Standard Operating Procedure **SOP008**

Title: MANAGEMENT AND CONTROL OF INCOMING BIOLOGICAL MATERIAL TO PREVENT ENTRY AND SPREAD OF MYCOPLASMA CONTAMINATION

Location: CBE Laboratories

1. PURPOSE

To describe the procedure for the acquisition and safe receipt, identification, quarantine, testing, and disposition of biological materials in CBE laboratory facilities.

2. SCOPE

This SOP applies to containment level 2 (CL2) CBE laboratories, including the CBE Laboratory Unit (located in the Holywell Park) and the CBE Tissue Engineering Laboratory T208B (located in the Wolfson School). The SOP applies to personnel who procure, purchase or otherwise acquire biological materials from external vendors for use in CBE laboratory facilities. The SOP covers the acquisition, receipt and handling of incoming biological material (as defined below) to prevent the entrance and spread of mycoplasma in CBE laboratories.

Definitions:

(i) **Biological material:** Material consisting of or containing Biological Agents (HG1 & HG2) and Genetically Modified Organisms (Class 1) defined according to COSHH (6th edition) and GMO (contained Use) Regulations 2002. Includes cell lines and tissues (and their derivatives) and where applicable, associated culture reagents such as conditioned media, animal or human sera.
Note: Incoming HTA relevant material is covered in CBE/HTA-PR-SOP004 receipt and storage of HTA material.

(ii) **Quarantine:** A status for the material inventory that cannot be used in the CBE until released by the Quality Management team.

(iii) **Released:** A status for the material inventory that permits its use in the CBE

(iv) **CofA:** Certificate of Analysis

(v) **Receiver:** The person whose name appears on the address label or an authorized person designated to receive and inspect shipments of hazardous or potentially hazardous biological materials. May also be the recipient.

(vi) **Recipient:** The person who procured, purchased or acquired the material – the material owner. May also be the receiver.

(vii) **Material Management team:** Person(s) responsible for the quarantine, storage, traceability, and testing of incoming materials at the CBE facility.

3. RESPONSIBILITIES

3.1. Receiver shall:

(i) Receive, inspect and store materials & associated documentation in accordance with this SOP.
(ii) Return materials to vendors as required under the conditions detailed in by this SOP
(iii) Ensure incoming material is traceable to a designated quarantine location.
(iv) Wear Personal Protective Equipment (PPE) including gloves, lab coat, and eye protection when opening packages containing potentially infectious substances

3.2. Recipient shall:

(i) Where possible, procure, purchase or acquire materials from quality vendors.
(ii) Ensure all quality documentation is available from the vendor.
(iii) Ensure risk assessments are in place before ordering or acquiring any hazardous biological material.
(iv) Receive, inspect & store materials and associated documentation in accordance with this SOP
(v) Return materials to vendors as required under the conditions detailed in by this SOP
(vi) Ensure incoming material is traceable to a designated quarantine location and regular storage areas when released.
(vii) Perform corrective actions as required by this SOP.
(viii) Shall wear Personal Protective Equipment (PPE) including gloves, lab coat, and eye protection when opening packages containing potentially infectious substances
(ix) Make sure an FS008.1 Receipt and Acquisition of Potentially Hazardous Biological Materials and is passed onto the Quality Systems manager.

3.3. Material management team shall:

(i) Locate an appropriate quarantine area for incoming materials.
(ii) Ensure that materials are identified in accordance with the material disposition.
(iii) Maintain material inventory database to ensure incoming material is traceable to a location within quarantine and regular storage areas.
(iv) Arrange internal & external mycoplasma testing, as required. Ensure results are reported to QM.
(v) Ensure that corrective actions are taken and implemented in accordance with this SOP.

3.4 Quality Systems Manager shall:

(i) Review the Material Receipt Checklist to authenticate the origin of the material and determine

its conformity to established specifications with respect to the risk of cross-contamination or contamination with micro-organisms and mycoplasma.

(ii) View and sign off FS008.1 if appropriate or instruct on further actions.

(iii) Assign the final disposition of the quarantined material.

4. EQUIPMENT AND MATERIALS

(i) Cryostorage Units, CBE Cryostore Banks located in H30, H31 and H34 (***this does not include the Experimental Bank***).

(ii) Fridges, Freezers, -80C Freezer.

(iii) Spill Kit

(iv) Personal Protective Equipment including disposable gloves and lab coat.

(v) Aluminium spill tray

5. PROCEDURE

5.1. Material Acquisition

5.1.1. Risk Assessments for hazardous biological material

5.1.1.1. Before ordering or acquiring any materials containing **Biological Agents and GMOs** check that the required risk assessments have been completed and available in the CBE office.

5.1.1.2. Biological agents designated HG3 or HG4 and GMOs of Class 2 or above **MUST NOT** be brought into the CBE Laboratories. GMOs of any Class **MUST NOT** be brought into the Tissue Engineering Laboratory (T208B), located in the Wolfson School.

5.1.1.3 HTA relevant material see: **CBE/HTA-PR-SOP004 receipt and storage of HTA material.**

5.1.2. Evaluation Criteria of Vendors to ensure quality

5.1.2.1. The vendor should be selected based on of the ability to meet quality system and any quality assurance criteria based on the level of service provided and registrations or certifications held.

For example: Vendor operates under regional standards bodies such as the European Committee for standardisation (Cen,) Human Tissue Authority (HTA) or international

standards organisations such as the international standards organisation (ISO) or equivalent. Vendor meets nationally certified standards such as those of the National Institute for Science and Technology (NIST), United States Pharmacopeia (USP), American Type Culture Collection (ATCC), the National Institute for Biological Standards and Control (NIBSC), the National Collection of Type Cultures (NCTC) or the European Collection of Cell Cultures (ECACC) etc.

5.1.2.2. Select vendors that can provide records of origin and history and certificates of the quality control (QC) testing performed to ensure materials are free of contaminants and particularly tested negative for mycoplasma. (Certificate of Analysis)

5.1.2.3. Where the level of service provided and registrations or certifications held by the vendor and/or the level of evidence available may not be sufficient to authenticate the material, if possible arrange for material to be tested for mycoplasma at source.

Note: In some cases the selection of vendors may be restricted because of the specification in the methods or collaborating laboratories being used or even cost for example,

5.1.2.4. In these cases, if no mycoplasma testing has been performed by the supplier or provider, incoming cells and where applicable, cell culture reagents, particularly conditioned media, sera and animal-derived products (*historically a common source of mycoplasma contamination*) will require quarantine and be tested negative for mycoplasma before use in the CBE.

5.2. Receipt and inspection of incoming Biological Material

On receipt and before accepting the package:

5.2.1. Check the integrity of the secondary (outer) packaging for leakage or any other damage.

5.2.2. Check the goods against the delivery note to ensure that the consignment is intended for a person who works within the CBE laboratories.

5.2.3. If the packaging is damaged so as to compromise safety or the integrity/quality of the enclosed material or the identity of material or person the consignment is intended for is uncertain, instruct the shipper to return the package to the sender.

5.2.4. If the packaging and documentation is in order, notify the recipient for pick-up or transfer to CBE laboratories.

Note: Deliveries may be made to the Wolfson. These need to be collected by CBE personnel upon notification from the Goods Inwards Department in the Wolfson. They may also be delivered to ChemEng or Herbert Manzoni.

5.2.5. Prior to taking goods into CBE laboratories, if practicable remove all fibrous packaging material and dispose of any additional outer layers of protective packaging.

5.2.6. Transfer the package to the storage location as described on the packaging (e.g., fridge, freezer, -80 freezer, cryostore).

5.2.7. Check to make sure the material has a Certificate of Analysis (C of A) from the vendor. If the C of A is not received in advance or did not accompany the shipment, notify the material owner immediately so the vendor can be contacted and the appropriate C of A obtained.

5.2.8. Check that identification and quantity or amount received matches the amount ordered, as specified on the Purchase Order (if available).

5.2.9. Inspect the material to make sure that all containers or packaging are clearly and accurately labeled and that the lot number on each package or container(s) matches the Lot Number on the accompanying Certificate of Analysis (if available). Annotate received documentation with the initials of the recipient.

5.2.10. Check that the condition of the containers and packaging is satisfactory, making sure that there are no punctures, leaks or other damage and that all containers are adequately sealed.

5.2.11. If the package or any container is leaking or damaged in any way, follow the instructions detailed in Annex 1

5.2.12. If any of the following applies, the material should be either discarded or returned to the vendor.

- If the packaging or container is damaged so as to compromise material integrity and quality

- Material received is different in identity from what was ordered
- Material identity is uncertain
- Materials cannot be safely handled

5.2.13. If the received material is rejected as defective, deficient or otherwise not fit for purpose and cannot be immediately returned to the vendor, that material is considered Out of Specification (OOS). Label the Material with a material 'Out of Specification' – Reject label.

5.1.14. Transfer the OOS material to the appropriate rejected goods storage area pending action regarding its disposition.

5.1.15. Contact the vendor to arrange for return and / or credit for the OOS material and arrange for reordering if necessary.

5.3. Transfer to storage/Quarantine location

All Materials that are received in satisfactory condition should be handled as follows:

5.3.1. If inspection and release can be carried out immediately follow instructions in section 5.3.2. If material requires additional checks or paperwork you must transfer the material to the identified quarantine area of the appropriate storage location. E.g. Cryostore 8 is the Quarantine bank. If it is to be stored in a fridge or freezer, it should be put into a secondary container.

5.3.2. If the item is to be stored in liquid nitrogen (vapour phase):

- Choose a location within the cryostorage unit by checking the cryostorage bank database .This can be found on the CBE-QUAL workspace . **Quarantine locations are located in Cryobank 8. There are quarantine boxes for both HTA relevant material and non-HTA relevant material.**

  Note;There are Cryo stickers avaliable, which can be written upon and stuck to frozen vials. See Jen Bowdrey (CBE Technician) to get stickers. These should be put onto vials to allow identification of groups and other information.

- Choose an empty box in the cryostorage unit in the above location. Please note space has been allocated per group.

- Open the cryostorage unit and place vials in the empty box. **Under no circumstance should any other vials be stored in this box.**

Note: Before placing any items in quarantine storage inspect the items to ensure they are still completely frozen. Notify the recipient if any degree of thawing is observed.

5.3.3. Complete the Cryostorage Transfer, which can be found in the internal lab corridor on the wall or there is a version on the website which can be emailed. Submit to the member of the material management team responsible for the cell banking database who will update the database. Also enter the information onto Procuro, so that there is a record of the sample(s) on this database.

5.3.4. Once in storage location/ quarantine, complete Material Receipt Checklist (FSOP008.1), attach the C of A and all associated/supporting documentation and submit to the Quality Manager (QM). If any supporting information is missing please inform the Quality Manager that items are in quarantine until documents/further testing can be arranged.

5.4. Authentication and Release from Quarantine

5.4.1. Upon receipt, the QM will review the Material Receipt Checklist to authenticate the origin of the material and determine its conformity to established specifications with respect to the risk of cross-contamination or contamination with micro-organisms and mycoplasma and the ability to detect them.

5.4.2. The QM will assign the final disposition of the material to one of the following:

- Approve the item for release (if all documentation has been received)
- Approve the item for release **subject to testing negative for mycoplasma** (using internal PCR or external Mycoplasma Test Cell Screen PCR Test (via 'Mycoplasma Experience' - turnaround time of 2 days)). *See Annex 2 for generic performance specifications for detection methods.*
- ***NOTE****: If quarantined material is cultured to allow internal mycoplasma testing to be performed follow procedures in section 5.6.*

- Approve the item for release **subject to testing negative for mycoplasma** (using external microbiological culture method – via 'Mycoplasma Experience - turnaround time of 28 days). *See Annex 2 for generic performance specifications for detection methods.*

- Reject and destroy.

5.4.3. If the material is approved it can be released and either used or placed into storage into the relevant groups storage areas, e.g. the groups cryoboxes.

5.4.4. In accordance with the materials disposition – rejected material should be pulled from the quarantine area and destroyed.

5.5. Removal of material from Quarantine

Following QM approval for release of material:

5.5.1. Remove the items from the quarantine area and transfer the released material to the regular storage area.

5.5.2. Identify the appropriate location to store the material. If applicable, review the cell banking database available on CBE -QUAL workspace to find locations that are not currently being used.

5.6. Sampling and testing of incoming Biological Materials for mycoplasma

5.6.1. Remove a representative batch(s)/lot(s) of material from the quarantine area and transfer to a designated Biological Safety Cabinet (BSC) located in a defined laboratory within the CBE. Check with the relevant Lab Leader.

5.6.2. In the BSC, prepare a sample of the material as specified by the designated mycoplasma test method in accordance with the material disposition e.g. actively growing cell and conditioned media.

Note: Sampling must be conducted at defined locations and by control procedures designed to prevent contamination of the material sampled and contamination of other materials or equipment.

5.6.3. Ideally, a separate quarantine laboratory should be available for this purpose. The next best approach is to have a Class II BSC and an incubator dedicated for quarantine. If

this is not possible, other steps should be taken to minimise the risk of spreading contamination, including:

- Materials in quarantine should be handled only after all other cell culture has been completed that day,
- The new cultures should be placed in a dedicated incubator or into a sealed secondary container before going into a general incubator,
- The Incubator should be thoroughly cleaned after use with a suitable disinfectant (Virkon or Mycoplasma Ex)
- After use the BSC should be run with the UV light activated for at least 30 minutes. The BSC should then be thoroughly cleaned with a suitable disinfectant (Virkon or Mycoplasma Ex) followed by a second UV disinfection cycle before before shut down.

5.6.4. If any mycoplasma contamination is detected following internal or external mycoplasma testing, initiate corrective action according to SOP158.

6. DOCUMENTATION

The following records are outputs of this SOP:

- FSOP008.1 Record of the Receipt and Acquisition of Hazardous Biological Material
- FSOP31.1 Cryostorage Transfer Sheet
- Procuro- electronic database where all incoming and current samples are entered.

These records shall be filed in the CBE Office or otherwise archived for future review or retrieval.

ANNEX 1. Leaking or Damaged Packages

Leaking or damaged packages **MUST NOT** be accepted. If evidence of leakage is found subsequent to acceptance, receiving personnel shall:

(i) Not handle the package - it should only be handled by personnel trained in spill clean-up procedures and wearing appropriate personal protective equipment.

(ii) Isolate the area around the package.

(iii) Notify the Responsible Person and the CBE Laboratory Manager/Departmental Safety Officer (DSO).

(iv) Ensure that anyone that has handled the package and may have exposed their skin to the leaking material washes the affected area with antiseptic soap and warm water or antiseptic/germicidal wipes (provided in the spill kit).

(v) Notify the responsible person so that they can clean up any spill as directed in the Biological Risk Assessment. The Responsible Person shall decontaminate the area according to the following procedure:

1. Wear appropriate PPE, i.e. gloves lab coat. disposable apron, safety goggles
2. Surround package with absorbent
3. Place leaking package in another box and seal
4. Place sealed box in plastic bag and seal bag
5. Place entire package in an outer carton, label for return to sender or for proper disposal
6. Cover the spill area with sufficient powdered Virkon and leave until all liquid is absorbed

CAUTION: Wear a disposable facemask to prevent inhalation of powder.

CAUTION: Virkon is corrosive to metals; do not allow Virkon to contact metals for periods exceeding 10mins.

7. Scrape the soaked powder into a dustpan and place into a biohazard bag/container.
8. Wipe the spill area with the paper towels soaked in 1% Virkon solution and place the used towels in the biohazard bag/container
9. Remove gloves and other contaminated garments and place them in a biohazard bag/container for incineration. DO NOT AUTOCLAVE WASTE
10. Thoroughly wash hands, face, and other apparently contaminated areas again with soap and water

NOTE: If the responsible person is unavailable, the receiver shall clean up the spillage.

ANNEX 2. Available mycoplasma detection methods:

Option A: External testing

External mycoplasma testing regime conducted through the company called Mycoplasma Experience (www.mycoplasma-exp.com/). Samples can be sent to Mycoplasma Experience as per the instructions provided by the Company. Mycoplasma Experience can conduct one or more of the following tests:

1. **PCR Test** where DNA from a 200µL of the sample is extracted using the Roche MagNA Pure Compact robot. RT-PCR amplification of the samples is carried out in optical reaction plates by TaqMan methodology using the mycoplasma detected kit VenorGen-qEP from Minerva Biolabs in a Roche LC480 cycler. This is an indirect test and can give a false positive but the results are back within 2 days.

2. **Microbiological Culture Test**. This test is a combination of direct culture on mycoplasma media and an indirect or non-cultural method. This is widely regarded as reliable for examination of cells for mycoplasma infection. The test can take longer than a month for the final results.

3. **Cell Screen Test.** Lower cost compared with (2) but retains most of the QC elements. Samples are transported overnight to the Mycoplasma Experience laboratories and cultured as cell suspensions or monolayers in plates. There is an inspection after one week which can give a preliminary indication of contamination with mycoplasma. The cells are allowed to culture in Agar for up to 28 days and then a final result is returned. The samples are checked daily during this time for any contamination.

Option B: Internal testing

4. **Internal Mycoplasma PCR test.**

CHOOSING THE APPROPRIATE MYCOPLASMA DETECTION METHOD

ID	Method	Internal/ External	Relative Sensitivity	Specificity	Turnaround Time	Sample types	When to use
1	Mycoplasma Exp PCR Test	External	High	Medium (will not detect all mycoplasma species)	1 day (+ shipping)	Cell cultures	Release testing
2	Mycoplasma Exp Micro culture test	External	High	High	28 days (+ shipping)	Cell cultures	Certifiable testing
3	Mycoplasma Exp Cell Screen Test	External	Medium	Medium (will not detect all Mycoplasma species)	28 days (+ shipping)	Cell cultures preferred but supernatant possible.	Routine/regular screening

4	Mycoplasma PCR Test	Internal	Medium	High	Very rapid.	Cell culture supernatant.	Release testing/ Urgent testing

External testing for routine QA under SOP036:

The CBE Lab Manager will send selected samples from actively growing/continuously maintained cultures to Mycoplasma Experience for testing at defined regular intervals (minimum 6 months). Detection method 3 (Cell Screen Test) is selected as the primary test but if results are required urgently, samples can be also be tested using detection method 1 (PCR Test). In circumstances where fully certified testing is required detection method 2 (culture test) should be selected.

Internal QA testing under SOP036:

As a minimum, internal mycoplasma testing (detection method 4) should be applied to the following:

- Release testing of quarantined adherent cell lines or other relevant biological material acquired (supplied, purchased or transferred) from non-QA certified sources or where administrative assurance cannot otherwise be verified e.g. by provision of Certificates of Analysis or other relevant documentation that indicate that the risk of mycoplasma contamination is low.
- Release testing before transfer of relevant material from CBE to other external laboratories
- Testing of actively growing cultures that are behaving suspiciously.
- Testing of Master and Working cell banks at the time of freezing.
- Regular testing of randomly selected growing culture specimens to support the external mycoplasma testing regime.
- Testing of commonly used constituents of complete media (*where applicable*).

SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
001	04Jan2010 Revised by K. Brosnan	**Section 2 –** scope altered to specify types of biological materials permissible in the CBE, also included note regarding requirement for BRA for all biological materials and highlighted prerequisite to read SOP036 and 048 prior to reading this SOP. **Section 3 –** added references to university policies **Section 4-** Altered definition of receiving to receiver and recipient to responsible person to clarify roles. **Section 5** – altered responsibilities of receivers, responsible persons and the QSM to reflect changes in procedure. **Section 6** – included cryostorage unit CBE042 and CBE043 to equipment list and removed reference to dedicated quarantine	**002**

		fridge in T030 **Section 7.1** – altered to reflect new procedure adopted by CBE Unit, main body of text has remained as is, with slight changes in arrangement. Included specific details regarding quarantining of materials **Section 7.2** – included new section to detail procedure to follow when removing items from quarantine. **Section 7.3** – previously section 7.2 now listed as 7.3, slight amendments made but main body of text as before. **Section 7.4.1** – Included section to detail to procedure to be followed when assigning a cell bank record database reference number. **Section 7.4.2** – included section to specifically detail the locations of each vial within the cryostorage unit. **Section 8.1** – Alter the receiving form to include specific quality control checks before removal from quarantine. **Section 8.2** – included a print screen shot of the Cell Bank Record to illustrate entry points of the database reference number and location numbers.	
002	01/02/2011 K. Brosnan	Annual Review – No amendments required	Not Issued
002	10/02/2011	Revised scope and records sections to include the CBE Tissue Engineering Laboratory (T208B), located in the Wolfson School	003
003	01/10/12 P.Hourd	Annual Review – revision of format	004
004	09/12/2014 P. Hourd	Major review of the SOP following CBE/CAPA/072	005
005	04/07/2018 J.Bowdrey	Review of the SOP, removal of Internal MycoAlert test and replaced with the internal mycoplasma PCR test. Added HTA relevant material and directed to the relevant SOP for this kind of material. Removed mention of SOP031 and SOP032 as no longer relevant.	006
006	09/06/2020 J.Bowdrey/C.Kavanagh	Addition of entering Sample information in Procuro.Cryo stickers for adding information onto frozen vials. Minor editorials	007