Section 6.1. Implementing Cold Chain for Safe Sample Transport and Storage

Prepared by
David Wolking, University of California, Davis,
and the PREDICT One Health Consortium

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.

This document was made possible by the generous support of the American people through the United States Agency for International Development (USAID) Emerging 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.

For more information about the contents of this guide, please contact predict@ucdavis.edu.

Suggested Citation Form: PREDICT One Health Consortium 2016. PREDICT Operating Procedures: Implementing Cold Chain for Safe Sample Transport and Storage.

Table of Contents: Implementing Cold Chain for Safe Sample Transport and Storage

Section 6.1.1. 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 (Section 8.4.14.)***.

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.

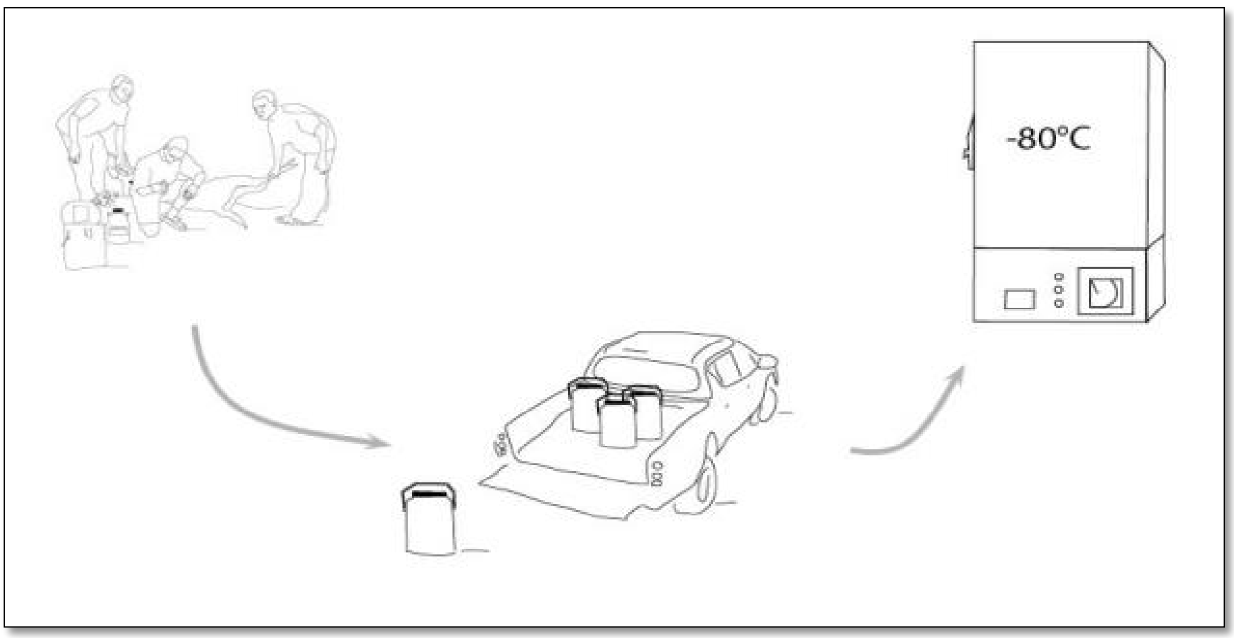

Figure 1: Illustration of a typical cold chain from field to lab storage for PREDICT biological samples. Field teams sample an animal and place specimens in liquid nitrogen dewar for storage. The dewar with specimens is transported in the back of a project vehicle to long-term storage at a PREDICT laboratory or field station, inventoried, and archived until testing in an ultra-low temperature freezer (<-80°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 -80ºC suitable for the preservation of targeted PREDICT pathogens and viral detection.

Section 6.1.2. Implementing the Cold Chain

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

Section 6.1.2a. 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.

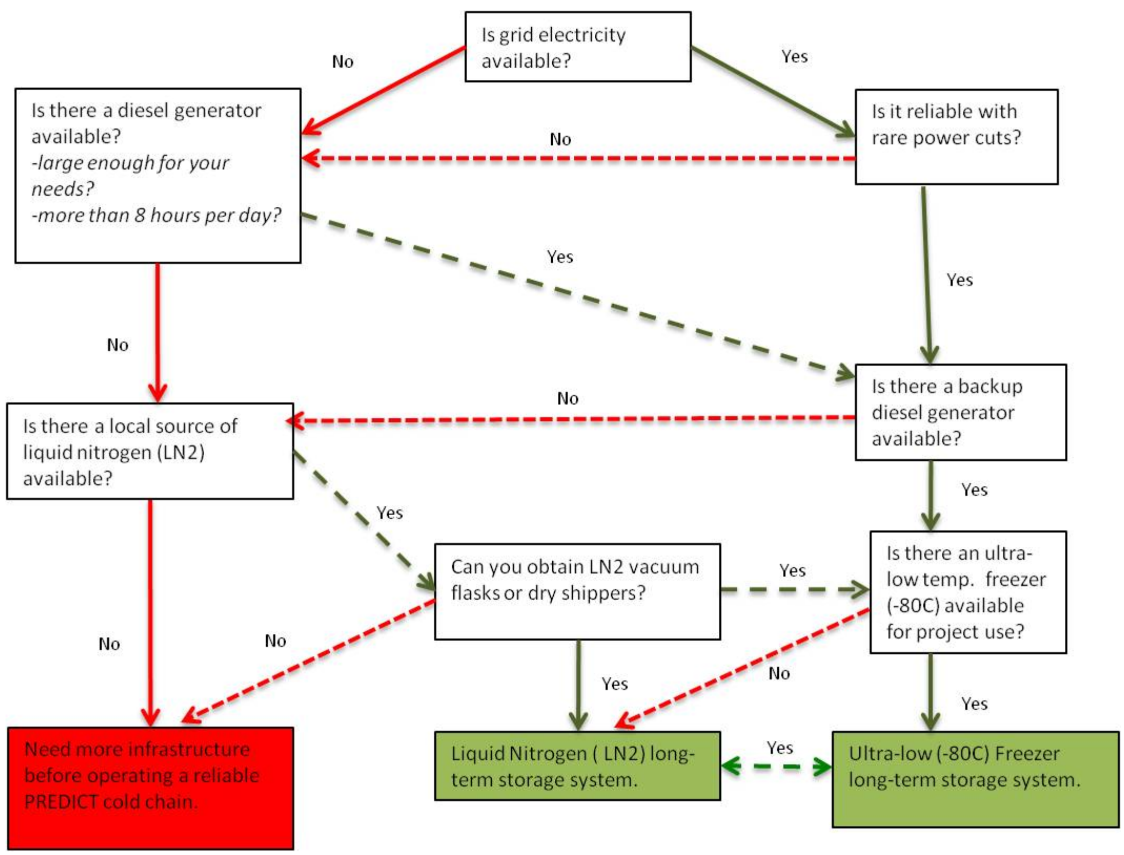

Figure 2: A decision tree for cold chain planning. Based on United Nations World Food Program Logistics Cluster "Logistics Operational Guide".

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 -80ºC 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 to* ***Biosafety and PPE Use (Section 4.)***.

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 -78.5ºC. In ambient conditions, dry ice is unstable and evaporates quickly. Therefore, samples packed in dry ice should be transferred to a <-80º 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 spilt.	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.

<u>Containers for Cold Chain Transport and Shipping</u>

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.

<u>Dry shippers (vapor shippers)</u>

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.

<u>Vacuum (dewar) flasks</u>

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.

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.

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). [kWh / 24 h] x [kWh costs in your location] x [365 days] = Operational Cost / 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 6.1.3.). 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 6.1.3.. 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, CO_2 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 6.1.2b. 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 6.1.2c. 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 *Commercial Refrigerator or "on-ice"*	-70°C *Dry ice*	-80°C or colder *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 CO_2 (release of CO_2 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 sublimate (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 6.1.2d. 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 ***Section 6.2 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 6.1.2e. 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 6.1.3.. 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 (Section 6.2)***.

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 6.1.2f. 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.

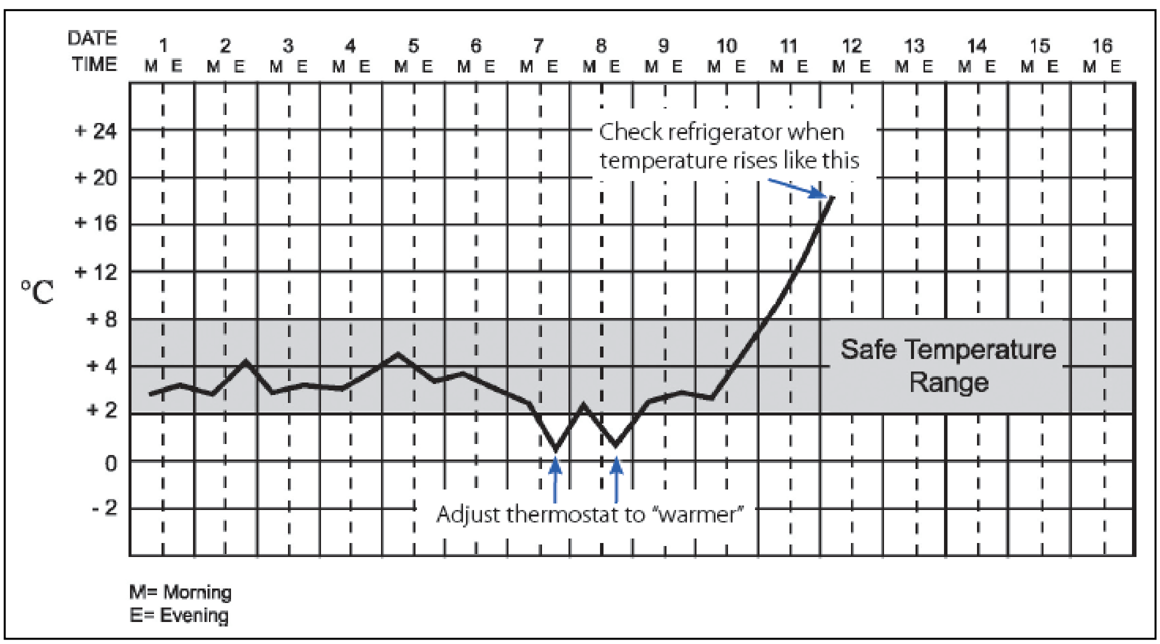

Figure 5. An example of a cold chain temperature monitoring chart. Source: WHO, 2004.
Note: this chart is for a cold chain optimized for vaccines at 2-8ºC. Use the chart included in the Appendix for the PREDICT cold chain at lower storage temperatures optimal for viral isolation.

Section 6.1.3. 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	Dave of reset: July 12, 2010	Time of reset: 11:12 AM

Section 6.1.4. References

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

Commonwealth of Australia. 2005. *National Vaccination Storage Guidelines: Strive for 5*. ISBN: 0 642 82750 8.

United Nations World Food Program Logistics Cluster. "Logistics Operational Guide: Response: Cold Chain." Available online: http://log.logcluster.org/response/cold-chain/index.html

Vaccine Preventable Disease Program, Niagara Region Department of Public Health. 2007. *Cold Chain and Influenza Information for Private Sector Clinics*.

World Health Organization Epidemic and Pandemic Alert and Response. 2006. *Collecting, preserving and shipping specimens for the diagnosis of avian influenza A(H5N1) virus infection: Guide for field operations*. WHO/CDS/EPR/ARO/2006.1

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.

World Health Organization Department of Immunization, Vaccines, and Biologicals. 2004. "Ch. 3. The Cold Chain Module." In *Immunization in Practice: A practical resource guide for health workers (CD)*. World Health Organization.

Section 6.1.5. Appendix I. Datasheets and Checklists for Cold Chain Planning and Implementation

Equipment Inventory Template

Item	Specifications (brand, model, SN, date of acquisition)	Current Location	Current Condition (working, in repair, out of commission)	Date of Purchase	Estimated Replacement Date	Notes

Equipment Maintenance Record Template

Model No.	Serial No.	Purchase Date	Last Service Date	Work (Maintenance) Performed

**Note: It is also recommended that teams catalog recommended maintenance forms, registries, and schedules that accompany equipment to help plan for equipment maintenance and minimize interruptions in the cold chain. It may also be helpful to keep a record of responsible team members so staff are aware of equipment maintenance duties.*

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 of 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 (-80ºC 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																				
-50																				
-52																				
-54																				
-56																				
-58																				
-60																				
-62																				
-64																				
-66																				
-68																				
-70																				
-72																				
-74																				
-76																				
-78																				
-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						
18						
19						
20						
21						
22						
23						
24						
25						
26						
27						
28						
29						
30						
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.*