Standard Operating Procedure **SOP158**

Title: CORRECTIVE ACTION RESPONSE TO A POSITIVE MYCOPLASMA TEST RESULT

Location: CBE Laboratories

1. PURPOSE

The intent of this SOP is to describe the procedure for responding to a potential mycoplasma infection in the CBE Laboratories.

2. SCOPE

This SOP applies to the Centre for Biological Engineering Laboratories and the Cell Therapy Manufacturing Facility (CTMF) located in Area GH, Garendon Wing, Holywell Park and the CBE Tissue Engineering Laboratory in the Wolfson School. The SOP applies to personnel responsible for the quarantine, testing, and disposition of biological materials in CBE laboratory facilities. This SOP describes the corrective action response that personnel must take in the event of a positive Mycoplasma result being confirmed as a result of internal or external mycoplasma test regimes.

3. RESPONSIBILITES

3.1. **Operator:** Person performing the mycoplasma test (see SOP010 for Internal Mycoplasma testing). In the event of a positive result for a mycoplasma infection the operator shall liaise with the Laboratory Manager/QM to establish, record and implement this SOP. The operator shall implement a CAPA procedure in accordance with this SOP.

3.2. **Owner:** Owner of the test sample and culture. In the event of a positive mycoplasma test result, the owner of the test sample/culture shall liaise with the Laboratory Manager to organise the implementation of laboratory decontamination procedures according to this SOP.

3.3. **Responsible Person (RP)/Laboratory Manager (LM):** Shall liaise with the operator, the owner and the QM to ensure all necessary corrective actions are immediately implemented.

3.4. **Quality Manager:** Shall eensure that all corrective actions are taken and implemented in accordance with this SOP.

4. EQUIPMENT AND MATERIALS

Materials	**Equipment**
70% IMS	Autoclaves
Mycoplasma Ex Disinfectant	BSC
Aquaguard 2	Waterbath
Lifeguard disinfectant	Mop and bucket
Paper towels	
Disposable lab coats/Coloured lab coats	
Gloves	
Shoe covers	

Autoclave bags, Non autoclave bags	
Yellow bags	

5. PROCEDURE

5.1. Once mycoplasma has been detected, the affected laboratory rooms/areas must be isolated to contain and prevent the spread of contamination: As a minimum, includes the following:

- Check where the infected material came from by tracing the steps of the owner and their work activity.
- Place signs on the door to prevent people from entering the identified rooms/areas.
- If applicable, place a sign on the relevant cryostore unit where infected cells may have been stored to prevent any further access to the cryostore unit.
- Send an email to all CBE Personnel (cbe-everybody@lists.lboro.ac.uk) to inform laboratory users of the potential contamination risk and the resultant steps taken to isolate rooms/areas at risk.

NOTE: Controlled access to the isolated rooms may be available after consultation with QM or deputy. Under controlled conditions, a regime of separate lab coats, gloves and shoe covers shall be initiated for each isolated area. Disposable lab coats must be autoclaved before disposal. If a different coloured lab coat regime is initiated, then lab coats must be changed along with gloves and shoe covers on entry and following exit of the isolated room/area.

5.2. Create a sampling plan to ensure the systematic selection of samples from areas considered at greatest risk of contamination. Where relevant, this should include the following:

- Select at least two samples from the cryostore bank. One should be from the same rack location as the sample with the positive mycoplasma test result. The other should be selected from a different but adjacent rack location. Other samples can be selected from racks located above and/or below.
- Select samples from cell lines in current culture, targetting those which have used the same equipment used to (or in) the culture from which the postive test sample was identified eg BSC, incubator, water bath etc.
- Prepare all selected samples for testing according to internal or external procedural test requirements. Prepare all samples under the controlled conditions in a designated and segregated area – See Annex 1.

5.3. Removal and dispose of all materials and consumables from laboratory rooms/areas at risk:

- Remove and dispose of any cultures in the area.

- Dispose of any open reagents (e.g., media, serum, aliquots).
- Dispose of all tissue paper stocks.
- Dispose of laboratory consumables, using the yellow stream bags for unopened items and the orange stream bags (ie for autoclaving) for opened items. As a minimum, disposable stock includes the following items:
    - Pipette tips (all boxes)
    - Stripettes (all sizes)
    - Eppendorf tubes (all sizes)
    - Syringes
    - Polypropylene centrifuge tubes (15 ml and 50 ml)
    - Opened packs containing Tissue culture flasks.
    - All opened PBS containers.

NOTE: In certain circumstances, operators may wish to retain valuable cultures and/or vials. Retention of suspect flasks/vials will be considered for exceptional cases but must be approved of the QM and Biological Safety Officer.

5.4 Cleaning of laboratory rooms/areas at risk

5.4.1 Before cleaning begins open a new roll of tissue paper towels.

5.4.2. Clean both the inside and ouside of the incubators, wipe down using Mycoplasma Ex followed by 70% IMS. Run the decontamination cycle if the incubator has one. Replace the water in the bottom of the incubator and add Aqua-guard 1 in the place of copper sulphate. (See the relevant SOP for each incubator for full instructions on cleaning and decontamination cycles)

5.4.3. Activate the UV in the BSC for a minimum of 30 minutes, immediately clean the BSC using Mycoplasma Ex. Once complete initiate a second UV disinfection cycle. (See the relevant SOP for cleaning BSC‘s)

5.4.4. Clean and replenish the water bath, substituting Aqua-guard 2 in place of Sigma bath. (See relevant SOP- for waterbath cleaning)

5.4.5. Clean all surfaces in the laboratory with Mycoplasma Ex followed by 70% IMS.

NOTE: **Everything must be fully cleaned, including all equipment, shelves, stools, cupboards door handles etc**.

5.4.6. Disinfect the floors by generously spraying the floor with Mycoplasma Ex. Allow the disinfectant to remain on the floor for 5minutes and then and then mop the floor using Lifeguard disinfectant. After cleaning, the mop head should be disposed as yellow bag waste.

5.4.7. Collect all Mycoplasma Ex and 70% IMS contaminated waste in a clear non-autoclave bag. Spray generously with mycoplasma ex and transfer to the lab change area. Once in the change area, place these bags into yellow bag(s) and double bag. Spray generously with 70% IMS before **transfering directly to the biohazard waste bin in Gas Pod 2**.

5.4.8. Collect all autoclavable waste (including contaminated flasks) and transfer to the laboratory change area. Within the change area**, double bag** everything before transfering to the autoclave. These bags must be autoclaved immediately. If the autoclave is in use, keep bags in the labs changing area until the autoclave becomes available.

5.4.9. Before leaving the lab, complete the cleaning checklist and attach to the CAPA report.

5.4.10. Cleaning of the Second Change Room for the affected laboratory area should be performed as a deep clean by washing the walls, doors, coat hooks, floors and any other equipment with Mycoplasma Exs Spray, followed by wiping with 70% IMS wipes.

5.4.11. To disinfect and clean the First Change Room, the following procedure should be used:

- All hanging lab coats should be changed. This is done by emptying the pockets and bagging the lab coats in autoclavable bags and sterilising by autoclaving.
- All pocket items from lab coats are disinfected with Mycoplasma Ex and 70% IMS.
- All walls, doors and coat hooks are disinfected with Mycoplasma Ex and 70 % IMS.
- Disinfect the floors by generously spraying the floor with Mycoplasma Ex. Allow the disinfectant to remain on the floor for 5minutes and then and then mop the floor using Lifeguard disinfectant. After cleaning, the mop head should be disposed as yellow bag waste.
- Clean lab coats can now replace the old lab coats.

- Once the old lab coats have been autoclaved, these are placed in the external corridor to await collection for cleaning.

6. DOCUMENTATION

The following records are outputs of this SOP:

- QS-FORM-022 – Cleaning checklist.

These records will be filed in the Mycoplasma testing file in the CBE office and will be kept for 10 years.

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ANNEX 1: Preparation of test samples for Mycoplasma testing

1. Liaise with the respective lab leader to identify and segregate the laboratory in which the test will be carried out.

2. Restrict access to laboratory during the sample preparation and test period

3. Isolate and label all supplies and consumable to be used during the sample preparation and testing.

4. If possible, handle the selected cell line in a dedicated BSC for the duration of the test procedure. Isolate and label the BSC to prevent access. If not practicable, follow the cleaning procedure detailed in Section 5.4 of this SOP between each transfer of culture vessel(s) and after final use.

5. If possible, use a dedicated incubator for the duration of the culture and test procedure. Isolate and label the incubator to prevent access. If not possible, If not practicable, place culture vessels inside a secondary container within the incubator. Do not use unsealed dishes or plates. Label both the incubator and the secondary container with *`infection risk culture`, name and date.*

6. Spray the outer surfaces of all vessels (eg flasks, eppendorf tubes etc) with Mycoplasma Exs disinfectant before transferring into or out of the BSC.

7. Follow the respective procedure (i) for preparation of samples for provision of external testing or (ii) for preparation and testing of samples using internal test (SOP010).

8. Place all autoclavable waste associated with the procedure into the appropriate waste bags. Place the bags into yellow secondary containers. Spray and wipe the containers with Mycoplasma Ex and transfer to the autoclave room. Autoclave waste immediately. If the autoclave is unavailable, seal the containers using tape and store in the autoclave room. After use, disinfect all containers used to transport the waste with Mycoplasma Ex disinfectant.

9. Place all non-autoclavable waste in yellow bags. Spray the outside of the bags with Mycoplasma Ex and transfer to the waste cage in Gas Pod 2.

   NOTE: This includes waste contaminated with Mycoplasma Ex (alcohol based), which should be placed in yellow bags for incineration.

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SOP Version History

Version Reviewed	Date Revised/ Reviewed	Revision Summary	New Version Number
001	**16th March 2020 by C.Kavanagh**	None	**No new version**