Standard Operating Procedure **SOP049**

Title: Use and Maintenance of the – 80 °C Freezers

Location: CBE Laboratories

1. PURPOSE

To describe the procedures for use and maintenance of the four -80°C Freezers in the CBE Laboratories.

Revco Elite Plus (CBE H34)

TSX Series (CBE H34)

New Brunswick U101 (CBE H34)

FRYKA B35-85 (T208B)

National Lab PMU-0680-1

2. SCOPE

The scope of this SOP is to outline the use and maintenance of the five -80°C Freezers located in the CBE laboratories. This SOP describes the general maintenance requirements to ensure the safe storage of biological agents and ancillary reagents such as cell culture stock items e.g. media and serum, as well as general laboratory reagents. **To ensure compliance with COSHH regulations, this SOP must be**

read in conjunction with SOP003; "Storage and Transport of Biological Agents" and SOP039; "Disposal of Waste Chemicals and Solvents". To ensure compliance with HTA regulations, this SOP must be read in conjunction with HTA-PR-SOP004 "Receipt and Storage of HTA Licensable Material". This SOP does not cover the temperature monitoring of the equipment, or liquid nitrogen stores, which are described elsewhere in SOP028 & SOP013 respectively.

3. RESPONSIBILITES

CBE Laboratory Users

It is the responsibility of the laboratory staff to:

(i) ensure that all stored material is labelled correctly and has the appropriate risk assessment.

(ii) maintain the inventory of all stored material that requires a risk assessment. This inventory system should include details of location for each sample and records of removal for use or disposal. It is important that all these items can be traced back to a risk assessment.

(iii) if nominated by the Responsible Person or Laboratory Manager, to ensure that the steps outlined in this SOP are followed when defrosting and cleaning -80^{0}C freezers which may contain biological materials.

(iv) report any leakages/spillages of stored material, overcrowding or excessive buildup of ice to the Responsible Person or Laboratory Manager or DSO.

Responsible Person (RP)/Laboratory Manager (LM)

It is the responsibility of the Responsible Person to:

(i) ensure the safe storage of all material in the -80^{0}C freezers designated in this SOP.

(ii) ensure that the routine cleaning and defrosting of the designated -80^{0}C freezers is carried out according to this SOP.

(iii) inspect the -80^{0}C freezers every 6 months to identify and remove any substances that are unlabeled, out of date or without an appropriate COSHH assessment.

(iv) To perform and record monthly maintenance for the equipment as detailed in HTA-PR-SOP011 Freezer Maintenance and Manual Challenge of Freezers.

4. EQUIPMENT AND MATERIALS

i) New Brunswick Scientific Benchtop Ultra Low Temperature Freezer (Model No. U101)
ii) Thermo-Scientific Revco Elite Plus -80 Freezer (Old)
iii) Thermo-Scientific TSX Series Ultra Low Temperature Freezer (New)
iv) FRYKA Cold Box B35-85
v) National Lab PMU-0680-1
vi) 2% Detergent Solution (Neutracon)
vii) 1% Virkon
viii) 70% IMS

5. PROCEDURE

The -80^{0}C freezers are designed to provide precise, ultra-low temperature environments.

NOTE: For more specific information for each - 80°C Freezer please refer to the respective manuals.

5.1 General Use of the -80°C Freezers

5.1.1 Temperature control

The -80°C Freezers is maintained at temperatures outlined in SOP028, “Temperature Monitoring of Refrigerators and Freezers”.

5.1.2 Storage of study related substances

(i) Where practicable, each study should have designated areas in the -80°C Freezers which will be segregated as either sealed bags, boxes or separate shelves (within the requirement for safe storage of BAs & GMOs).

(ii) All study related substances should be labeled according to SOP007 and SOP005.

(iii) All study related substances stored in the -80^{0}C freezer should be recorded in the individuals laboratory workbook. They must also be recorded on the inventory log (see Section 6.) located on or close to the freezer. This will allow potentially hazardous items to be traced back to the risk assessment.

(iv) **NOTE:** At six monthly intervals the -80^{0}C freezer shall be inspected by the Responsible Person to identify any substances that are un-labelled, out of date or without an appropriate COSHH assessment. The Responsible Person shall notify all relevant personnel before any such substance is removed and disposed of.

5.1.3 Storage of general use non-hazardous substances

(i) General use substances should be stored in areas of the -80°C Freezers not assigned to specific studies.

(ii) General use solutions and reagents must be labeled according to SOP007 “Labelling of “General Use” Reagents and Solutions.

(iii) All general use substances stored in the -80^{0}C freezer should be recorded on the inventory log (see Section 6) located on or close to the designated freezer. This will allow potentially hazardous items to be traced back to the risk assessment.

(iv) **NOTE:** At six monthly intervals the -80^{0}C freezer shall be inspected by the Responsible Person to identify any substances that are un-labelled, out of date or without an appropriate COSHH assessment. The lab manager shall notify all relevant personnel before any such substance is removed and disposed of.

5.1.4 Storage of hazardous substances

(i) Hazardous substances should be stored according to procedures and conditions identified in their risk assessment.

(ii) All potentially infectious materials containing BAs/GMOs must be clearly labelled. The storage space (e.g., freezer, refrigerator) should also be labelled with the universal biohazard symbol, if

a risk assessment has identified this as necessary. Additional information including contact name and emergency numbers should be visible in case of emergency, i.e. freezer breakdown.

(iii) All GMOs stored in the freezer must be clearly identifiable with the GMO name, classification (risk assessment number), date of storage and the name of the research group. The box containing the GMOs must also be labelled. Refer to the local Code of Practice for further details (Reference 8).

(iv) All cultures of BAs/GMOs being stored inside the facility must be sealed during storage to prevent dissemination of the BAs/GMOs. **NOTE:** The type of container necessary to prevent the BAs/GMOs from escaping will vary depending on the type of organisms being stored.

(v) All cultures and samples of HTA relevant material should be stored in the designated HTA labelled secondary container within the -80 freezers. The location of this container should never change unless in an emergency, any change of location must be logged. All HTA relevant samples must have their details logged in the procuro sample tracking software and be labelled with a unique procuro reference number before being stored. All movements of HTA relevant samples must be tracked and logged (in to an area and where from, out of an area and where to) to ensure full traceability. This includes temporary storage. Follow CBE/HTA-PR-SOP004 for full details on storage of HTA licensable material.

(vi) Storage of flammable or volatile liquids reagents, chemicals, poisons or samples must **NEVER** be stored in a fridge or freezer (or cold room) unless they are spark free (domestic refrigeration is not spark free i.e. have spark proof compressor and lighting units. **It is highly dangerous to store solvents with low flashpoints e.g. acetone, diethyl ether etc. in a non-spark proof refrigerator or deep freeze.**

NOTE: Warning notices must be placed on all refrigerators/freezers which are not suitable for storing flammable materials.

(vii) Expired and other unwanted material must be decontaminated properly. Materials for long-term storage must be annually inspected and each container must be checked for cracks and other damages and properly disposed or replaced.

(viii) In the event of freezer failure (**NOTE:** an alarm will sound), all materials that are unable to be salvaged must be properly treated by autoclaving or chemical disinfecting.

5.1.5 Security

(i) BAs/GMOs or organisms containing BAs/GMOs may be stored outside the facility in a storage unit (freezer, fridge, controlled temperature room or other container). A biohazard symbol must be posted on the storage unit. The storage unit must be locked when not in use, unless access is restricted to the room or area where the storage unit is located. Access to the storage unit

must be restricted or controlled to prevent unintentional release of BAs/GMOs into the environment.

(ii) BAs/GMOs or organisms containing BAs/GMOs being stored outside the facility must be double-contained. The primary container must be sealed to prevent the escape or release of BAs/GMOs and must be labelled. The primary container must be stored in an unbreakable secondary container. In the case of a small storage unit such as a fridge or freezer, the secondary container may be the storage unit.

5.2 General Maintenance of the -80^{0}C Freezers

The -80^{0}C freezer should be defrosted and cleaned every 12 months if practicable, or as deemed necessary. The appliance should be defrosted according to the steps described in Section 5.2.1, if any of the following conditions are observed:

(i) The thickness of the ice on the internal walls of the freezer reaches 5 to 6 mm in thickness.

(ii) In the event of leakage of biological materials onto the internal surfaces of the freezer.

(iii) The freezer compartments are overcrowded and unsafe.

5.2.1 Cleaning and defrosting the -80°C freezers

(i) A laboratory coat, safety spectacles and double gloves should be worn throughout the procedure. Protective gloves should be worn when handling ultra-low temperature items.

(ii) Switch the freezer off. **(**Refer to the individual operating manuals for information regarding alarms).

(iii) Remove the contents of the freezer and transfer to an operational freezer as appropriate.

(iv) Inform the study leader/study personnel responsible for each of the samples of the temporary location of their samples.

(v) If samples require disposal, record the disposal date on the inventory log. **NOTE:** Samples or material should not be disposed of without prior consent of the Responsible Person. Procedures for disposal of samples should be in accordance with the appropriate risk assessment.

(v) Place plenty of absorbent paper on the floor in front of the appliance. Wearing gloves try and remove as much ice as possible, using a disposable cloth and hot water. **CAUTION:** the ice may contain contaminated material if a leakage has occurred.

(vi) After 20 minutes replace the absorbent paper (discard into yellow biohazard bags) with a dry layer of paper. Clean the removable drawers with 2% detergent & hot water followed by 1%

virkon followed by 70% IMS. **NOTE:** For layers of thick ice, a bowl of hot water can be placed inside the appliance.

(vii) When the appliance is thoroughly defrosted, dispose of the absorbent paper into yellow biohazard bags. Wipe the area with 2% detergent followed by 1% virkon followed by 70% IMS. Place the used towels and gloves in the yellow biohazard bag/container.

NOTE: Abrasive cleaners or solvents should not be used.

(viii) Switch the appliance back on and re-activate any alarms.

(ix) Once the temperature has reached its specification (see SOP028 "Temperature Monitoring of Refrigerators and Freezers", return the samples to their original location (unless a reorganization has been agreed with the Responsible Person and Laboratory Manager) and inform the study leaders/personnel and the Laboratory Manager.

5.2.2 General Preventative Maintenance of the -80°C Freezers

(i) The intake air grill and filter must be cleaned regularly to keep it free from dust and debris. Under normal conditions, clean the grill once every three months using a soft brush. The filter can be washed with warm soapy water and left to dry before returning. **NOTE**: Do not obstruct the air grill at the front of the freezer.

(ii) Clean the door seal once a month with a damp cloth and lubricate the door hinges every 12 months with general purpose oil.

(iii) Regularly check the alarm (Refer to the respective manuals for details)

(iv) After closing the door, a vacuum may be created. Before the door can be opened, it may be necessary to wait a few minutes. DO NOT force the door.

(v) A troubleshooting guide and more information can be found in the operating manuals.

5.3 Equipment Malfunction

(i) If any part of the equipment fails or malfunctions, the user should contact the Responsible Person. With permission of the Responsible Person the user should consult the Operator Instruction Manuals to access fault finding, error displays and troubleshooting procedures.

(ii) All problems and corrective actions should be recorded in the Maintenance Log referenced in Section 6.

(iii) If the equipment fails to work or malfunctions and cannot be rectified according to troubleshooting procedures detailed in the Operator and Users Manuals the Responsible Person and/or Laboratory Manager must be informed and a "Do Not Use" notice should be posted on the

equipment. Contact the manufacturer for advice and coordinate with the Lab Manager for external maintenance and servicing.

(iv) External maintenance and servicing of the equipment can only be performed after it has been suitably disinfected (refer to SOP003 for further details) and a 'Decontamination Certificate' has been issued by the School/Building/Unit Safety Co-ordinator. The Decontamination Certificate is referenced in Section 6.

5.4 Freezer Decommissioning Checklist

If the freezer needs to be decommissioned, for example due to relocation or change of use, it is necessary to ensure no hazardous materials are left behind and that the unit has been decontaminated and made safe for future use or removal. A checklist that can be used to record that the freezer has been suitably decommissioned is referenced in Section 6.

On completion, the form should be forwarded to Department Safety Officer (DSO) or other responsible person to request its disposal. If multiple refrigerated items need to be disposed of together from the same room, it may be possible to use one form – as long as each item is listed and each has a "Safe for Disposal" sticker / note on it to confirm that appropriate cleaning / disinfecting has been carried out. A completed decommissioning checklist precludes the need for maintenance staff and contractors to be issued with a Laboratory Permit to Work.

6.DOCUMENTATION

The following records are outputs of this SOP:

- QS-FORM-009 Generic equipment Decontamination Certificate
- QS-FORM-010 Cleaning & Maintenance record for fridges/freezers
- QS-FORM-011 Inventory log sheet for stored material for fridges/freezers
- QS-FORM-012 Fridge/freezers decommissioning checklists
- HTA-PR-FORM-013 – Freezer Maintenance Schedule

These records will be filed in the equipment file & stored in the CBE office or otherwise archived for future review or retrieval. All forms can be found on the CBE website.

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SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
001	Annual Review carried out on 22 March 2010 by Carolyn Kavanagh	Minor administrative revisions: 1. A 'Responsible Person' has been added to the maintenance responsibilities 2. Section 3 (4) - Amended title to say 'Disposal of Biological Healthcare Waste' 3. Section 3 (6) - Amended title to say ' General Laboratory Housekeeping' 4. Section 7.1.4 – Clarified the labeling requirement : labeling of the freezer with biohazard or GMO signage is only required if this has been identified by a risk assessment	002
002	**21.09.10 Reviewed by C. Kavanagh**	**1.Section 4.4 –** A statement was added to say 'Cryogenic gloves must be worn when removing items from the -80°C freezer. They are located on a hook on the side of the equipment. 2.Minor editorial amendments**.**	**003**
003	20th November 2012 Reviewed by C. Kavanagh	i)Changed location ii)Added another two -80 freezers iii)Removal of forms from SOP. These are now available separately on the CBE website. iv)Minor Format changes v) Section 5.1.5 added a statement to say NOTE: For more specific information for each -80 Freezer please refer to the respective manual. vi)Removed specific detail of -80 freezer usage. vii)Minor amendments to section 5 with regards to the defrosting & cleaning of the – 80 freezer.	**004**
004	13th November	Minor editorial changes, no requirement for new	**004**

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	2015. Reviewed by K.Sikand	version number.	
004	J. Harriman 22/06/18	SOP reviewed. Added TSX series -80 freezer. Added section on storage of HTA relevant material. Minor editorial changes. Added pictures of freezers.	**005**
005	14th November 2019 by C.Kavanagh	Additon of new -80 freezer Addition of details regarding new monthly freezer maintenance schedule.	**006**