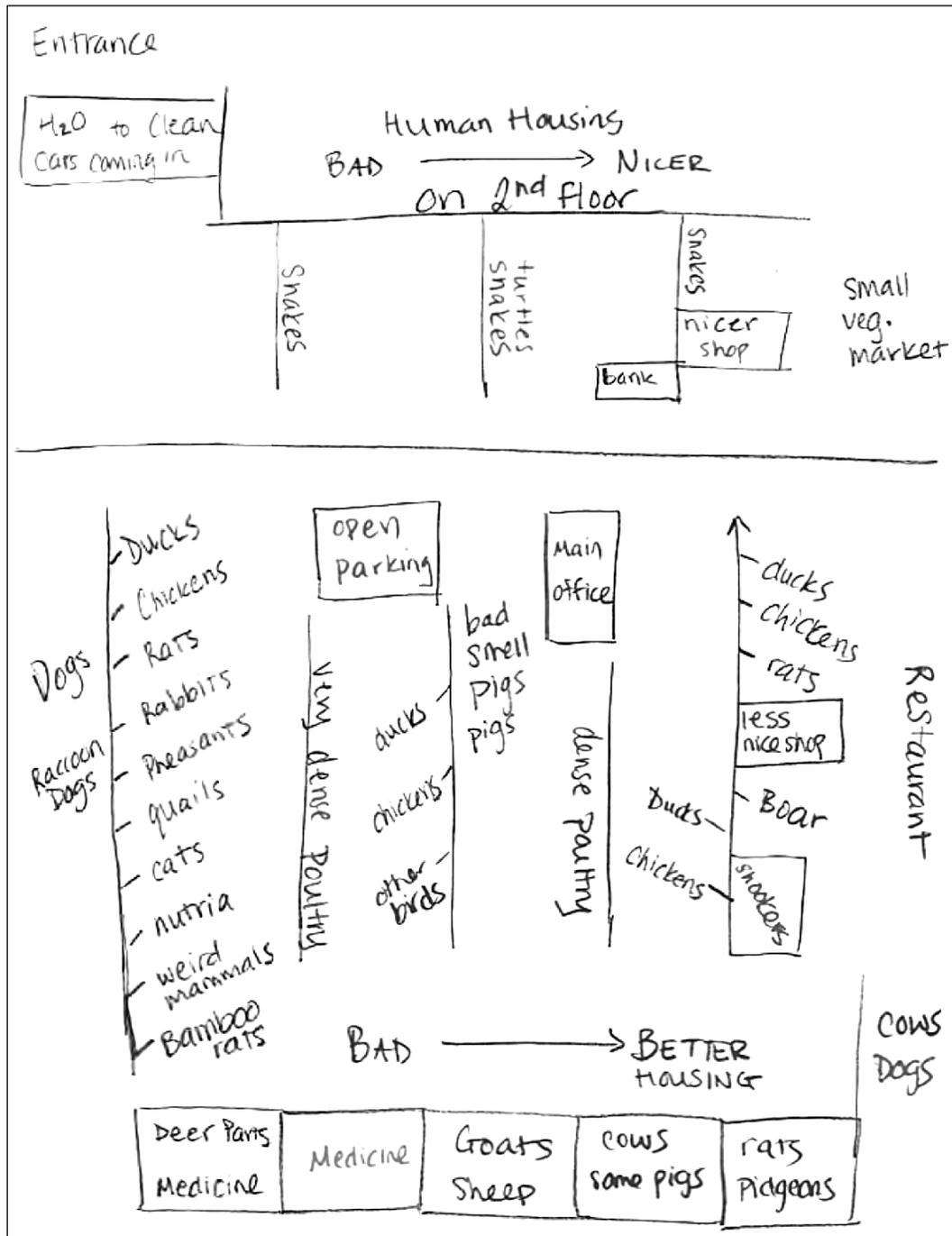


Figure 1: Example Community Map

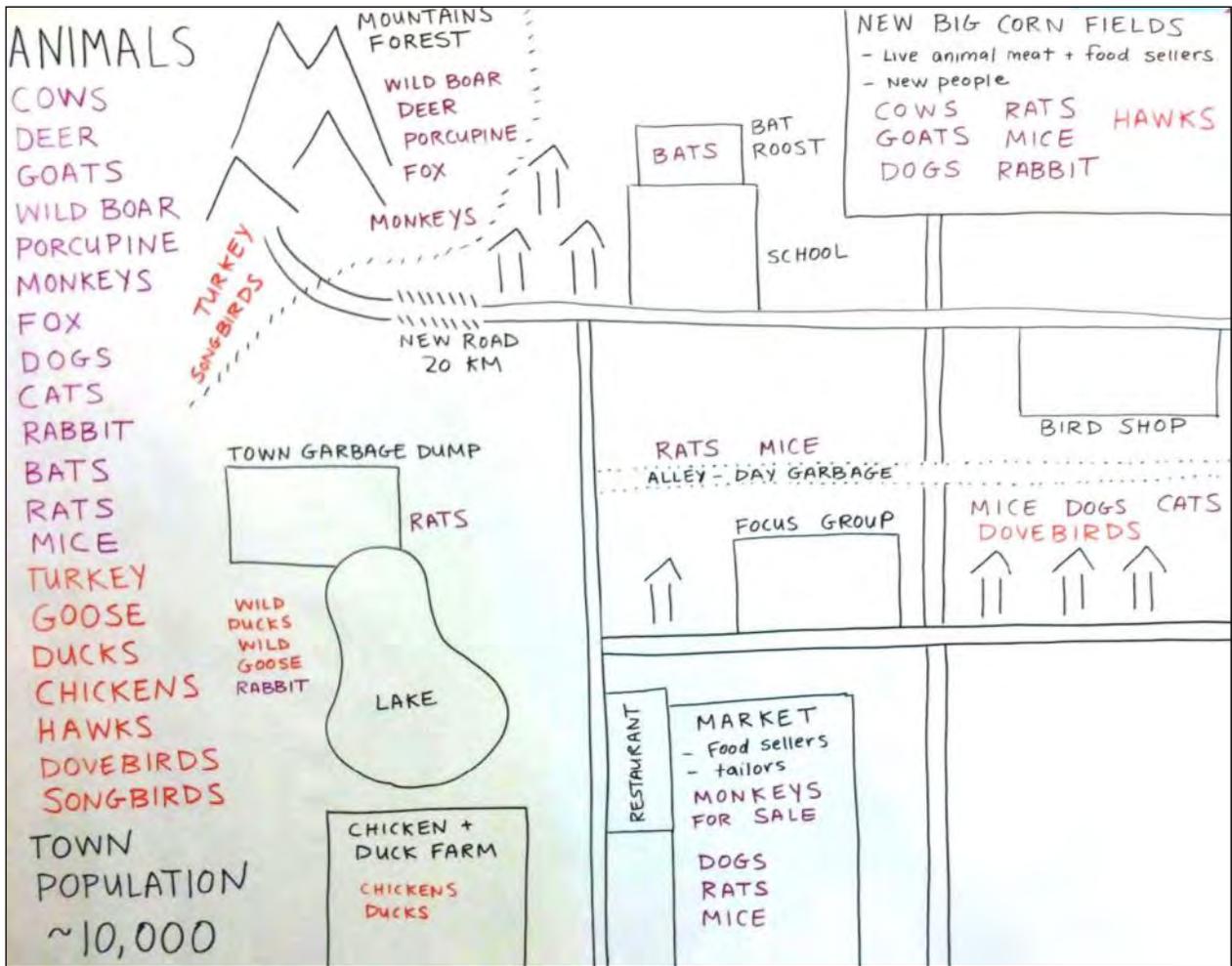


Figure 2: Example Community Map

Section 6. Appendix III. Example Focus Group Excerpt

Q: and what do you do? When you see that dead animal?

A5: when we see an animal in that state, if the animal is already decomposed, we cannot

A1, A4, A7: yes... it is spoiled...

A7: if it's still in the good state, if it's still in the good state we can consume the animal.

A6: good meat.

A7: and there are times you can go to the bush, somebody sets a trap

A6: yeah.

A7: you meet an animal there. That is maybe already dead. If you...From judging you can discover that the animal... maybe it is still...

A4: fresh

A7: fresh....

A5: yes

A7: not in the decomposed state. You can eat that

A5: why won't you eat?

A7: but when you look around and flies are already visiting. Heuh...you have maggots around

A5: yes

A7: you cannot eat

All: (laughing and noise)

A9: somebody like me I will

A7: when it is expose

A6: when we meet...(laughing) or that the animals fell into you must ask yourself questions before euh... thinking of eating such an animal.

Q: tell me, when you see a dead animal. How can you know that the animal is already... that you can cook it or you cannot cook it?

All: it is... (noise)

A6: they gave you the reason

Q: you can know that the animal is.....

A3: from the smell

A5: the smell

Q: the smell?

A8: yes the scent.

A5: they talk of the smell, the scent, flies,

A7: flies appears

A5: flies on the decomposing euh... euh... euh....

A3: situation

A5: situation. You can easily detect whether you can eat or not. We need the fowl that is already... smelling

Q: yes.

A5: and flies are already all over the whole place.

Q: hum euh.

A5: I am not sure a normal human being will eventually eat such an animal

Section 7. Appendix IV. PREDICT-2 Ethnographic Interview Guide

Core Themes

1. Human movement
2. Socioeconomics
3. Biosecurity in human environments
4. Illness, medical care/treatment and death of humans
5. Human-animal contact

1. Human Movement

To understand living environment and 'home range' (e.g., how far people travel and why).

Home

- Where do you live/what kind of dwelling? How many people are in the household? How many rooms? How many are children? Is everyone related? Sleeping arrangements?
- How often do you move? Any seasonality of movements?—e.g., for work, for food, for safety (e.g., against flood, drought, conflict)?
- What are the things you do to protect your home (against predators, animals, outsiders, bad weather)?

Work

- What kind of work or activities do you do? What do other household members do? Where do these activities happen?
- How do you protect your activities and business interests? (e.g., grazing or crop land, business competition, hunting territory, animal stock)

Travel

- How far do household members travel from home and why? (Follow up on animal related issues: shopping, selling/buying/trading, hunting, transport, etc.)
- How travel (by foot, bike, cart, truck, plane)? Is it ever for overnight? Where stay?
- Why traveling? (work/migrant, family, religion, holidays, to sell/trade/buy animals)
- Other family members in other areas of the country? Visit often?

Observed Environment

- Have there been any changes in the environment: new roads, more boats or ports, fields, buildings, population movement (in or out), land clearing or abandonment, new houses, other new buildings
- Who is responsible for the changes? Are the changes good or bad?

2. Socioeconomics

To understand a typical day and how money and social standing impact opportunity and risk.

Daily Routine

- Tell me about your daily routine (get description of work on a usual day, include purchasing and preparing food, timing of types of meals, responsibilities/duties related to animals, any changes by season)
- How do people in the household contribute to earning money and getting food (and water)?
- Where do the children play? Who takes care of the children when you are at work?

Animal Responsibilities

- Describe the animal related jobs and responsibilities for people at every age (i.e., young children, older children, young adults, adults, elderly).
- What are the skills/knowledge needed before moving to the next stage of duties/responsibilities?
- Are there differences in responsibilities between boys and girls, men and women, by ethnicity or class?

Education

- How many children are currently in school? Until what age do your children go to school? (boys and girls?)
- What is your level of education? Why did you stop?

Economics

- Do you make more money than other people who do the same things as you? Why do you think that is?
- Are there times of year when you make less money? What happens then?
- Are there times when food is more expensive than others? Tell me about that (eg, different food availability, seasonal, festival related).
- Do you think you and your household are better off than most people? Could you do things to make it better?

3. Biosecurity in Human Environments

To determine if any sanitation or hygiene factors could play a role in disease spillover

Water and Food

- Is there a central source of water? What is the source? (eg, pond, uncovered well, rainwater, taps, covered well)
- Is there a water source you like better?
- How far away is the water source? Do animals drink from the same source?
- Do you do anything to your drinking water to clean it before you drink it?
- How do you store your food? (e.g., open containers, covered, hanging, refrigerate)
- Do you eat or drink things where you suspect animal contact? (e.g., teeth/scratch marks, feces or urine seen)
- Do you regularly clean your food prep station/kitchen and tools? How?

Sanitation

- Are there toilets, latrines or other designated areas for human waste? Are these cleaned and used regularly?
- Are butchering and slaughtering areas separate? How often are they cleaned and how? Who does the cleaning?
- Are there any official rules or laws about human waste and garbage disposal?
- Are there any animal pest control laws? What do you do to control animal pests?

Hygiene

- When are the best times to wash your hands? Do you use soap? How much does soap cost and where get it?
- Do you wash your hands at home? at work?
- How often and where do you and your household members bathe?

4. Illness, Medical Care/Treatment, Death

To identify any unusual disease experiences—signs, symptoms and sources

Household Illness

- Is anyone sick right now?
- What do you do when someone in the household gets sick? Who takes care of that person?
- The last time someone was seriously sick what happened (explore when, with what, how did they get sick, who told/consulted, anyone else get sick after, final outcome)?
- Has anyone ever had an sickness that people don't usually get? What happened? Where did it come from?

Illness from Animals

- Do you know anyone who has gotten sick from an animal? What animal? What did they get? What happened? Do you know any other diseases/illnesses people can get from animals? How does the animal give the illness to the person? How often does it happen?

Medical Care/Treatment

- How sick would you have to feel to stay home and not do normal routine?
- Where do you go when you are sick?
- Do you prefer to use traditional medicine, western medicine or a combination?
- How sick would you have to feel to go to doctor/clinic/hospital? What does that cost? (in time, lost wages/business, transport costs, etc.) How far away?

Death

- What is the tradition when someone dies? (Explore if reported to authorities, differ by age or gender, what happens to the body, does the community come together or is it private.)

5. Human Animal Contact

To gain knowledge about interactions with animals, animal health and animal perceptions and knowledge

Encourage but don't lead discussion about which animals. Allow respondent to name the animals. If no birds or bats are mentioned, follow up by asking specific questions about birds and bats.

Indirect Contact

- What kind of meat do people in your household eat? How do you get it/where does it come from? What is furthest away an animal comes from?
- Is meat dead or alive when you get it? If dead(/prepared), how to tell if good/fresh?
- If alive, how long are live animals kept before being sold or eaten? How do you get live animals home?
- How is meat prepared (raw/undercooked)? Is meat prepared in the same place as other activities? (e.g., preparing vegetables, cleaning babies/changing diapers, where other food or drinking water is stored)
- Do animals come in or near the dwelling? How do you know animals are there? Which animals?

Direct Contact

- Do you or someone in your household handle live animals? In what context? (e.g. ranching/animal husbandry, hunting, wet markets, work, around dwelling/other building, pets)
- What are the animals that you keep/raise or sell? How many different kinds of animals? How many of each?
- For how long do you have the animals?
- Where do live animals come from? Where is the furthest away an animal comes from?

- Who buys/trades for your live animals? Where do the animals go?
- Have you been bitten, scratched or had bleeding after handling an animal? By a wild animal?
- Where are live animals slaughtered? butchered? Do people buy or sell parts?
- Do you travel with animals? Explore details of the process, specific routes and encounters (eg, with other animals, with animal transport supporting industries, such as holding areas, restaurants, hotels) along the way.
- Explore for differences over time in animal handling, eg, seasonality, legal, religious, animal reproduction

Animal Products/Rituals

- Other uses of animals—e.g., as pets, medicine, magic, fertilizer, for trading
- Rules for children around wild animals as pets, playing with wild animals or dead animals

Animal Health

- How do you care for your animals: how are they fed, what do they eat, where do they eat/graze and sleep? Are they segregated or all together? Differences by season? day/night? Does anyone live or stay with the animals?
- Is there a central area for animal waste? How often are animal cages, stalls, or penned areas cleaned? Who cleans them?
- Do the animals get veterinary care? Vaccinations?
- How do you know when an animal is sick? What's the first thing you do about a sick animal?
- Have you seen an animal outbreak or die-off? What happened?

Perceptions and Knowledge

- What are the most unusual animals anyone can buy?—seasonal? Expensive? Who buys?
- Are there any animals you avoid eating? Why? Ever heard of anyone eating/selling dead or infected animals?
- Do people ever eat non-domesticated animals/wildlife? Where do they get them?
- Who usually buys wildlife products? Have there been changes over time?
- What do you do when you find a dead animal?
- What laws about animals do you know? (e.g., limiting/outlawing hunting, reporting and culling of sick animals)

Section 8. Appendix V. Interview Checklist

Participant ID: _____

Interviewer: _____

INTERVIEW CHECKLIST

PREDICT-2 Spillover Pathways	
<input type="checkbox"/> Land conversion for commercialization <input type="checkbox"/> Intensification of animal production systems <input type="checkbox"/> Animal value chains	
CORE THEMES	
<input type="checkbox"/> Human movement <input type="checkbox"/> Home <input type="checkbox"/> Work <input type="checkbox"/> Travel <input type="checkbox"/> Observed environment <input type="checkbox"/> Socioeconomics <input type="checkbox"/> Daily routine <input type="checkbox"/> Animal responsibilities <input type="checkbox"/> Education <input type="checkbox"/> Economics <input type="checkbox"/> Biosecurity in human environments <input type="checkbox"/> Water and food <input type="checkbox"/> Sanitation <input type="checkbox"/> Hygiene	<input type="checkbox"/> Illness, medical care/treatment and death <input type="checkbox"/> Household illness <input type="checkbox"/> Illness from animals <input type="checkbox"/> Medical care/treatment <input type="checkbox"/> Death <input type="checkbox"/> Human-animal contact <input type="checkbox"/> Indirect contact <input type="checkbox"/> Direct contact <input type="checkbox"/> Animal products/rituals <input type="checkbox"/> Animal health <input type="checkbox"/> Perceptions/knowledge

Section 9. Appendix VI. Ethnographic Interview Excerpts

Two Examples of Good Ethnographic Interviews

1) WOMAN AGED 52

INT: What are the kinds of jobs that children have with animals. You were just talking about your first cow.

NYCQ01: I loved the cow. I told my mom I want this cow for myself and she said ok she was going to sell it and I said no. I said I want the cow. She gave it to me. She says your responsibility. When the cow first dropped, she gave me the little calf. I started...I didn't know how to milk and I started to shoot the milk into my mouth. It was funny but I enjoy it. I continue selling milk take the money put it away then I bought the other cow. Same thing I do and I started multiplying cows. Then my mom she decided to give me some money. If you want another cow you could buy. It was so cheap. Then I bought it. When I bought he cow I enjoyed doing the things I do. I had one little sheep that my uncle gived to me. I raised the sheep and sheep is not getting big and I started to plead with the sheep "get big." I got another sheep and I took the money from the milk and bought another one and started to raise them. So after I started to do that I ended up with about 15 cows and about 12 sheep no goat sheep. I started to selling because I am selling tomatoes, spinach, mangoes. I am selling all kinds of things and making the money. So I finally don't want to go back to school.

2) MAN AGED???

INT: Do you know who got an infectious disease from an animal?

CHY26: I don't know, I also have no education, I haven't heard of that kind of things.

INT: Do you know animals can spread diseases?

CHY26: No, I used to hear from veterinarian, but I don't know

Later in the interview...

INT: How do you kill cows, introduce the entire process to me.

CHY26: Tie the rope and with one stroke of the hammer the cow will fall down. Then take out the blood, and then from the chest kill like a pig kill. It will be similar to killing a pig.

INT: What do you do once the cow is dead?

CHY26: Some people want to take skin, use hot boiling water directly. Some don't, then put the skins, put out in the garbage. In addition there is ox hair, cow excrement, other things that can be used. Nothing will be wasted.

INT: How do you treat the skin of the cows?

CHY26: They buy them to make leather. Cowhide can sell for three to four hundred yuan.

INT: How long does it take to kill a cow?

CHY26: Depends on the size of the cow, if you need to peel the skin off. Small one maybe two hours, if bigger, it will take more than three hours.

INT: How many people do you need to kill a cow?

CHY26: At least three people, one person is not enough. We have to pay attention to health, keep the meat clean.

Example of Bad Ethnographic Interview

INT: How often do you kill a cattle?

CHY24: About ten days, thirty-one a year.

INT: Do you usually buy it in local market?

CHY24: We prefer to buy cattle in local farm, because they are large-scale farms, Well, we trust it. In contrast, the beef in the market may have problems.

Interviewer should have asked: WHAT KIND OF PROBLEMS? WHY ARE LARGE SCALE FARMS BETTER/ ARE THERE TIMES WHEN LARGE SCALE FARMS COULD BE BAD?

INT: How do you treat the polluted water after slaughter?

CHY24: There are some special place to treat them.

Interviewer should have asked: WHAT SPECIAL PLACES? WHAT EXACTLY IS DONE?

INT: Burn it?

Interviewer should recognize: STILL NOT ANSWERED. THE INTERVIEWER SHOULD CONTINUE ASKING ANYTHING ELSE?

CHY24: Ah, offal could be the beast manure.

Interviewer should have asked: IS IT USED WITH ANY SPECIAL CROP? WHAT OTHER THINGS ARE USED AS MANURE?

INT: You have opened restaurant for so many years, have you contacted with any other animals?

CHY24: Well we are Muslims, so cattle, sheep, chickens, fish, geese and ducks are rare for us.

Interviewer should have asked: WHY ARE THESE FOODS RARE?

INT: Sheep?

CHY24: We don't buy it.

Interviewer should have asked: WHAT ANIMALS DO YOU HAVE AT THE RESTAURANT? ARE THEY LIVE ANIMALS? DO YOU KILL THEM AT THE RESTAURANT? WHERE? KILLED IN SPECIAL WAY BECAUSE YOU ARE MUSLIM? THERE ARE TONS OF QUESTIONS TO BE ASKED. INSTEAD, THE INTERVIEWER ASKS ABOUT TRAVEL...

INT: Do you travel every year?

CHY24: Child is too young, only three or four, he-he, so we did not go out anymore, only the local neighborhood around it, there is no time.

INT: Do you have holiday?

CHY24: Annual Eid Well, just the same as your Spring Festival.

INT: Any rituals?

CHY24: If I told you, you will also not understand it.

EVERY TIME SOMEONE SAYS 'YOU WILL NOT UNDERSTAND' IT IS THE INTERVIEWER'S RESPONSIBILITY TO ASK MORE QUESTIONS. FOR EXAMPLE ANY OF THESE SENTENCES COULD WORK: I AM VERY CURIOUS, I WOULD LIKE TO LEARN MORE ABOUT THIS. I WOULD LIKE TO UNDERSTAND.

Section 10. Appendix VII. Suggested Coding Key Words

Human Movement
Home
Dwelling, living quarters, sleeping quarters
Children, family
Daily movement/travel
Flood
Drought
Conflict
Protection from predators/ animals
Safety
Religion
Work
Work activities
Agriculture areas
Grazing areas
Hunting territories
Boundaries
Livestock areas
Markets
Crops
Business
Travel
Traveling to Shop/buy/sell/trade
Hunting trips
Transporting animals
Transportation: Walking, biking, cart, truck, plane, boat, trains
Overnight trips
Reasons for travel
Travel destinations
Border crossings
Travel obstacles/issues
Transportation of resources/moving
Observed Environment
Town roads/ports/ trains
New buildings/roads/construction
Route changes
Abandoned land

Socioeconomics
Daily routine
Meal preparation
Shopping
Childcare
Market trips
Groceries
Purchases
Errands
Animal responsibilities
Animal duties/responsibilities
Feeding/grazing
Tasks/roles by age or gender
Sick animals
Slaughtering/Butchering
Education
School/education/graduation
Reading/understanding numbers
Dropping out
Economics
Livelihood
Earning/earning changes throughout year
Large purchases
Income
Purchases for event/holiday
Social standing (compared to Neighbors/others)
Expenses
Number of jobs/activities

Biosecurity in Human Environments
Water and food
Water source (where does it come from?)
Water taste/quality/purification
Rain/rainwater/water taps/well
Storing food/storing water
Pests/rats/pesticides/cockroaches/insects
Kitchen
Cleaning
Water usage
Sanitation
Waste management/garbage
Toilets/latrines/bathroom
Cleaning bathroom/kitchen
Feces
Urine
Pesticides
Hygiene
Washing hands
Showering/bathing
Soap
Leave shoes/footwear outside

Illness, Medical Care/ Treatment and Death

Household illness/Wellness

Sick relatives
Caretaking of sick
Types of sickness
Unusual illness
Symptoms of illness (fever, bleeding, difficulty breathing, etc....)
Ebola
SARS
MERS
(Other endemic zoonotic diseases)
Dispensaries/medication
Births

Illness from animals

Illness from animals

Medical Care and Treatment

Doctor/clinic visit
Medicine/Treatment
Cost of medicine/doctor/treatment
Professionals (doctor, nurse, religious leader, healthcare worker etc...)
Traditional medicine
Ethno botany
Healthcare protocols

Death

Reporting death
Burial/ burial rites
Funeral tradition/rites
Dead body/corpse
Body preparation

Human Animal Contact

Indirect Contact/Food:

Meat/animal consumption
Acquisition of meat
Preparing meat
Meat/animal storage
Butchering
Animal taboos
Infected animals
Wildlife consumption
Purchasing meat or wildlife
Cleaning up after animals
Meat/dead animal markets
Animals around dwelling/pests
Signs of animals (hear, smell)
Feces
Animal tracks
Garbage disturbance
Observed animals
Hunting

Direct Contact

Ownership of animals
Live animals
Pets
Playing with animals (wild or domestic, alive or dead)
Animal caretaking
Feeding animals
Grazing animals
Working with animals
Live animal markets/wet markets
Ranching
Animal husbandry
Buying/selling/trading live animals

Bite
Scratch
Animal handling
Killing live animals/slaughtering
Handling of wildlife

Animal products/rites

Animal byproducts (milk, leather, magic, medical)
Magic involving animals
Fertilizer

Animal health

Animals eating/sleeping/grazing
Sick animals
Animal caretaking activities/roles
Animal waste
Cleaning animal areas
Veterinary care
Vaccinations
Outbreak
Die off

Perceptions and knowledge

Exotic or expensive animals
Wildlife consumption
Regulations/laws regarding animals (e.g., Hunting, eating, poaching regulations)
Danger from animals
Conservation
Taboos
Special occasions/holidays
feasts/ holy days

Section 11. Appendix VIII. Examples of Coded Text

Example 1:

INT: Do you live in local ? How old are you? And how many people in your family?

CHY22: Yes, i am 45 years old. There are four people in my family, two girls, my wife and I. The older girl is selling water filter in Chuxiong, Yunnan Province. And the young girl is in grade 3 in middle school, and she is the top one in her class.

INT: That's great, at what age does she start school? And how about her tuition?

CHY22: Seven years old, and we paid for her tuition several years ago, school sponsored her these years.

INT: Should you your child to school? And how?

CHY22: When she was in primary school, she got to school and back by herself, and we sent her to school when she got to middle school by motor.

INT: How long do you live in here? When was this house built? And where is the material from?

CHY22: For all of my life. The house was built 3 years ago. We save the material each year, and it take a whole year to complete, get help to build the house, dig the foundation, the structure is armored concrete, all of it take about 85,000 yuan,.

INT: That's not a decimal, what's your work in detail? And do you have farmland?

CHY22: Part-time job, such as carrying bricks, constructing and so on, I have 2.8 mu farmland at home.

Aleksei MacDurian 7/11/2015 11:36 AM
Comment [1]: Biosecurity in Human Environments – Water and Food – Water Source

Aleksei MacDurian 7/11/2015 11:36 AM
Comment [2]: Human Movement – Work – Work activities

Aleksei MacDurian 7/11/2015 11:36 AM
Comment [3]: Socioeconomics – Daily Routine - Childcare

Maureen Miller 7/17/2015 10:34 AM
Comment [4]: Human movement: observed environment: new buildings

Aleksei MacDurian 7/11/2015 11:40 AM
Comment [5]: ~ one half of an acre or ~ one fifth of an hectare

Example 2:

INT: What kind of wild animals have you ever contact with in your work, and what kind of wild animals have you grabbed?

CHY30: Rodents are mainly to be grasped,rats including house ones and wild ones.Main species are yellow brown rats, brown rats, Gao Shanji mouse, the mouse, the older kyi mouse, younger kyi mouse and so on, other special kinds are such as squirrel and weasel and some other climbing kinds.We have caught wild animals all voer the Yunnan province .

INT: Where did you catch the bats ?

CHY30: So many, we have been to the caves of Anning, Jinning, Baoshan and Mojiang to grab the bats. We also have been to Xishuangbanna .We grab the bat with a mist net.The bat like living in damp cave and like the poly group life.I have been to several times, among the bats, Hipposideridae and Rousettusleschenau are the most.Rousettusleschenau were caught in in Ruili.After catching bat ,we need not only sample, but also cut the vessel of wings to sample the blood bats can not fly after sampling ,and died.Some people also use torches to burn when grasping, or with a bamboo pole,set off firecrackers to scare the bats.There are no bats in the place where we have ever grabbed the bats .They also take the bat's brain, feces and urine.

INT: Have you ever seen someone live in the cave or near the cave where to the bats live in?

CHY30:No,I haven't, but there are mine workers getting in and out of a cave in a small town of Honghe. They work inside the cave during the day ,but they doesn't live there at night , they probably contact with the bats. But I don't know whether they fall ill.

Maureen Miller 7/17/2015 10:42 AM
Comment [6]: Economics:livelihood

Karissa Whiting 6/30/2015 9:44 AM
Comment [7]: HA Contact: direct contact: working with animals, animal handling, handling of wildlife

Karissa Whiting 6/30/2015 9:43 AM
Comment [8]: HA Contact: direct contact: working with animals, animal handling, handling of wildlife

Maureen Miller 7/17/2015 10:40 AM
Comment [9]: HA Contact: direct contact: working with animals, Economics: livelihood

Maureen Miller 7/17/2015 10:38 AM
Comment [10]: Illness from animals

Example 3:

INT: Which animal raised in your family?

CHY22: Ten hen and cocks raised by my wife.

INT: Injected in vaccine?

CHY22: Yes.

INT: Raised for chicken?

CHY22: Yes.

INT: And when do you have chicken?

CHY22: The time when relatives visited and the Spring Festival.

INT: How long can the cocks been eat?

CHY22: About eight months.

INT: Where to get the young chicken?

CHY22: Bought on the market.

INT: Do you raise other animals?

CHY22: A dog, it was three years old.

Aleksei MacDurian 7/11/2015 11:48 AM
Comment [11]: Human Animal Contact – Direct Contact - Ownership of animals

Aleksei MacDurian 7/11/2015 11:49 AM
Comment [12]: Human Animal Contact – Animal Health – Vaccinations

Aleksei MacDurian 7/11/2015 1:10 PM
Comment [13]: Socioeconomics – Economics – Purchases for event/holiday

Aleksei MacDurian 7/11/2015 1:11 PM
Comment [14]: Socioeconomics – Economics – Purchases for event/holiday

Section 12. Appendix IX. Ethnographic Interview Summary Document Examples

INTERVIEW ID: CHY26
MAN AGED ??

Summary

Human Movement p 2

The interviewee takes the cattle to town in a lorry for slaughter. He usually takes about 5-6 large ones and 8-10 smaller ones. The cattle market is about 1 kilometer away.

Socioeconomics pp 1-2

He finished one grade, while his wife graduated from primary school. He considers himself to be of a middle economic level as compared to others in his village. His income varies throughout the year with the rise and fall in prices of meat. He considers wild animals to be too expensive to eat.

Biosecurity in Human Environments pp1-2

They drink from a local spring and use the tap water to cook. Depending on the amount of people in the house, they will collect garbage anywhere from 1-3x a week.

He carries a water tank with him in order to wash his hands.

Very hot water is used during the slaughtering of animals.

Illness, medical care/treatment and death p 7

His hand was seriously injured. He sawed off 4 fingers. He was treated at the best orthopedic hospital in the province.

He has heard of animal infectious diseases from his veterinarian, but does not really know what they are.

He will go to the hospital and take medication if necessary.

He briefly explains the burial practices surrounding his father's death. His body remained in the house for 5 days.

Human Animal Contact pp 2, 8, 10-11

He raises about 20-30 cattle and chickens. They will kill cows for special occasions (e.g. weddings).

They only consume "regular" animals (especially pork), not wildlife. He feels wildlife is too expensive.

They slaughter their own chickens and cattle. It usually takes 3 people to slaughter a cow because of the necessary health precautions that need to be taken. He has previously been hurt during the slaughter process.

He has also been bitten by a dog on the leg a long time ago.

He will not purchase cattle that have not been vaccinated. He refers to the governmental regulations that exist on vaccinating animals.

SPECIFIC QUOTES

Page 2: **Human animal contact:** *Indirect contact/food:* acquisition of meat, purchasing meat or wildlife;
Socioeconomics: *daily routine:* purchases; **Human animal contact:** *direct contact:* buying/selling/trading live animals; **Socioeconomics:** *daily routine:* purchases

CHY26: Both, mostly I buy the killed ones. Some are not suited to eat, for example-calf, then I would make a change-buying some sheep. If the big cattle are not fat, we would buy them back to raise for some days.

Page 7: **Illness, Medical Care, Treatment and Death:** *Illness from animals:* Illness from animals

INT: Do you know someone got animal infectious disease?
CHY26: I don't know, I also have no culture, I haven't heard of that kind of things.
INT: Do you know animals can spread diseases?
CHY26: No, I used to hear from veterinarian, but I don't know

Page 10: **Human animal contact:** *direct contact:* killing live animals/slaughtering; **Biosecurity in human environments:** *water and food:* cleaning

INT: How many people do you need to kill a cow?
CHY26: At least three people, one person is not enough, we have to pay attention to health, keep the meat clean.

Page 8: **Human animal contact:** *indirect contact:* meat/animal consumption, wildlife consumption; **Human animal contact:** *perceptions and knowledge:* exotic or expensive animals; **Socioeconomics:** *economics*

INT: Eat wild animals' meat?
CHY26: Do not eat, expensive!
Page 12: **Human animal contact:** *indirect contact/food:* meat/animal consumption
INT: What meat your family don't eat?
No, eat any kind of meat!

Page 10: **Human animal contact:** *direct contact:* animal handling, killing live animals/slaughtering **Human animal contact:** *direct contact:* bite

INT: Have you been hurt in the process of killing cattle?
CHY26: Yes, we do cattle business, some cattle temperament is bad
INT: Have you been hurt by other animals?
CHY26: Bitten by a dog
Page 11: **Human animal contact:** *animal health:* vaccinations; **Human animal contact:** *perceptions and knowledge:* regulations/laws regarding animals
INT: Usually play the vaccine?
CHY26: Yes, when we go to the farmers to buy we will ask whether the cattle ever been play with a vaccine, if the cattle haven't been treat with any vaccine, we don't buy, afraid of an accident, if the government pursue, we will be in big trouble



PREDICT QUIZZES

TABLE OF CONTENTS

General Information

- Basic Laboratory Safety
- Biosafety and PPE Use
- Emergency Preparedness
- Implementing Cold Chain for Safe Sample Transport and Storage
- Packing and Shipping Biological Samples

Field Sampling Guides

- Avian Sampling Methods
- Bat Sampling Methods
- Bushmeat Sampling Methods
- Livestock Sampling Methods
- Non-Human Primate Sampling Methods
- Orangutan Sampling Methods
- Rodent Sampling Methods
- Safe Animal Capture and Sampling
- Small Carnivore Sampling Methods

Qualitative Research Guides

- Qualitative Research: Introduction & Observational Research Methods
- Qualitative Research: Focus Groups, Ethnographic Interviews, & Data Analysis

This document was made possible by the generous support of the American people through the United States Agency for International Development (USAID) Emerging zoonotic and Pandemic Threats PREDICT program. It was drafted to support activities conducted under PREDICT and is intended for an audience of qualified professionals trained in standard, associated best practices. This guide is not intended for use by untrained individuals.

All quizzes in this document are accompanied with answers based on information shared throughout the training guides. Contact predict.ucdavis.edu to access the *PREDICT One Health Surveillance Solutions Manual*.

Quiz – Basic Laboratory Safety

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Basic Laboratory Safety* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. PREDICT laboratory principles include:

- a. Comply with the PREDICT Environmental Compliance Protocol and local and national safety and health requirements
- b. Ensure all PREDICT personnel understand relevant safe and healthy work practices
- c. Never use glassware
- d. Periodically review and evaluate plans, facilities, equipment and activities to ensure that safety and health objectives are achieved

2. If you use PPE kits, it is a good idea to have a contents list with each PPE kit.

- True False

3. Broken glass should be disposed of in a sharps container.

- True False

4. After you have removed PPE is it not necessary to wash your hands.

- True False

5. In the laboratory, gloves are the only required PPE.

- True False

6. Dry ice, if handled improperly, poses the following risks:

- a. Asphyxiation
- b. Zoonoses transmission
- c. Burns
- d. Toxic exposure

7. Caution must be exercised when using bleach as a disinfectant because bleach:

- a. May freeze the skin
- b. May explode when mixed with water
- c. May burn the skin
- d. May cause respiratory irritation

e. Bleach reacts with Trizol to form a toxic gas and the two chemicals should not be mixed

8. Highly hazardous materials are chemicals, toxics, and reactivities that have the potential to cause immediate and permanent harm at feasible exposure levels.

True False

9. The components of Personal Protective Equipment (PPE) required for a task depend on the exposure and other hazards associated with the tasks.

True False

10. It is not necessary to read the Material Safety Data Sheet for a chemical you use, as long as it is on file as a reference in the office.

True False

11. Country Coordinators must ensure that personnel have reviewed the MSDS for materials they will be using.

True False

12. An MSDS contains the following information:

- a. A list of local hospitals that can handle a chemical accident victim
- b. First aid measures for exposure the material
- c. Physical and chemical properties of the material
- d. Disposal considerations for the material

13. Other information contained in a MSDS regarding a material includes:

- a. Exposure controls and personal protection
- b. Transport information
- c. Identification information including the manufacturer names and emergency phone numbers
- d. A list of materials that are safer alternatives

14. There are online sources for downloading MSDS for materials.

True False

15. Needlestick injuries are not a risk if you wear two pairs of nitrile gloves.

True False

16. After using a needle and syringe, it is a good safety procedure to bend the tip of the needle.

True False

17. If a needlestick injury occurs, you must report it to your PREDICT supervisor.

True False

18. Biosafety Level 1 (BSL-1) is required for the most hazardous pathogens.

True False

19. Biosafety Level 2 (BSL-2) is more restrictive than BSL-1 and is suitable for work involving agents of moderate potential hazard to personnel and the environment.

True False

20. BSL-2 laboratories include the following special practices:

- a. Access to the laboratory should be restricted when work with infectious agents is in progress
- b. A high degree of precaution must be taken when handling sharps such as syringes, slides, pipettes, capillary tubes and scalpels
- c. There should be no eating area in the laboratory
- d. Broken glassware must be picked up quickly

21. PREDICT samples should be handled and RNA/DNA extraction performed in a Biosafety Level 2 (BSL-2) laboratory in a biosafety cabinet.

True False

22. Containers for sharps disposal should be easily accessible in the laboratory.

True False

23. Handling TRIzol has a moderate risk associated with illness or injury.

True False

24. PPE should be worn when handling TRIzol.

True False

25. TRIzol should be aliquoted into vials for use in the field in a biosafety cabinet or fume hood.

True False

Quiz - Biosafety and PPE Use

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Biosafety and PPE Use* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. With regard to biosafety and PPE use, individuals:

- a. Have primary responsibility for their own health and safety
- b. Are required to follow PREDICT safety protocols for activities that involve potential exposure to infectious pathogens
- c. Are required to use appropriate PPE
- d. Are required to take PPE home to wash after each use

2. PREDICT Country Coordinators are responsible for the following:

- a. Documenting and reporting all training activities to PREDICT Management
- b. Ensuring activities are conducted in compliance with PREDICT Environmental Mitigation and Monitoring Plan
- c. Ensuring that all personnel are trained on the safe use of equipment that they must use for PREDICT activities
- d. Reporting injuries and accidents to PREDICT Management

3. General zoonotic pathogen biosafety precautions include:

- a. Informing all persons who may enter a potential zoonotic pathogen risk area of their potential for exposure
- b. Reusing all PPE supplies
- c. Washing hands after removing PPE
- d. When seeking medical advice for any illness, informing your doctor of your work with humans and animals

4. One of the most important biosafety precautions is:

- a. Washing your laboratory coat at home every night
- b. Proper and frequent hand washing
- c. Washing foods carefully before eating in the laboratory
- d. Avoiding handling animals

5. When assessing biological risk to determine the necessary PPE one factor that is not included is the following:

- a. Pathogenicity of likely pathogens
- b. Routes of infection
- c. Time available to perform the activity
- d. Availability of effective prophylaxis or treatment interventions

6. Primates do not pose significant risk of zoonotic pathogen transmission because they have similar immune systems as humans.

- True False

7. A person may work many hours in PPE coveralls, mask and goggles in warm environments because the white suits reflect heat.

- True False

8. The following are important considerations when using PPE:

- a. A designated decontamination area should be established before putting on PPE
- b. Personnel should carry a water bottle when wearing PPE so they do not have to remove PPE when they are thirsty
- c. When workers are heat stressed, uncomfortable or unable to see out of fogged goggles, they are more likely to remove PPE in an unsafe environment
- d. N95 respirators may only be used following proper fit testing

9. Supplies to bring when planning to use PPE in the field should include:

- a. Disinfectants
- b. Large disposal bags for contaminated PPE
- c. Bottled drinking water for drinking before and after the use of PPE
- d. Adequate sets of PPE for all personnel that may participate in the field activities that have a risk of exposure to infectious pathogens

10. The N95 respirator is another name for a surgical mask.

- True False

11. Checking the seal of a N95 respirator before each use is also called fit testing.

- True False

12. Rubber boots and plastic goggles may be reused if they are disinfected between uses.

- True False

13. The head strap of goggles should be checked for fit prior to putting on PPE.

True False

14. After placing used PPE into a medical waste bag, the bag should be left open to allow ventilation.

True False

15. The N95 mask should always be put on after the hood and goggles.

True False

16. When ordering PPE supplies, it is important to order fog-free goggles because it is difficult to draw blood and do other activities with goggles that tend to fog up.

True False

17. Proper hand washing procedures after removing PPE include rubbing hands in soap and water and using a towel to turn off the faucet.

True False

18. The supervisor should make sure that any staff wearing a N95 mask has no difficulty breathing.

True False

Quiz - Emergency Preparedness

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Emergency Preparedness* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. Steps for emergency planning should include:

- a. Understand the hazards and issues
- b. Evaluate the risks
- c. Develop an emergency communications plan
- d. Monitor the local situation in the event something changes

2. An emergency communications plan should include information regarding:

- a. Local emergency number
- b. Nearest clinic or human health care provider
- c. Nearest airport
- d. Local points of contact not with the field team

3. Field personnel emergency information records should include information on:

- a. Allergies
- b. Blood type
- c. Health insurance
- d. Medical conditions

4. All personnel health records must be guarded with the strictest confidentiality as directed by institutional requirements.

- True False

5. All PREDICT partner institutions are responsible for appropriately managing general occupational health programs for their staff both domestically and abroad.

- True False

6. With regard to the Emergency Communications Plan, it is critical to have a well-informed understanding of what communications will be available at the field site and having a back-up plan for communication in the event of an emergency.

- True False

7. The following are important components of emergency planning:

- a. Field personnel emergency information records should be prepared prior to field activities
- b. Field teams should all have at least two members who are properly trained in basic First Aid techniques including cardiopulmonary resuscitation (CPR) and wound management
- c. Hazards should be identified and evaluated before EACH field activity, and plans should be developed appropriately
- d. The Country Coordinator or field supervisor should ensure that personnel have consulted with a human health worker with regard to the immunizations required prior to participating in fieldwork

8. On the job accident or injury requiring only basic medical attention does not need to be reported.

- True False

9. Only a human health professional can recommend and provide vaccination and immunizations to personnel.

- True False

Quiz – Implementing a Cold Chain for Safe Sample Transport and Storage

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Implementing a Cold Chain for Safe Sample Transport and Storage* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. What is the goal of having a cold chain for biological materials?

- a. To keep a sample or material within a certain temperature range during delivery
- b. To keep a sample or material within a certain temperature range during processing
- c. To keep a sample or material within a certain temperature range during storage
- d. All of the above

2. What are the different ways to maintain cold chain for biological materials? (Select all that apply)

- a. Cooler with dry ice
- b. Liquid nitrogen
- c. Ultra low freezer (-80°C and colder)
- d. A domestic kitchen freezer

3. The following are important steps in using cold boxes and coolers:

- a. Never rotate ice/gel packs in the cold box or cooler
- b. It is okay to store the cold box or cooler in your kitchen
- c. It is okay to continuously open the cold box or cooler to check the temperature inside
- d. None of the above

4. The following are important steps when using dry ice:

- a. Place dry ice and sample containers as close together as possible
- b. Always pack samples in a good insulated container
- c. Dry ice blocks take longer to evaporate and are better at maintaining samples frozen for longer storage/transit periods
- d. All of the above

5. What is the recommended ratio of dry ice to samples to ensure effective temperature control?

- a. Use a minimum 2 kg of dry ice for each 1 kg of samples for every 48-hour transit period
- b. Use a minimum 1 kg of dry ice for each 1 kg of samples for every 24-hour transit period
- c. Use a minimum 1 kg of dry ice for each 2 kg of samples for every 12-hour transit period
- d. None of the above

6. The following are important steps for filling dry shippers/vacuum flasks:

- a. Always use the appropriate PPE
- b. It is okay to overfill the container
- c. Remove all free liquid nitrogen from the container prior to transport
- d. None of the above

7. It is important to work with dry ice and liquid nitrogen in well ventilated areas due to the gasses they give off which can cause asphyxiation.

- True False

8. Dry ice and liquid nitrogen can cause an explosion if the gasses are not allowed to vent properly.

- True False

9. All samples stored in VTM and Trizol should be:

- a. Frozen in liquid nitrogen immediately in the field, if possible
- b. Stored in an ice box with gel packs at -20°C in the field for no longer than 48 hours
- c. Stored at room temperature for one week
- d. Transferred to a -80°C freezer within 48 hours, once back in the lab

10. It is not important to have a backup generator for a -80°C freezer if there is a good source of electricity.

- True False

11. What are some of the important steps in responding to a cold chain breach?

- a. Contact your PREDICT Country Coordinator
- b. Define the incident (discuss possible explanations for the breach)
- c. Evaluate performance of cold chain equipment
- d. If temperatures approach -30°C, begin planning for sample transfer to temporary cold boxes or coolers, or other laboratory facilities
- e. All of the above

Quiz – Packing and Shipping Biological Samples

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Packing and Shipping Biological Samples* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. The definition of “Infectious Substances, Category A” is: Samples collected from sources that are known to be infected with a pathogen.

True False

2. “Biological Substances, Category B” are biological materials that do not include any pathogens.

True False

3. Samples of Category A and Category B are legally considered to be “Dangerous Goods” and are regulated by the International Air Transport Association (IATA) and the United States Department of Transportation (DOT).

True False

4. Principles of safe packing and shipping of biological samples include:

- a. Minimizing the probability of inadvertent exposure to an infectious agent through shipping, importing or exporting samples
- b. Always reusing shipping materials to reduce waste
- c. Shipping only on weekdays
- d. Preventing human injury, as well as damage to the samples, the environment and property that can be caused by improper handling and packaging of storage and shipping materials that are flammable and/or toxic, and/or volatile

5. When exporting biological samples to the USA from another country you may need:

- a. An export permit for the country you are exporting from
- b. An import permit for the USA
- c. A UNFAO export permit
- d. A valid CITES I, II, III and/or Migratory Bird permit

6. PREDICT activities will involve primarily two hazard classes: Class 6.2—Infectious Substances, and Miscellaneous Dangerous Goods (dry ice).

True False

7. All biological samples are in the same hazard class under the United Nations classification system.

True False

8. The UN Number for a hazardous sample is based on the year and month of the shipment.

True False

9. The UN Number assigned to Infectious Substances, Category A is UN 2814, and its proper shipping name is "No Risk to Humans."

True False

10. One of the most common UN Numbers that will be used for samples collected and shipped for PREDICT is UN3373, Biological Substance, Category B.

True False

11. The proper shipping number and name for dry ice is UN 1845 "Carbon Dioxide solid" or "Dry Ice".

True False

12. Class 6.2 infectious substances may be packed in single packaging if it is a dry substance and double packaging if it is a liquid.

True False

13. According to IATA and DOT, dry ice is a safe substance and requires no special precautions.

True False

14. The outer shipping box for a biological sample must be able to drop from a height of 1.2 meters without suffering any damage.

True False

15. It is best to obtain the appropriate outer packing materials used by your carrier, such as DHL or Fedex.

True False

16. Always tape shut the lid of Styrofoam cartons to contain dry ice.

True False

17. The secondary packaging should be a leak-proof container.

True False

18. You should place an itemized list of the package contents between the Styrofoam packaging and the outer cardboard box.

True False

Quiz – Avian Sampling

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Avian Sampling* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. Samples to be collected for birds include:

- a. Two oral swabs, whole blood, serum, and tissue samples
- b. One oral swab, cloacal swab and/or fecal sample, whole blood, serum, and tissue samples
- c. One oral swab, cloacal swab and/or fecal sample, whole blood, serum
- d. Two oral swabs, cloacal swabs and/or fecal samples, whole blood, and serum samples, and sets of three tissue samples (in the case of dead birds).

2. The maximum safe quantity of blood that can be collected from live birds is:

- a. 0.6-0.9 cc of blood per 100 g of body mass
- b. 1 cc of blood per 100 g of body mass
- c. 0.3-0.6 cc of blood per 100 g of body mass, although collect as little as possible to conduct necessary testing
- d. 0.3-0.6 cc of blood per 100 g of body mass

3. Sites for venipuncture in birds include:

- a. Jugular vein
- b. Medial metatarsal vein
- c. Wing vein
- d. All of the above

4. Dead birds should be sampled only following all safety measures including proper use of PPE, proper work-station decontamination, and proper carcass disposal.

- True False

5. Serum samples should be stored in VTM and Trizol.

- True False

6. Tissue samples should be stored in:

- a. 1 mL Trizol
- b. One pea sized sample in 500 μ L VTM and the other in 1 mL Trizol
- c. No storage media; samples to be stored as is
- d. One pea sized sample frozen in 500 μ L VTM, one sample frozen in 1 mL Trizol, and one sample at room temperature in 10% buffered formalin.

7. Samples for histopathology should be collected at the abnormal margins to include both normal and abnormal sections in the same piece of tissue.

- True False

8. If a centrifuge is not available, you can allow clots and cells to settle as much as possible and then collect serum and save remaining blood clot in VTM and Trizol.

- True False

9. If there is no short-term access (i.e., within 24 hours) to cold chain such as in an emergency situation, then samples can be collected in 500 μ L of RNAlater and stored (check as many as apply):

- a. 1 day at 37 °C (i.e. ambient temp)
- b. 1 week at 37 °C (i.e. ambient temp)
- c. 1 week in the refrigerator
- d. Within one week freeze at -80 °C for storage until analysis

10. Cloacal cavities of small birds can be very shallow.

- True False

11. Birds are very susceptible to physiological shock and neurological inertia in response to stress of capture and handling.

- True False

12. Which of the following are important principles to adhere to in wild bird capture and sampling? (Check all that apply)

- a. Permits are not needed for capture of wild birds
- b. Capture and restraint techniques vary for different habitats and bird types
- c. Sampling near nesting sites is not a problem
- d. Traps and nets should be checked regularly
- e. Monitoring weather forecasts is important

Quiz – Bat Sampling

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Bat Sampling* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. The minimum PPE for bat sampling includes:

- a. Nitrile gloves
- b. Tyvek-like clothing
- c. N95 facemask
- d. Eye protection
- e. All of the above

2. Bats may be anesthetized with:

- a. Isoflurane
- b. Chloroform
- c. Medetomidine and Ketamine
- d. Ether

3. For larger bats, recommended bleeding sites include:

- a. Cephalic vein
- b. Jugular vein
- c. Cardiac
- d. Tail vein

4. For small bats, recommended bleeding methods include:

- a. Cardiac puncture
- b. Radial vein puncture
- c. Wing clip
- d. All of the above

5. For larger bats, three people are preferred for handling and sampling.

- True False

6. Following an injury in a person caused by a bat, the wound should be washed for at least 15 minutes and post-exposure rabies vaccination should be obtained as soon as possible.

- True False

7. For bats <100g, the maximum amount of blood that should be obtained per gram of body weight is:

- a. 2 μL
- b. 4 μL
- c. 6 μL
- d. 10 μL

8. All people handling bats or their blood products should be vaccinated for rabies and be aware of appropriate post exposure prophylaxis in the case of bites.

- True False

9. When obtaining a rectal swab, Trizol, but not VTM, can be used as a lubricant.

- True False

10. A bat is classified as an adult if the individual has the following characteristic:

- a. pregnant or lactating
- b. complete fusion of phalangeal symphysis
- c. clinging to the dam
- d. all the above

Quiz – Bushmeat Sampling

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Bushmeat Sampling* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. Proper procedures for collection of bushmeat samples include:

- a. Using sterile disposable or sterilized sample collection utensils before collecting each sample type
- b. Obtaining whole blood samples from the abdominal cavity of the carcass
- c. Collecting swab samples from fresh carcasses only
- d. Targeting samples from animals that have been dead for less than 24 hours to maximize pathogen detection

2. Important considerations for collection of bushmeat samples include:

- a. For animals too small to collect blood for both whole blood and serum, serum and the remaining clot can be utilized
- b. Freeze all samples (except tissue in formalin) in liquid nitrogen immediately in the field and transfer to -80°C freezer once back in the lab
- c. During sampling, be very careful not to contaminate carcasses with hazardous chemicals (e.g. Trizol or formalin) or to touch bushmeat with potentially contaminated gloved hands or non-sterile utensils
- d. After placement of swabs into the collection vials, keep them at room temperature indefinitely

3. Minimum PPE recommended for bushmeat sampling includes (Check all that apply)

- a. Double gloves
- b. Protective glasses
- c. N95 face mask
- d. None of the above

4. The preferred organ to sample for bushmeat is bladder.

- True False

5. PREDICT ethical policy does **not** permit payment or trade for obtaining bushmeat samples.

- True False

Quiz – Livestock Sampling

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Livestock Sampling* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. The Personal Protective Equipment that must be worn when collecting biological samples from animals includes:

- a. Nitrile exam gloves
- b. Dedicated clothing
- c. Safety glasses or other eye protection
- d. Large hats to protect against the sun

2. Animal handling must be done in a respectful and careful way, and animals should be monitored at all times for signs of excessive distress. If an animal is noted to be in excessive distress, staff should:

- a. Continue with the sampling, but exercising greater caution in handling the animal
- b. Blindfold the animal
- c. Provide adequate care to the animal and continue sampling when the animal has recovered.
- d. Stop all procedures, provide adequate support to the animal, and release the animal upon recovery

3. The following samples should be collected from each live animal whenever possible (select ONE best answer):

- a. Two fecal samples/rectal swabs, two nasal swabs, two whole blood samples, two serum samples, and two urogenital swabs/urine samples
- b. Two fecal samples/rectal swabs, two nasal swabs, and two whole blood samples
- c. Two serum samples, two urogenital swabs/urine samples, and two fecal samples/rectal swabs
- d. One whole blood sample, one serum sample, one nasal swab, and one fecal sample/rectal swab, and one urogenital swab/urine sample

4. Blood samples from cattle can be taken from the jugular vein (in cattle of all ages) or the lateral thoracic vein (in older cattle).

- True False

5. Sheep and goats should be bled at the tail (coccygeal) vein.

- True False

6. Blood samples can be taken from camels at the jugular vein, lateral thoracic vein, or caudal epigastric ("milk") vein.

- True False

7. Blood samples from swine can be collected from the external jugular vein, the cranial vena cava, or the caudal auricular ("marginal ear") vein.

- True False

8. What is the acceptable standard for fecal sample collection?

- a. Two ~200mg (pea-sized) samples, fresh from the rectum
 b. Two ~200mg (pea-sized) samples, fresh from the rectum or from the top part of a freshly passed fecal pat
 c. Two ~500mg (marble-sized) samples, fresh from the rectum
 d. Two ~500mg (marble-sized) samples, fresh from the rectum or from the top part of a freshly passed fecal pat

9. Which of the following is NOT part of the recommended technique for nasal swab collection?

- a. Use sterile, cotton-tipped swabs with a cardboard shaft
 b. Thoroughly saturate the swab tip against the walls of the animal's nares, 1-2" from the opening
 c. Place one swab in a cryovial with 500 μ L VTM and one swab in a cryovial with 500 μ L Trizol
 d. Store cryovials in a liquid nitrogen dry shipper or dewar and transfer to a -80°C freezer when possible

10. Which of the following is/are part of the preferred approach to urine/urogenital sample collection?

- a. Free-catch any urine the animal passes during handling. Add 500 μ L urine to each of two vials, one containing 500 μ L of VTM and the other containing 500 μ L Trizol
 b. If the animal passes urine under observation, pipette 1000 μ L urine from the puddle. Add 500 μ L urine to each of two vials, one containing 500 μ L of VTM and the other containing 500 μ L Trizol
 c. If urine is not available, collect two urogenital swabs, wrapping them in sterile plastic wrap to prevent desiccation
 d. If urine is not available, collect two urogenital swabs, placing one in a cryovial filled with 500 μ L VTM and the other in a tube containing 500 μ L Trizol

Quiz – Non-Human Primate Sampling

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Non-Human Primate Sampling* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. Which of the following is/are priority samples to collect from NHP for PREDICT:

- a. Feces or rectal swabs
- b. Hair
- c. Blood
- d. Oropharyngeal swabs

2. Which of the following PPE items are recommended when collecting samples from dead or live NHP?

- a. Nitrile gloves
- b. Eye protection (goggles or face shield)
- c. N95 (or better) respirator
- d. Tyvek-like suits
- d. All of the above

3. Which of the following statements about B virus is/are true?

- a. Exposure of PREDICT staff is not possible when handling live macaques
- b. Macaques can shed B virus in their oral, gingival, and genital mucosa
- c. If exposure is suspected (due to a bite, needlestick, facial splash, etc), work must stop immediately to initiate the B virus emergency exposure protocol
- d. All of the above

4. Live NHP should be chemically restrained during invasive sample collection.

- True False

5. Handling NHP involves special consideration because:

- a. NHP are typically very social animals and may defend other individuals in their groups
- b. NHP can carry pathogens that can cause fatal illnesses in humans including Ebola virus and B virus
- c. NHP may use their grasping hands and feet to grab (and then bite) handlers

d. Due to their close genetic relationship with humans, NHP are susceptible to many human pathogens such as respiratory viruses

6. The femoral vein is typically the best venipuncture site for small NHP.

True False

7. Allowing NHP to chew on oral swab ropes dipped in an attractant like jam is a safe and effective method for collecting non-invasive saliva samples from semi-habituated NHP.

True False

8. The upper limit for a safe quantity of blood that can be collected from NHP is:

- a. 2 ml of blood per 100 g of body mass
- b. 1 ml of blood per 100 g of body mass
- c. 0.5 ml of blood per 100 g of body mass
- d. 0.25 ml of blood per 100 g of body mass

Quiz – Pangolin Sampling Methods

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Pangolin Sampling Methods* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. The following samples should be collected from each live animal whenever possible (select ONE best answer):

- a. Two fecal samples/rectal swabs, two oral swabs, two whole blood samples, two serum samples, and two urogenital swabs/urine samples
- b. Two fecal samples/rectal swabs, two nasal swabs, and two whole blood samples
- c. Two serum samples, two urogenital swabs/urine samples, and two ectoparasite samples
- d. One whole blood sample, one serum sample, one nasal swab, one fecal sample/rectal swab, and one urogenital swab/urine sample

2. The maximum safe quantity of blood that can be collected from live pangolins is:

- a. 0.1 cc of blood per 100 g of body weight
- b. 1 cc of blood per 100 g of body weight
- c. 10 cc of blood per 100 g of body weight
- d. 0.5 cc of blood per 100 g of body weight

3. Which of the following is an acceptable site for venipuncture in pangolins:

- a. Jugular vein
- b. Ventral coccygeal vein
- c. Femoral vein
- d. All of the above

4. Due to their inability to bite, ease of manual capture, and relatively docile nature, gloves are not required when handling pangolins.

- True False

5. Pangolins are capable of rolling into a defensive ball when threatened, which can make it challenging to obtain samples. Which of the following methods is an appropriate way of sampling pangolins that have rolled into a defensive ball:

- a. Pangolins should never be unrolled from a defensive ball, even if no samples can be obtained
- b. Hold the base of the tail and use the centripetal force of a downward swing, similar to that of a yo-yo, to unravel the body.
- c. Use proper anesthetic protocol to relax the pangolin and gently unroll them.
- d. B and C

6. Which of the following is an acceptable method of anesthetic restraint in pangolins:

- a. Induction with 2-5% isoflurane gas followed by 0.5-1.5% maintenance
- b. 9 mg/kg ketamine and 1 mg/kg xylazine given orally
- c. 1 mg/kg ketamine and 9 mg/kg xylazine given intramuscularly into the thigh muscle
- d. All of the above

7. Respiratory distress is a possible complication of anesthetic restraint in pangolins. If a pangolin stops breathing under anesthesia, non-invasive intubation may be performed by inserting a flexible oro-tracheal tube:

- True False

8. Pangolins are especially prone to health complications due to stress from handling. Which of the following are best practices to minimize stress while handling and sampling pangolins:

- a. Keep your distance, speak with a soft voice, and avoid sudden movements while maneuvering around captured or recovering pangolins.
- b. Keep capture boxes covered with a tarp/towel and in dark, quiet locations while an animal is recovering.
- c. Cover the eyes of anesthetized pangolins with a towel or cloth to reduce visual stimuli.
- d. All of the above

Quiz – Rodent Sampling

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Rodent Sampling* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. All are requirements of minimum PPE for rodent sampling EXCEPT:

- a. Nitrile gloves
- b. Open-toed shoes
- c. Tyvek-like suits
- d. N95 facemask
- e. Eye protection

2. Rodent traps should

- a. Be set up and baited only during the night
- b. Be closed if bad weather and rain are expected
- c. Include bedding so animals can be protected from hypothermia
- d. Be placed in sheltered location
- e. Be protected from predator access

3. Rodents may be anesthetized with:

- a. Isoflurane
- b. Chloroform
- c. Halothane
- d. Ether

4. For larger rodents, the recommended bleeding site is:

- a. Tail clip
- b. Lateral saphenous vein
- c. Lateral tail vein
- d. Cardiac puncture

5. Bleeding methods in small rodents include:

- a. Facial vein
- b. Lateral saphenous vein
- c. Tail clip
- d. Retro-orbital sinus

6. Scruffing (pinching) the skin where the rodent's spine meets the head between the handler's forefinger and thumb is the best way to handle a rodent.

True False

7. Cardiac puncture may be used to collect blood in live small rodents.

True False

8. Facial vein bleeding includes which of the following steps:

- a. Choose the proper lancet point length
- b. Bleed from a live, anesthetized animal
- c. Locate vascular bundle at the rear of the jaw bone
- d. Insert only the tip of the lancet
- e. Apply gentle pressure with a gauze pad to stop bleeding when done

9. Subcutaneous fluids should be administered when

- a. Animals are visibly dehydrated
- b. Preventatively prior to blood sampling
- c. At a rate of 20% body weight
- d. Sampled rodents are 200g or less

10. Urine samples may be collected using pipettors.

True False

11. Rodents often defecate during sampling, providing a sample collection opportunity.

True False

12. The safe quantity of blood that can be collected from rodents is:

- a. 0.6-0.9 cc of blood per 100 g of body mass
- b. 1 cc of blood per 100 g of body mass
- c. 0.3-0.6 cc of blood per 100 g of body mass
- d. 0.3-0.6 cc of blood per 100 g of body mass, although collect as little as possible to conduct necessary testing

13. In dead rodents...

- a. Sterile collection of samples is not necessary
- b. Three samples of each tissue should be collected
- c. Only formalin must be used as a preservative
- d. All of the above

14. Generally, it is possible to classify rodents into one of the following EIDITH age classes, EXCEPT:

- a. Fetus (in utero)
- b. Neonate (newborn)
- c. Juvenile (dependent on dam)
- d. Subadult (immature, independent)
- e. Adult (reproductive)

Quiz – Safe Animal Capture and Handling

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Safe Animal Capture and Handling* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. Which of the following are precautions that should be considered for fieldwork that involves handling animals or diagnostic samples?

- a. Field teams must have contingency plans to respond to accidents or injuries
- b. Personnel should work in teams of at least two people when handling animals
- c. Chemical restraint should never be used
- d. If one person is capturing animals alone, they should have a mobile phone

2. Once captured, an animal should never be released until all sampling is completed.

- True False

3. If bite wounds are washed within five minutes, they do not pose a risk of infection in humans.

- True False

4. Which of the following are precautions for handling animals:

- a. Handlers should have a basic understanding of the animal's typical behavior
- b. Handlers should make quick deliberate movements when restraining the animal
- c. All animal handlers should be trained in handling techniques
- d. Personnel should use extreme caution when giving injections and handling sharps around animals

5. Animal anesthetic drugs do not pose a risk to humans.

- True False

6. The rules and regulations regarding drugs and specialized animal capture equipment are the same in every country.

- True False

7. When using darts to capture animals, darts that missed their target should be collected whenever possible because if left in the field they may pose a hazard to humans or animals.

- True False

8. If personnel who handle vertebrates become sick more than two weeks following animal capture activities, they should assume their illness is not related to the animal handling activities.

True False

9. Personal Protective Equipment (PPE), other than leather gloves, is not necessary when handling animals that appear healthy.

True False

10. Personnel should consult a human health professional regarding immunizations prior to conducting fieldwork that involves handling animals.

True False

11. If personnel will be working with bats, carnivores, or other wild mammals, pre-exposure rabies vaccination is recommended.

True False

12. Which of the following must be considered when determining what type of restraint should be used for animals:

- a. Animal species and condition
- b. Animal safety
- c. Restraints should be plastic rather than natural materials
- d. Safety of personnel

13. When using nets to capture animals, the animals should not be handled until they have stopped moving.

True False

14. Chemical restraint is preferred over physical restraint if the drugs are available.

True False

15. Anesthesia should be planned to sedate an animal for a minimum of an hour.

True False

16. Animals should be released after having recovered as fully as possible from anesthesia.

True False

Quiz – Small Carnivore Sampling

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Small Carnivore Sampling* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. The minimum PPE for sampling small carnivores includes:

- a. Designated clothing and nitrile gloves
- b. Designated clothing
- c. N95 facemask
- d. All of the above

2. Special considerations in the handling of small carnivores include:

- a. Zoonotic diseases such as rabies and leptospirosis
- b. Staff vaccinations
- c. Safe capture and handling
- d. None of the above

3. Urine can be collected:

- a. Free catch, since some animals will urinate as a fear reaction
- b. Via bladder expression
- c. Both of the above
- d. None of the above

4. All personnel handling small carnivores should be vaccinated against rabies beforehand.

- True False

5. PREDICT personnel are not expected to have detailed capture/immobilization protocols for target small carnivore species.

- True False

6. Trizol may be used as a lubricant while collecting rectal swabs.

- True False

7. If animals are too small to collect two blood tubes (for whole blood and serum), collect:

- a. Whole blood and freeze at -80°C
- b. Whole blood and store in 500 μL VTM or 500 μL Trizol
- c. Serum and save remaining clot in 500 μL VTM after serum separation
- d. Serum and discard remaining clot

8. Whole blood should be collected in EDTA prior to transfer in VTM and Trizol.

- True False

9. In case of dead or euthanized small carnivores, it is not advisable to consider the bushmeat sampling protocol.

- True False

10. The maximum safe quantity of blood that can be collected from small carnivores is:

- a. 0.6-0.9 cc of blood per 100 g of body mass
- b. 1 cc of blood per 100 g of body mass
- c. 0.3-0.6 cc of blood per 100 g of body mass
- d. 0.3-0.6 cc of blood per 100 g of body mass, although collect as little as possible to conduct necessary testing

Quiz – Qualitative Research: Introduction and Observational Research

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Qualitative Research: Introduction and Observational Research* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question.

1. Which of the following are the five main themes for PREDICT human qualitative research?

- a. Animal movements and migrations
- b. Socioeconomics and daily living
- c. Educational systems
- d. Human movement
- e. Human-animal contact
- f. Animal markets and economics
- g. Biosecurity in human environments
- h. Community development
- i. Illness, medical care/treatment, and death of humans

2. What are PREDICT's three key pathways of disease emergence and spillover risk?

- a. Intensification of animal production systems
- b. Animal value chains
- c. Land conversion
- d. Water systems

3. What is the target population of PREDICT's qualitative research?

- a. People who frequently travel into and out of the community
- b. People who work or are treated in hospital or health care settings
- c. People who are actively exposed to animals along one or more of the key pathways
- d. People who have studied animals in school

4. What is the most important thing to keep in mind when selecting individuals from the target population for participation in PREDICT's qualitative research?

- a. That a uniform/similar group of people should be selected
- b. That mostly elderly people should be selected
- c. That mostly women should be selected
- d. That a diverse group of people should be selected

5. Which of the following is NOT a goal of PREDICT's observational research program?

- a. To map a setting/site of possible future research
- b. To prepare for future qualitative research activities, such as focus groups
- c. To begin to collect blood specimens for viral testing
- d. To identify key informants

6. Which of the following are key activities in observational research?

- a. Passive observation of a site
- b. Structured, in-depth interviews with key informants
- c. Note taking
- d. Mapping of land and community

7. The Observer should never reveal too much about the PREDICT study to people in communities where future PREDICT research will take place.

- True False

8. The Observer should try to identify and seek introductions to members of the target community, particularly community leaders.

- True False

9. In order to learn about a community, to whom should an Observer speak?

- a. People who are interacting with animals
- b. People with a lot of influence in the community
- c. People in the community who are easy to approach
- d. Men only

10. Data that is collected through observational research...

- a. ...includes only the information gathered from key informants
- b. ...should be documented as "Field Notes"
- c. ...can help researchers understand behavior in the community
- d. ...can include drawings and maps

11. The time for conducting observational research ends when focus groups begin.

- True False

12. An ethics review board or committee ("IRB") must officially approve a qualitative research plan that includes focus groups and ethnographic interviews before research can begin.

- True False

Quiz – Qualitative Research: Focus Groups, Ethnographic Interviews, & Data Analysis

This knowledge confirmation quiz should be completed and reviewed with the trainer after you read and understand the important information in the *Qualitative Research and Data Collection: Focus Groups, Ethnographic Interviews, & Data Analysis* training guide.

Quiz: Multiple-choice questions may have more than one correct answer. Check the correct answer(s) for each question (select all correct answers for the question unless instructed otherwise).

FOCUS GROUPS

1. What are key characteristics of a focus group?

- a. They are groups of 50-100 people
- b. Group members come from different households/families
- c. Group members share key characteristics
- d. Groups meet 4-8 times to discuss their topics

2. Gender should never be a consideration when inviting people to participate in a focus group.

- True False

3. The location in which a focus group will be held should be decided by agreement when all members of the group arrive.

- True False

4. On what topics will the PREDICT focus groups collect information?

- a. Rules and restrictions related to wild and domesticated animals
- b. The distribution and overlap of animals in the community setting
- c. Illnesses in humans and animals
- d. Animal contact and context

5. Which of the following is/are NOT a goal of the PREDICT focus groups?

- a. To explain the PREDICT animal sampling plan to communities
- b. To identify how groups of people think or feel about behaviors and practices that may be linked to disease transmission
- c. To give personal advice to community members on avoiding disease transmission
- d. To explore reasons why certain opinions are held

6. The Moderator of a focus group...

- a. should be charismatic and friendly, not timid, authoritarian, or judgmental
- b. introduces each question or activity to the group and encourages everyone to answer or participate
- c. asks follow-up questions until a topic is exhausted
- d. should, above all else, teach and correct the group when it misunderstands an important topic

7. The Recorder/Observer of a focus group...

- a. times the speech of each participant
- b. photographs each participant and records his/her personal details, including name, age, address, and occupation, in the focus group notes
- c. documents the highlights of the discussion, particularly when new information is shared
- d. discretely points out topics the Moderator may wish to probe and/or suggests probing questions the Moderator may have missed

8. True or False: PREDICT focus group meetings will be filmed so that they may be transcribed, coded, and analyzed.

- True False

9. Which of the following are NOT an aim of the community mapping exercise?

- a. For the Moderator to observe all group dynamics
- b. For the Observer/Recorder to note where each participant lives
- c. For the group to map key geographic features in the country
- d. For the group to note where all animals are located in the community

ETHNOGRAPHIC INTERVIEWS

10. Which of the following is/are NOT a characteristic of ethnographic interviews?

- a. They consist of small group (8-10 people) interviews
- b. They consist of one-on-one interviews
- c. They usually last all day (6-10 hours)
- d. They are semi-structured, guided interviews

11. True or False: Ethnographic interviews will be audio-recorded, with permission, for late transcription, coding, and analysis.

- True False

12. What people must be present for an ethnographic interview to occur?

- a. A respondent
- b. A timekeeper
- c. An observer/recorder
- d. An interviewer

13. True or False: The interviewer must ask every question in the Ethnographic Interview guide.

- True False

14. How can the person conducting the interview decide which topics in the Interview Guide to discuss? He/she can...

- a. ask the person being interviewed what he/she wants to talk about
- b. consider the interviewee's age, gender, etc., and from that imagine what the interviewee is likely to know
- c. select questions in advance for different kinds of people who are likely to be interviewed that day
- d. only ask questions that other interviewees haven't answered

15. What are the essential characteristics of a good interview location?

- a. It is selected in advance of the interview
- b. The person being interviewed may not be heard by outsiders
- c. There should be food and water available
- d. The person being interviewed may not be seen by outsiders

16. Which of the following are key to a successful interview?

- a. Telling the respondent what you want them to say before the interview
- b. Being as prepared as possible before the interview
- c. Being flexible during the interview
- d. Taking frequent breaks during the interview

17. Why is an Interview Checklist used?

- a. To ensure all core themes are being covered during an interview
- b. To ensure that the person conducting the interview has all of his/her materials ready prior to the interview
- c. To aid in data coding and analysis after the interview
- d. To indicate which themes were discussed in an interview

DATA ANALYSIS

18. Which of the following is NOT a goal of the qualitative data analysis process?

- a. To review information from observational research that may help contextualize research findings
- b. To remove information that is incorrect
- c. To systematically review and prepare data for analysis
- d. To uncover new information that was shared during the interviews and focus groups

19. What data is the coding process designed to identify?

- a. Information linked to the three key PREDICT pathways of disease emergence
- b. Information associated with the five core PREDICT themes and subthemes
- c. Information about issues the participant thinks are important
- d. Information on the best ways to avoid disease emergence

20. Why are coding keywords useful?

- a. They help the coder identify places in the interview where key topics were discussed

- b. They help the person transcribing the interview to interpret the interviewee's ideas
- c. They give the interviewee an idea of what is expected from the interview
- d. They standardize themes across reports

21. True or False: Data should be coded using the Highlight and Comment functions.

- True False

22. What kind of quotes should be selected for inclusion in the final summary document?

- a. Quotes that provide new information about a core theme
- b. Quotes that verify the interviewer's beliefs about the core themes
- c. Quotes that show differences of opinion around the core themes
- d. Quotes that stand out because they clearly express one of the core themes in an interesting way

23. How should quotes of interest be identified and treated?

- a. They should be highlighted in the transcribed document
- b. They should be copied to the final summary document
- c. Their page number should be noted in the final summary document
- d. The researcher should identify which core theme(s) each quote represent(s) and give an opinion as to why the quote is a good example of the theme

24. Why are brief summary notes written?

- a. To summarize the most important information that the interview revealed in the interviewers opinion
- b. To briefly explain why some interview questions, and not others, were asked
- c. To briefly summarize the situations and experiences of the individual(s) as they relate to the core themes
- d. To describe the community in which an interview/focus group occurred

25. What material is included in the Summary Document?

- a. Descriptions of the community in which the interview/focus group occurred
- b. Quotes that are good examples of each of the core themes
- c. Brief summary notes from each of the core themes discussed in the interview
- d. Opinions about which key interfaces are most important in the community in which the interview/focus group occurred

26. True or False: preliminary data coding and data analysis of qualitative data do not need to be conducted by local research staff in every country.

- True False