Standard Operating Procedure **SOP005**

Title: Storage and Transport of Biological Agents

Location: CBE Laboratories

1. PURPOSE

The intent of this SOP is to adhere to COSHH regulations which require the safe storage and transport of Biological Agents. This SOP describes procedures for the storage of biological agents within the Centre for Biological Engineering (CBE) laboratories and procedures for the on-site transport between and within laboratories of the CBE.

2. SCOPE

This SOP applies to containment level 2 (CL2) CBE laboratories and personnel, including the CBE Laboratory Unit (located in the Holywell Park) and the CBE Tissue Engineering Laboratory T208B (located in the Wolfson School). This document describes the storage and transportation requirements and procedures for Biological Agents (BAs) and Class 1 GMOs, including guidelines for the on-site transportation of BAs/GMOs within the CBE Laboratory Unit (See Annex 2 for definitions).. This SOP applies to cell cultures (including primary and early passage cultures and finite/continuous cell lines), animal/human sera and media or reagents that may contain human or animal derived constituents i.e. that may contain microorganisms that may cause infection, allergy, and toxicity or otherwise cause a hazard to human health. This SOP does not cover the storage and transport of biological waste, which is described elsewhere (SOP003).

Important Restrictions: The SOP does not cover the storage and transport of Genetically Modified Organisms within the CBE Tissue Engineering Laboratory (T208B). **The storage and use of GMOs in the T208B laboratory is prohibited**

NOTE: Please note the Storage of HTA relevant material is documented in a separate SOP (HTA-PR-SOP004 Receipt Storage of HTA licensable material and HTA-PR-SOP006 Acquisition and transfer of HTA licensable material.

SPECIAL NOTES – HEALTH & SAFETY

(i) Under the COSHH regulations and the University Biological Safety Policy all hazardous Biological Agents require the safe and secure storage. In addition, storage of GMOs is a 'contained use' activity and as such is subject to the requirements of the Contained Use Regulations namely: Risk assessment, application of appropriate control measures, classification of the activity, notification where appropriate. An up to date register of all hazardous biological material kept in storage must be maintained. The University Health, Safety & Environment Office must be notified concerning receipt, possession and storage of Hazard Groups 2 organisms (or above) or GMO.

(ii) Acquisition and Storage of GMOs: All GMOs stored in the CBE Laboratory Unit must be registered with the BHGMSC. This is achieved partly through acceptance of the risk

assessment by the committee; however each group is required to provide an inventory of GMOs currently being stored. This inventory must be kept up to date.

(iii) **The storage and use of GMOs in the Tissue Engineering Laboratory (T208B) located in the Wolfson School is prohibited**

3. RESPONSIBILITES

CBE Laboratory Users

(i) It is the responsibility of CBE Laboratory Users to ensure that all work activities involving hazardous biological material has an approved risk assessment before receipt of the material and before any work commences. This includes responsibility for the safe and secure storage of the biological agent.

(ii) In the event of a deviation from procedure CBE Laboratory Users should initiate a CAPA, contact their supervisor, and notify a member of the lab management team.

Responsible Person (RP)/Laboratory Manager (LM)

(i) It is the responsibility of the Laboratory Manager/Quality Manager to notify the University University BSO of any proposals to acquire biological agents in Hazard Group 2 or above or any GMO. This is achieved partly through the submission and approval of the risk assessment for work activities involving the use of biological agents or GMOs.

(ii) It is the responsibility of the Laboratory Manager or designated person to monitor the receipt, possession and storage of Hazard Groups 2 or any GM organisms. An up to date register of all hazardous biological material kept in storage must be maintained (see below).

(iii) The Laboratory Manager or designated person must maintain an inventory of all organisms stored within the laboratories. This inventory system should include details of location for each sample and records of removal for use or disposal. It is important that every individual vial/plate etc can be traced back to a risk assessment.

4. EQUIPMENT AND MATERIALS

1. Primary and secondary storage vessels/containers
2. Biohazard labels
3. All 4°C Fridges/Cold Rooms
4. All -20°C Freezers
5. All -80°C Freezers

6. Cryostorage Units – Biorack 750;
7. Liquid Nitrogen Dewars- Cryolab 25- 25L.

5. PROCEDURE

Refer to ANNEX 3 for recommendation for storage of Biological Agents/GMOs in Containment Level 2 CBE Laboratories.

The storage and transport requirements must be determined within the risk assessment for the work activity. The acquisition, storage and transport requirements must therefore be identified prior to the work commencing . Biological Risk Assessment form can be found at https://www.lboro.ac.uk/services/health-safety/forms/.

5.1. Quarantine of New Biological Agents in CBE Laboratories

(i) Primary or early passage cultures and finite/continuous cell lines new to the laboratory should be quarantined; i.e. kept entirely separate from existing cell line stocks, until the origin or provenance of the cells has been authenticated (or a DNA fingerprint or profile defined) and it is confirmed that they are negative for microorganisms (according to SOP036).

NOTE: The advice of the local BGMSA should be sought where there is any doubt about the introduction of new materials or procedures.

(ii) If separate quarantine is not available and the use of a dedicated Class II BSC and an incubator is not practicable the following procedure is recommended to minimise the risk of contamination:

- The biological agents in quarantine should be handled only after all the other manipulations e.g. cell culture has been completed that day
- The new cultures should be placed in a sealed container before going into a general incubator
- The BSC should be cleaned after use with a suitable disinfecting agent and run for at least another 5 minutes prior to shutdown.

5.2 Storage of Biological Agents in CBE Laboratories

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

(ii) On receiving, check the instruction for storage, place in the correct storage conditions and inform the person who ordered the item (Refer to SOP008).

(iii) Ensure that every item containing BAs/GMOs is clearly labelled or coded. Researchers should be able to explain their coding system and identify all samples. Storage units housing HG2/GMO agents or biological toxins should display a biohazard sticker or sign, if identified as necessary in risk assessments.

(iv) Ensure that the selected storage space (e.g., freezer, refrigerator) has the appropriate label i.e. contact name and emergency numbers should be visible in case of emergency, i.e., freezer breakdown.

(v) Ensure that all cultures of BAs/GMOs being stored inside the facility are 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.

(vi) Secure all liquid reagents in racks or place in suitable secondary containers to reduce risk of spillage. Clean up spillages immediately according to procedures described in SOP038; "Biological Spill Response".

(vii) Ensure that expired and other unwanted material is decontaminated properly.

(viii) Regularly inspect (at least annually) all containers in long-term storage. Check for cracks and other damage. Damaged containers should be replaced or disposed of.

(ix) In the event of a freezer failure, ensure that all materials that are unable to be salvaged are properly treated by autoclaving or chemical disinfection.

5.2.1 Security

(i) Keep all storage facilities containing biohazardous materials locked at all times if they are located outside the laboratories.

(ii) If BAs/GMOs or organisms containing BAs/GMOs are stored outside the facility in a storage unit (freezer, fridge, controlled temperature room or other container), ensure that a biohazard symbol is posted on the storage unit. Keep the storage unit locked when not in use, unless access is restricted to the room or area where the storage unit is located i.e. access to the storage unit must be restricted or controlled to prevent unintentional release of BAs/GMOs into the environment.

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

5.2.2 Acquisition & Storage of Class 1 GMOs in the CBE Laboratory Unit

(i) GMO work can only commence if a risk assessment has been approved by the BHGMSC.

(ii) Ensure that all GMOs stored in the CBE Laboratory Unit are registered with the BHGMSC. This is achieved partly through acceptance of the risk assessment by the committee; however each group is required to provide an inventory of GMOs currently being stored. This inventory must be kept up to date.

(iii) Ensure that all GMOs stored in the CBE Laboratory Unit are 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 and the storage system (fridge /freezer etc) should also be identified as containing GMOs, if identified as a requirement in the risk assessment. Refer to the local Code of Practice for further details.

(iv) Ensure that all cultures of GMOs being stored inside the CBE Laboratory Unit are sealed during storage to prevent dissemination.

5.3. General Storage Requirements

(i) Maintain an inventory of all stored organisms and ensure that every individual vessel/vial is traceable to the risk assessment e.g. according to SOP003 & SOP031 for cell culture material.

(ii) Store early passage cultures or GMO material in the dedicated storage area. If not practicable to dedicate an entire storage area for this purpose, a shelf or specified area should be designated.

(iii) Use sealed culture vessels whenever possible, especially for long term cultures. Unsealed culture plates and dishes, as well as flasks with loose caps provide a common way for contaminants to enter cultures.

(iv) Seal multiwell plates with labelling tape or alternative, or place in sealable bags.

(v) Place smaller unsealed dishes (e.g. 35 and 60 mm dishes) inside larger (e.g. 150 or 245 mm) dishes.

(vi) Use vented cap flasks whenever possible. These have hydrophobic filter membranes that allow sterile gas exchange but prevent the passage of microorganisms or liquids.

5.4 Storage at Room Temperature

(i) Where a risk assessment has identified that short-term storage is acceptable – ensure that containers e.g. samples containing liquid cultures, are stored securely in such a way that they cannot be knocked over or spilled

5.5. Storage in 4°C Fridges

(i) Ensure that all stocks of reagents, such as medium, sera etc, are properly labelled according to SOP007. Stocks of reagents stored in fridge compartments should be labelled with name and date of opening

(ii) Ensure that plates are sealed with parafilm and securely stacked.

(iii) Ensure that liquid cultures or reagents are secured in racks or placed in suitable outer [secondary] containers to reduce risk of spillage.

(iv) Segregate viable organisms from other non-viable materials in the fridge.

5.6 Storage in the -20°C or –80°C Freezer

5.6.1. Culture reagents

(i) It is recommended that reagents and sera are purchased from suppliers who issue certificates of analysis or results of quality control (QC) testing with each batch of products.

(ii) Reagents may be purchased in bulk to avoid variation between batches, depending on shelf life.

(iii) Store serum at -20^{o}C, but not in frost-free freezers as temperature cycling may crack the bottles. The shelf life of serum is 12-18 months and longer term storage is not recommended as any advantages gained by a single batch may be offset by deterioration. The shelf life of single strength medium is approximately 9-12 months, concentrated medium (10X) approximately 12-24 months and powdered medium approximately 24-36 months.

5.6.2 Cell line stocks

(i) Cell line stocks are usually contained in small ampoules of 2ml or less. Secure the ampoules in trays within boxes. Ensure that the box is sealed and leak-proof to contain liquid in the event of freezer failure. Label the box with the nature of the content and the identity of the person responsible for it.

NOTE: Cell stock viability may be progressively lost within a few months at -80^{o}C. Long term storage should be kept below -130^{o}C.

5.7 Ultra-low Temperature Storage

(i) Keep cell stocks below -130^{o}C as viability may be progressively lost within a few months at -80^{o}C.

NOTE: For liquid nitrogen storage it is a legal requirement in the UK to store potentially infectious material in the vapour phase. This reduces the risk of transfer of contaminating organisms and eliminates the hazard of liquid nitrogen penetrating ampoules which may then explode on warming (Refer to SOP013 & SOP031).

(ii) For security, divide important material (e.g. Master Cell Banks) into more than one storage vessel.

5.7.1 Storage and Banking of Cell lines

(i) Establish a Master Cell Bank of 10-20 ampoules to ensure a reliable and reproducible supply of cells.

(ii) Use one ampoule from the Master Cell Bank to generate a Working Cell Bank or Distribution Cell Bank. The Working Cell Bank should contain sufficient ampoules to provide at least one ampoule for every 3 months of the proposed experimental period plus sufficient ampoules for contingencies and distribution.

NOTE: Incorrect or serial banking (as occurs for cultures passed from one laboratory to another in a chain) results in a progressive increase in the population doubling number and additional risk of contamination or loss of key characteristics.

5.7.2. Liquid Nitrogen Cryopreservation

The aim of cryopreservation is to enable stocks of cells to be stored to prevent the need to have all cell lines in culture at all times. It is invaluable when dealing with cells of limited life span. The other main advantages of cryopreservation are:

- Reduced risk of microbial contamination
- Reduced risk of cross contamination with other cell lines
- Reduced risk of genetic drift and morphological changes
- Work conducted using cells at a consistent passage number (refer to cell banking section below)
- Reduced costs (consumables and staff time)

(i) Consult SOP031 & SOP032 for procedures to cryopreserve cells in a stable state for limited or prolonged periods. These include manual procedures for freezing, storage and recovery of cells.

(ii) All ultra-low temperature storage should be supported by accurate record keeping and inventory control incorporating the following procedure:

- Individually label, using “wrap around”, liquid nitrogen resistant labels with identity, lot

number and date of freezing

- Record the location of each ampoule on cryostorage record slip.. This record must be given to the CBE Technician to update the cryostorage units database.
- Update Pro-Curo when adding or removing material from the cryostorage units.
- **Ensure that no ampoule is deposited or withdrawn without updating the records**

5.8 Archive material

(i) Wherever possible, dispose of material associated with experiments that are no longer 'active' and are not covered by a current risk assessment.

(ii) Where archive material has to be kept and is not covered by a current assessment, physically segregate it from currently used material. Label the outer container to the effect that the material inside must not be used unless a risk assessment is carried out.

NOTE: At the end of the research project, consult the Laboratory Manager/Principal Investigator to determine the treatment plan and schedule for the destruction of stored and archived material.

5.9 Transport of Biological Agents

5.9.1. Off-site transport – outside CBE Facilities

Certain biological samples, cultures and other materials fall within the description of dangerous goods for carriage and both national and international legislation demand stringent requirements must be met is the goods are transported by any means, including primary receptacles, packaging, labelling and documentation. All CBE personnel MUST ensure Regulations applicable to the transport of biological materials are complied with for each particular consignment.

Before transporting hazardous or non-hazardous materials off-site CBE personnel **MUST** consult the following:

1. The relevant Biological Risk Assessment;

2. Sections 7 & 9 of the 'CBE Code of Practice for guidance on the Transport of Infectious Substances. The CoP gives full details of the requirements for transport of BA's/GMOs in the UK and abroad. These guidelines MUST be read in conjunction with the Loughborough University Biological Safety Policy;

3. The CBE Quality Manager and/or local BGMSA to confirm the requirements for transportation of biological agents in the UK or abroad.

NOTE: Further information can be obtained from Guidelines available on the CBE website.

NOTE: Biological materials which are not classified as dangerous goods for transport MUST still be packaged safely to ensure they do not leak in transit since this is likely to trigger safety/security alerts. The package must be appropriately labelled to avoid any ambiguity over the contents and should ideally show emergency contact details that can be used in the event of a query. This can be achieved by following the procedures given below.

NOTE: Any problems occurring during transport, such as leakage or breakage, should be reviewed in order that corrective measures can be taken to prevent any recurrence.

5.9.2. On-site Transport – within and between University Buildings

Any hazardous material (including modified) must be transported in appropriate containers ie lidded, leak-proof (or sift proof) containers that can be easily disinfected. Material must not be carried in hands, open trays, pockets or loose in plastic bags.

NOTE: If material is transported on foot, then the carriage of dangerous goods regulations do not apply, but appropriate containers should still be used (see below). In addition, consideration should be given to the route used to transport the material (eg how many roads need to be crossed, avoidance of crowded public area where possible). Those transporting the material should inform the receiving area when they are leaving and how long they expect to take.

NOTE: For transport by road, the transport regulations apply if the goods are transported on the public highway by any means. However, they do not apply to carriage between one part of University premises and another part situated in the immediate vicinity even if a public road separates them. Therefore the regulations do not apply to transport of dangerous goods within the University site. However, even if the regulations do not apply because the journey is as described above, the materials must still be packed in such a way to ensure they do not leak in transit and be appropriately labelled with emergency contact details.

5.9.2.1. For liquids consigned at ambient temperatures.

(i) Place the leak-proof primary receptacle inside leak-proof secondary packaging. Each primary receptacle should not contain more than 1 L

- Use primary receptacles consisting of glass, metal or plastics.
- Ensure that the container has a leak proof seal e.g. a heat seal, a skirted stopper or metal crimp seal.
- If screw caps are used, reinforce with adhesive tape

(ii) Place absorbent material in sufficient quantity to absorb the entire contents between the primary receptacle(s) and the secondary packaging

(iii) If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated so as to prevent contact between them

5.9.2.2. For solid substances consigned at ambient or higher temperatures:

(i) Place the siftproof primary receptacle inside secondary siftproof packaging. Each primary receptacle should not contain more than 4 Kg

(ii) If multiple fragile primary receptacles are placed in a single secondary packaging, they must be either individually wrapped or separated to prevent contact between them.

(iii) If there is any doubt as to whether or not residual liquid may be present in the primary receptacle during transport a packaging suitable for liquids, including absorbent materials must be used.

5.9.2.3. For refrigerated or frozen material: Ice, dry ice and liquid nitrogen:

(i) Place ice or dry ice outside the secondary packagings or in the outer packaging or an overpack.

(ii) Interior supports should be provided to secure the secondary packagings in the original position after the ice or dry ice has dissipated.

(iii) If ice is used, the outside packaging or overpack must be leak proof.

(iv) If dry ice is used, the packaging must be designed and constructed to permit the release of carbon dioxide gas to prevent a build-up of pressure that could rupture the packagings.

(v) The primary receptacle and the secondary packaging must maintain their integrity at the temperature of the refrigerant used as well as the temperatures and the pressures which could result if refrigeration were lost.

5.9.2.4. For substances consigned in liquid nitrogen:

(i) Only consign biological materials in liquid nitrogen if there is no suitable alternative means and do so only in a liquid nitrogen shipper that is UN type-approved.

(ii) Use plastic primary receptacles capable of withstanding very low temperatures.

(iii) Ensure the secondary packaging is also capable of withstanding very low temperatures, and in most cases will need to be fitted over the primary receptacle individually.

(iv) The primary receptacle and the secondary packaging must maintain their integrity at the temperature of the liquid nitrogen.

NOTE: Provisions in the regulations for the consignment of liquid nitrogen must also be fulfilled.

(v) Contact the University Biological Safety Officer for further advice.

5.9.2.5. For lyophilised substances:

(i) May be carried in primary receptacles that are flame-sealed glass ampoules or rubber-

stoppered glass vials fitted with metal seals.

5.9.3 Transport between laboratory areas within the CL2 CBE Laboratory Unit (Holywell Park) and within the CBE Tissue Engineering Laboratory (T208B).

(i) Aliquot BA's/GMOs intended for storage in liquid nitrogen or –80ºC freezers in non-breakable, cryovials. Surface decontaminate the vials with 70% ethanol after sealing and transport to the freezers in non-breakable, impermeable, closed containers.

(ii) For transport of Hazard Group 2 or GM frozen cells and tissues, including cells potentially infected with Hazard Group 2 biological agents (e.g. viruses), place the sealed containers (such as sealed screw top ice containers) into tube racks and trays to carry cultures around the laboratory.

(iii) For the transportation of small volumes of culture that can easily be carried, place container within a secondary outer container with a lid. If doors need to be negotiated, use a trolley or get assistance from a colleague.

(iv) For the transportation of larger cultures use additional trays and tube racks as required and transport using a trolley.

(v) Do not carry glass flasks containing viable organisms unless they are contained within a secondary container with a lid. Always carry unsealed cultures in trays or boxes to minimize contact with airborne contaminants.

(vi) Ensure that enough absorbent material and gloves readily available to deal with any spillage when transporting biological agents.

(vii) Ensure that containers for transportation are labelled with the nature of the content and the identity of the person responsible for them.

5.9.4 Transport to lower containment laboratories e.g. for Non Cell-Culture Procedures involving Human & Primate Cell Lines, HG2 Cell Lines or GMOs

(i) 'Make safe' material before transfer for experimental work in a lower containment laboratory: egg. Lysing, fixing, homogenization in strong detergent (such as SDS) of cells within the Containment Level 2 Laboratories, before being removed.

NOTE: These procedures may require additional safety measures and should be identified in a risk assessment before the work commences i.e. The use of the common fixative para-formaldehyde, which is a strong carcinogenic and volatile compound, the usage of a fume cupboard requires an externally vented fume hood.

(ii) Transport all biological agents in a sealed container, which must be opened within a fume hood and removed only after fixation has taken place.

6. DOCUMENTATION

The following records are outputs of this SOP:

- FSOP005.1 Cryostorage record slip

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SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
1.0	25.03.10 P.Hourd	Annual Review – Minor editorial revisions and surname of reviewer (CK) updated. The attachment of biohazard signage to storage units is not needed unless a risk assessment identifies a requirement i.e. biohazard signage on entrance is considered sufficient. This SOP has been revised to reflect a more judicious use of hazard signs. New version issue not required	Not Issued
001	23/02/2011 P.Hourd	Revised scope and records sections to include CBE Tissue Engineering Laboratory(T208B), located in the Wolfson School. Addition of off-site transport section; 7.9.1	**002**
002	27th April 2020 by C.Kavanagh	Addition of statement to say storage of HTA material is documented in a separate SOP. Added a reference to use of Pro-curo for updating when material is added or taken out of the cryostorage units,	**004**