Environmental Toxicology Laboratory
Toxicology Centre
University of Saskatchewan

STANDARD OPERATING PROCEDURE

Maintenance of eDNA Sample Integrity, and Proper Usage of Refrigerators and Freezers

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APPROVAL PAGE

Revisions to an existing SOP, addition of a SOP change form, or preparation of a new SOP must be reviewed, approved, and signed by the following:

Authored By: Yuwei Xie, Markus Hecker, Paul D. Jones and John P. Giesy Date: 04/15/2018

Supervisor Review By: John P. Giesy Date: 04/30/2018

Reviewed By: _____ Date: _____
(QA Coordinator)

DEFINITIONS AND ACRONYMS

ETL Environmental Toxicology Laboratory (University of Saskatchewan)

DQO Data Quality Objective

DHSE Department of Health, Safety and Environment (University of Saskatchewan)

QA Quality Assurance

QC Quality Control

QAPP Quality Assurance Project Plan

SOP Standard Operating Procedure

eDNA Environmental DNA

GWF Global Water Futures

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1.0 PURPOSE

Guidelines have been established to ensure that refrigerators and freezers are used in a safe, clean, and efficient manner.

2.0 SCOPE AND APPLICATION

This SOP applies to the ETL for eDNA samples supplied from the Global Water Futures (GWF) program titled "Next generation solutions to ensure healthy water resources for future generations" (eDNA project).

The following procedure outlines proper usage of refrigerators and freezers by the members of the Environmental Toxicology Laboratory (ETL), as well as sample logging and treating procedures.

This procedure describes guidelines for safe storage of samples and standards, sample storage locations, restrictions on the types of materials that may be stored in certain units, sample access, temperature monitoring, protection of sample integrity and alarm systems.

3.0 SAFETY CONSIDERATIONS

Safety training and medical monitoring requirements are described in the Health and Safety Plan for eDNA project of the GWF program.

In addition, there are various safety concerns regarding the receipt, storage, and disposal of sample containers at the ETL. Upon receipt, the sample containers will be monitored for breakage. If sample containers are broken, the appropriate personnel will be immediately notified and the Department of Health, Safety and Environment (DHSE) will be called in order to assess the hazard. DHSE will also be contacted in the case of chemical spills and will be responsible for the disposal of hazardous wastes.

4.0 EQUIPMENT, MATERIALS, AND REAGENTS

4.1 SAMPLE STORAGE LOCATIONS

A. Water samples are sometimes kept in short-term storage (12 to 48 hours) in the walk-in cooler #1, Room 118, Toxicology Building, University of Saskatchewan (U of S).

B. The walk-in freezer in Room 116 and the walk-in cooler in Room 118, Toxicology Building, U of S, are used to store tissue and whole organism samples from field and laboratory studies. The walk-in freezer is maintained at -20° C and the walk-in cooler is maintained at 4° C.

C. The Thermo Scientific refrigerator/ freezer unit (FRZ-10) in Room 261, Toxicology Building, U of S (Serial # 682-381-00 REV B) is used for storage of samples to be analyzed. The refrigerated areas are maintained at 4 °C, and the freezer areas at -20 °C.

D. The Thermo Scientific Forma -86C Ultra Freezer (Serial # 812712-2490) in Room 261, Toxicology Building, U of S, is used to store biological samples for analysis. It is maintained at -80 °C.

4.2 SAMPLE ACCESS

All rooms and laboratories are locked at all times and are considered very secure. Each employee authorized to work there has a key to enter the rooms. The walk-in freezer and cooler are locked and access to these rooms is limited to ETL personnel.

4.3 SCHEDULED MONITORING

All refrigerators and freezers used by the ETL group are examined frequently due to constant use and monitored weekly by reading the temperature from a thermometer located in each unit and recording the temperature in the Maintenance Log for Refrigerators, and Freezers. Freezer temperatures are maintained at -20 °C. If the freezer temperature rises to -15 °C such that it triggers the alarm of the fridge temperature rises over +7 °C corrective action must be taken. Actions include adjusting the thermostats, having the unit serviced, or moving the samples to another unit.

Any incidents requiring corrective action are recorded in the Maintenance Log, a three-ring binder in the Biochemistry Lab, Toxicology Building, Room 261.

Maximum-minimum thermometers are located inside the walk-in cooler and inside the walk-in freezer. These thermometers are checked weekly and reset, and the maximum temperature for each unit is recorded in the logbook. Directions for use of the maximum-minimum thermometers are located in the Additional Notes section of the Maintenance Log binder.

4.4 ALARM SYSTEM

The walk-in freezer and walk-in cooler can tolerate brief power outages or malfunctions without compromise of the samples they contain because of their large volume and large stored mass. However, it is important that any such malfunctions or power outages are recognized promptly; therefore, the walk-in freezer, walk-in cooler and Fisher Scientific Ultra Freezer are protected by temperature and power failure phone alarm systems. Instructions for these systems are posted with each unit and an additional emergency phone list is provided at the door of each laboratory/room. In case of a building power outage the freezers are all connected to the emergency generator. Be sure that the cause of the power outage is identified and corrected.

FREEZER ALARM

If the above alarms are triggered, it means that this freezer / refrigeration has a serious problem. Please take one of the actions listed below to save valuable research materials.

1. Consult the Laboratory On-Call List posted on the wall by the nearest telephone and call the responsible person.

2. Contact one of the following people:

    A. Yuwei Xie at 966-4978 (office) or at 370-4108 (cell)

    B. Paul D. Jones at 966-5062 (office) of at 517-281-5666 (cell)

    C. Markus Hecker at 966-5233 (office) or 220-5757 (cell)

    D. John Giesy at 966-2096 (office) or at 517-614-6123 (cell))

    E. Any member of the ETL Management team at 966-4680

3. During evenings or weekends, if no one answers at the numbers above, call the campus operator and request physical plant emergency service for a freezer malfunction.

4. Report electrical and freezer malfunctions to the Dr. Yuwei Xie, Toxicology Building, room 133

Any ETL personnel who respond to an alarm will visually inspect the unit that gave the alarm and comment in the Maintenance Log binder. An estimate of the amount of time the

unit was not functioning properly should be entered under Comments. If there is not enough space on the log sheet for a thorough description of the incident, refer the reader to the Additional Notes section and place a full report there. All electrical and freezer malfunctions should be reported to the ETL laboratory manager.

The walk-in freezer and walk-in cooler are connected to an audible alarm, phone alarm system and an auxiliary generator. If power from the main power grid is lost, the auxiliary generator automatically provides power and an alarm is called to U of S Public Safety. The alarm is then transferred to "on call" ETL personnel. There are two individuals on call at all times. These individuals can be contacted by telephone and carry pagers at all times. The Thermo Scientific Forma -86C Ultra Freezer in Room 261, also attached to the auxiliary power system and has an phone alarm system that in case of a power outage and/or temperature increase automatically calls the cell phones of ETL personnel on duty (a minimum of two persons are on duty at any time). Otherwise, the procedures for this freezer are the same as those for the walk-in freezer, and records for its maintenance will be kept in the Maintenance Log in the Toxicology Building room 261.

4.5 GUIDELINES FOR PROPER USE OF REFRIGERATORS/FREEZERS

A. All samples, standards, and reagents should be properly labeled with the identification of the substance in the container and the date. If appropriate, hazard warnings, concentration, and an expiration date should be added. Note: It is not acceptable to label only the rack holding many small tubes or vials.

B. Sample labels: All stored samples should be given new labels within 13 months past the date on the label. All samples must be in labeled sample boxes with a closing lid-preferably ECONSTOR 704 (Available from Fellowes Manufacturing Co., Perma Products, Atlanta, GA). Each individual sample in the box should be labeled with the following:

   Project name:
   Date collected:
   Date placed in freezer/ cooler:
   Sample type: (e.g. rainbow trout carcass)
   Client name:
   Client's sample ID:
   Sample tracking # (Chain of Custody):

   Samples should be labeled on the outside of the container or package as well as on a piece of paper placed inside the sample container. Labels should be written with pencil on paper. Do not use felt tip pens to write on glass or plastic. Use only permanent, waterproof markers to write on glass or plastic.

C. Sample Box Labels: Samples should be grouped by project and/or sample type and placed in the storage boxes. Boxes should be labeled with the following information:

Project name:
Sample type:
Date placed in freezer:
Name of person who collected sample:
Client Name:
Location where sample was collected:
List of sample #s in box:

A sample log sheet should be filled out and a copy placed in both the sample box and the sample log binder. Note that there are numbers on the shelves in the walk-in freezer and cooler and that there is a map of the -80 °C freezer in the sample log showing numbered storage areas. These numbers are the sample box location numbers. When a sample box is logged, the number corresponding to the area where it is stored should be recorded on the appropriate line in the logbook. This system makes the task of finding a sample box much simpler and minimizes the amount of time that the deep freeze door is open. An entry should be made in the Maintenance Log every time the freezer is entered so that in case of a malfunction, it can be determined when the unit was last known to be functioning properly.

D. Samples and analytical standards should be kept in separate refrigerators or freezers.

E. Food or beverages for human consumption should never be stored in a refrigerator or freezer where standards, samples, or reagents are stored.

F. Buffers such as TRIS, HEPES, and phosphate buffers should be kept for no longer than one month. The pH of a buffer should be checked regularly at the temperature at which the buffer is intended to function. Buffers with sucrose should be filtered before storage; filtering will increase the storage life to no longer than two weeks. Buffers should be marked with an expiration date and disposed of after that date.

G. Samples and standards should be kept in containers that will prevent them from spilling or otherwise contaminating other stored materials. Items easily tipped or without tight lids should be placed inside other containers to prevent spilling. For example, a flask with a Parafilm cover might be placed inside a wide beaker.

H. Each person using storage space in a refrigerator or freezer should check routinely (once a month) for old buffers, glassware, etc., that he/she has left behind and should remove unneeded items.

I. For reasons of cleanliness, safety, and limited space, each person using storage space in a refrigerator or freezer may remove any items not in compliance with the above guidelines to a designated area in another refrigerator or freezer. Every reasonable effort must then be made to contact the person responsible, including contact by mail and telephone, and a note describing the item(s) removed and the new storage location should be posted on the refrigerator or freezer in which the items were found. If the items are not claimed and dealt with properly, they will be destroyed one month after removal.

5.0 RESPONSIBILITIES

Project Manager — Dr. Yuwei Xie will oversee and approve all project activities, authorize necessary actions and adjustments, and act as liaison between the principal investigator and other U of S personnel and the sponsor Project Manager.

Principal Investigator — Prof. John P. Giesy will advise the Project Manager in overseeing and approving all project activities, authorize necessary actions and adjustments related to U of S activities to accomplish program QA objectives; and act as liaison between agencies, staff, and the sponsor Project Manager.

Study Team Leaders (STL) —Yuwei Xie, under the supervision of John P. Giesy, will oversee all research activities and supervise all personnel involved with the assemblage of the experimental exposure systems. The STLs will ensure that proper sample collection, preservation, storage, transport, and COC QC procedures are followed and will inform the Project QA Manager when problems occur, and will communicate and document corrective actions taken. The STLs will discuss study activities with the Project Manager.

Quality Assurance (QA) Manager — Prof. Paul D. Jones will initiate audits on work completed by project personnel. The manager will review program QA activities, quality problems, and quality-related requests. In response to experimental findings, the QA manager will approve corrective actions. The QA manager will report quality non-conformances to the Project Manager.

6.0 REFERENCES

Good Laboratory Practice Standards. 40 CFR Part 160. Environmental Protection Agency, 1989.
Steere, Norman V. CRC Handbook of Laboratory Safety. Chemical Rubber Co., Cleveland, 1967. pp 314-323.

APPENDIX A: ETL FREEZER/REFRIGERATOR TEMPERATURE LOG

UNIVERSITY OF SASKATCHEWAN

ETL Freezer/Refrigerator Temperature Log

Equipment Code: FRZ-

DATE	INITIALS	THERMOMETER SERIAL #	LOCATION	TEMPERATURE	NOTES