

BioEscalator Laboratory Safety Policy 0004: **Tissue Culture policy**

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BioEscalator Laboratory Safety Policy 0004: **Tissue Culture policy**

1) **Scope of policy**

This policy gives guidance on working practice in tissue culture (TC) laboratory and this is in addition to other policies all of which must be adhered to at all times when working in the TC laboratory. To ensure all tissue are performed to a standard that will prevent contamination from bacteria, fungi and mycoplasma and cross contamination with other cell lines.

2) **New users to the tissue culture laboratory**

- I. Access to the TC laboratory is restricted to authorised tissue culture users only.
- II. All new users in the TC laboratory must receive an induction to the laboratory by the BioEscalator Laboratory Manager or apprentice laboratory technician and have received appropriate training in good laboratory practice and good aseptic technique before commencing work.
- III. Following the laboratory induction new users will have access to Calpendo for TC laboratory booking.

3) **Tissue Culture Laboratory Local Rules**

- I. You must adhere to Good Laboratory practice and good aseptic technique for cell culture at all times (see Appendix 1).
- II. Good basic hygiene, including regular handwashing, must be practised at all times.
- III. White laboratory coat worn in other parts of the laboratory should be left outside the TC laboratory and a clean blue TC laboratory coat (provided) should be worn at all times.
- IV. Wearing of disposable laboratory gloves (not provided) and safety spectacles (provided) are mandatory unless a reason for not wearing them is identified by risk assessment.
- V. Equipment such as pipettes, disposable gloves, TC flask etc. and consumables for equipment etc. is not provided. The following consumables will be provided by BioEscalator which are 70% ethanol, blue paper roll, Virkon powder (5 Kg), autoclave bag (12 L), 60 L burn bin, yellow bag, disinfection solution for water bath & water tray in CO₂ incubator, autoclave tape, dispo jar (3 L) and bio-bin (6 L) for serological pipettes.
- VI. Risk assessments (RA) and standard operating procedures for the safe conduct of the work must be written beforehand, and strictly adhered to.
- VII. Work should be conducted at a work station which is clearly identified; has sufficient space for working safely; is not cluttered and working practices are not compromised due to lack of space.
- VIII. A microbiological safety cabinet (MSC) or other form of primary containment should be used for any procedure that may give rise to potentially infectious aerosols, for example, tissue homogenisation, vigorous mixing, etc. For those procedures requiring centrifugation, buckets with aerosol seals should be used and the bucket opened inside a microbiological safety cabinet.
- IX. The use of glassware and sharps should be avoided.
- X. The MSC, bench surface and any equipment used should be decontaminated immediately on completion of a session of work using 70% ethanol and a 1% Virkon solution should be used for decontaminating lab benches.
- XI. Equipment such as MSC, centrifuges and inverted microscope must be fully decontaminated prior to service maintenance work. A signed statement should be issued to this effect before service maintenance work is allowed.

4) **Online Booking system**

- I. To use one of the MSCs a booking must be made using the online booking software - Calpendo.
- II. To make a booking an active username and password is required, which need to be requested at start of work at the BioEscalator.
- III. You will be only given access to the TC laboratory once you have received your training by the BioEscalator Laboratory Manager or Apprentice laboratory technician.

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- IV. Each MSC should be booked for the duration of the work only and not to be booked out for the whole day including lunch breaks.
- V. MSC and users consumables in enclosed container **must** not be left on unattended at any times in the TC laboratory. This is bad practice!
- VI. If the user exceeds original booking time on Calpendo, they must immediately **STOP** their work or unless the next user has agreed (i.e. permission given) for them to continue until they finish their work (i.e. this is at the discretion of the next user).
- VII. User **must** cancel booking on Calpendo when not required even it is last minute as this will give other users to use the equipment.
- VIII. Please make sure to bring all consumables etc. in enclosed container with user name & company name and once finished must be removed from the TC lab so there is space for the next user.
- IX. Go to **calendars** on Calpendo then click on **equipment** then **Tissue Culture lab** then book individual equipment's for e.g. microcentrifuge, centrifuge Avanti J15, inverted microscope, cell counter, Zoe fluorescent cell imager, and MSC.
- X. Booking of equipment's can be made in 1 hour slots between 24/7 Mon-Sun.
- XI. All bookings should be made using the online booking system i.e. Calpendo as this provides a record of usage of the MSCs and prevents double booking.
- XII. MSC should be booked only for the time needed to complete the work and should not be block booked for extended times. Bookings must be edited on Calpendo if more or less time is found to be required to complete the work.

5) **Use of Microbiological safety cabinet**

- I. All MSCs in the laboratory are operated in the same way. Please read and sign RA form 0012 before use.
- II. Press & hold button 7 to move the window upwards.
- III. Press & hold the green LED button 1 to select the fan ON/OFF at normal velocity. The green LED light indicates that the fan is running at normal velocity, and that conditions are safe.
- IV. To switch the cabinet off press & hold the green LED button 1. Wait for the light to switch off and the air-flow indicator.
- V. There should be designated MSC for primary and viral work only if possible.
- VI. Personal protective equipment (PPE) (blue lab coat, gloves and safety spectacles) must be worn at all times.
- VII. Allow the air to circulate for a few minutes before using the cabinet.
- VIII. Before commencing work all accessible surfaces in the cabinet should be wiped down with 70% ethanol. Also to check underneath the metal work tray and the glass viewing panel (both inside and outside) are clean. If not to clean it and report in the TC incident log book located on bench next to the cell counter.
- IX. All equipment used inside the cabinet should also be sprayed and wiped with 70% ethanol before being placed in the cabinet.
- X. Work in the cabinet should be performed in the centre of the workspace avoiding blocking any of the air vents with equipment.
- XI. Loose material such as paper, tissue, etc. should be prevented from being sucked through the air vents and into the HEPA filters as this may compromise the air-flow.
- XII. At the end of a work session all items must be removed from the cabinet and the surfaces wiped down with 70% ethanol (refer BioE COSHH Form 0003); this includes underneath the metal work tray where liquid may have dripped through the air vent and the glass viewing panel (both inside and outside).
- XIII. Any spillages that occur while working in a cabinet should be disinfected immediately according to the disinfection policy (BioE Lab Safety Policy 0001). To report in the TC incident log book located on bench next to the cell counter.

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6) **Use of CO₂ Incubators**

- I. There are four CO₂ incubators (Make and model Binder C160) in the TC Laboratory. Please read and sign RA form 0010 before use.
- II. Each CO₂ incubator is programmed to run at 5% CO₂ and 37°C and these settings should never be adjusted unless discussed with BioEscalator laboratory manager beforehand.
- III. Each CO₂ incubators temperature are monitored 24/7 via T Scan system and the low alarm is set at 32°C and high alarm is set at 37°C. The alarm is managed by the BioEscalator laboratory manager.
- IV. There should be designated CO₂ incubator for primary and viral work only if possible. Each company will be allocated a shelf and there are three shelves per incubator. They must label & date the TC flask with initials and company name. Also on the front sheet of the CO₂ incubator they must write their company name and users name, in case of an emergency so they can be contactable.
- V. Water tray: All incubators require 2L of distilled water (up to the filling mark on the edge of the inner pan) and 1ml aquaguard-1 (refer to COSHH form BioE 0012) to each litre of autoclaved de-ionised water to be added to the tray at the bottom of the incubator to maintain correct humidity.
- VI. Cultures: All cultures placed in the incubators should be sealed with vented lids (flasks) or loose covers (tissue culture plates). All plastic-ware used should be clean and dry – inspect for drips of media/leaks before placing in the incubator. Cultures in plates/dishes with the potential for spillage should be placed on a clean metal tray rather than directly onto the shelf of the incubator.
- VII. Recording infections: Any infected cultures found in an incubator should be recorded on the Record of Infected Cultures Sheet (see Appendix 2) on the front of the incubator. Where possible provide details of the type of infection (mould, bacteria, yeast), the cell line affected, the type of plastic-ware used (vented/non-vented flask, plate, petri dish, etc.), the number of infected cultures and details of any antibiotics used. Infected cultures should be **decontaminated immediately using a 2% Virkon solution** and disposed of.
- VIII. The owner of any infected cells must inform all others currently using the same incubator and must arrange to clean and decontaminate the incubator.

7) **Use of shared equipment in tissue culture laboratory**

- I. There are a number of items of equipment that are provided for use by all TC users. These should never be removed from the tissue culture laboratory.
- IX. Water-bath: The water-bath should be set at 37°C and the temperature settings may be adjusted. Any tubes or flasks placed in the water-bath should be clean and sealed firmly. Please read and sign RA form 0023 before use.
- II. All items should be removed from the water-bath at the end of the day and the water bath should be switched off.
- III. Centrifuge: TC users must have received the necessary training (microcentrifuge & Avanti J15 centrifuge) before using the centrifuge. Please read and sign RA form 0007 & 0028 before use. The TC centrifuge can be used to spin 15ml and 50ml non-skirted tubes. It is primarily used for short, low rpm (1000 rpm) spins to pellet cells. Aerosol seals are provided for the buckets and should be used for samples which may give rise to potentially infectious aerosols. When aerosol seals are used the buckets should be removed from the centrifuge and opened inside a MSC. The seals/O-rings on the aerosol lids must be greased with vacuum grease periodically. When the centrifuge is not in use it should be switched off and left with the lid open.
- IV. Inverted Microscope: TC users must receive appropriate training before using the microscopes. Please read and sign RA form 0019 before use. Inverted microscope is fitted with cameras & iPad screen to allow photographs to be taken. After use the microscopes should be left clean and the light source and cameras & screen should be switched off.

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- V. Automated cell counter: TC users must receive appropriate training before using the automated cell counter. Please read and sign RA form 0036 before use.
- VI. Pipettes and pipette guns: TC users will need to provide their own pipettes, tips, tips with filters are recommended to minimise the risk of liquid being aspirated into the barrel of the pipette. Please read and sign RA form 0017 before use. BVC Vacuum systems are located next to each MSC for use with disposable sterile serological pipettes and tips. Care should be taken to avoid aspirating media into the filter of the BVC Vacuum system. If this does occur the filter inside the BVC Vacuum system should be replaced immediately.
- VII. Zoe fluorescent cell imager: TC users must receive appropriate training before using the Zoe fluorescent cell imager. Please read and sign RA form 0043 before use.
- VIII. Fridge and freezer: There is an upright (4°C) and underbench freezer (-20°C) for the storage of media and reagents. All items placed in the fridge and freezer must be clearly labelled with the owners name, company name, date and details of the reagent/chemical. It is recommended that permanent marker pens are used for labelling. Any unlabelled items are likely to be discarded without notice. Please ensure any old or out of date media or reagents are disposed of so as not to take up unnecessary space.

8) **Waste disposal**

- I. BioE waste disposal policy 0004: Waste Disposal, gives information about the available waste streams within BioEscalator.
- II. All waste generated in the TC rooms must be disposed of in the appropriate bin.
- III. Serological pipettes must be placed bio-bin (6 L) and pipette tips must be placed in a burn bin (60 L). These bins should be disposed of regularly when full.
- IV. The 6 L bio-bin must be autoclaved as waste discard at 134°C before disposing in black bin for incineration.
- V. Non-sharp, hazardous waste, that will not pierce the bag, must be placed in the yellow bag (waste should not exceed 10kg). Yellow clinical bag should not be overfilled and removed regularly when $\frac{3}{4}$ full.
- VI. All sharps (e.g. needles, scalpel blades) must be disposed of in sharps bins.
- VII. Any hazard group 2 material and waste generated from genetic modification (GM) work must be autoclaved prior to disposal. Autoclave bag $\frac{3}{4}$ (neck taped with autoclave tape) must be autoclaved as waste discard at 134°C before disposing in black bin for incineration.

9) **General disinfection**

- I. Disinfectants should be used in accordance with the BioE disinfection policy 0005.
- II. All work surfaces (including the microbiological safety cabinets) should be disinfected using 70% ethanol before and after each use. Please note that benches should be cleaned using a 1% Virkon solution at the end of each session.
- III. Liquid media waste should be collected in a plastic beaker containing 2% Virkon (final concentration) and treated for 1 hour. The liquid can then be disposed of down the tissue culture drain. The plastic beakers should be thoroughly rinsed with water until clean and left to dry on the sink.
- IV. Discarded cell cultures should be treated with 2% Virkon (final concentration) for 1 hour. The material can then be disposed of down the drain and the plastic ware discarded in the yellow burn bins.
- V. To disinfect blood treat with 2% Virkon (final concentration) for at least 1 hour before disposing down the drain.
- VI. Genetically modified material must be inactivated before being discarded. Solid waste must be autoclaved at 134°C and liquid waste must be treated with 2% Virkon for at least 1 hour.
- VII. Following a spillage the surfaces should be disinfected immediately according to the following guidelines:
 - i. Sprinkle powder Virkon onto the spill to cover it completely;

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- ii. wait for the Virkon to absorb the spill;
- iii. mop up with paper towels and place in the clinical waste bin;
- iv. Clean the surface with a 1% solution of Virkon.

10) Mycoplasma testing

- I. Mycoplasma are small micro-organisms that are resistant to many common antibiotics (e.g. penicillin and streptomycin) and therefore pose a considerable contamination risk to cell cultures. Mycoplasma infections often go undetected for extended periods of time as they are too small to be viewed by light microscopy and do not cause changes in the turbidity of the culture medium. The consequences of mycoplasma infections include slower cell proliferation rates and global changes in gene expression in the host cells.
- II. All cell lines in use are screened for mycoplasma infections quarterly by the end users. All tissue culture users in the laboratory are expected to provide mycoplasma report to the BioEscalator Laboratory Manager. Additional screens should be performed when a new cell line arrives in the laboratory or if a mycoplasma infection is suspected.
- III. Any cell lines found to be positive for mycoplasma should be quarantined immediately and disposed of.
- IV. Any frozen stocks of the cell line that may also be infected should be tested and then disposed of if positive.

11) Cleaning duties

- I. The BioEscalator cleaners have access to the TC laboratory for only cleaning the floor and removing black domestic bin so other cleaning must be undertaken by the TC users themselves with the help of the BioEscalator Laboratory technician.
- II. The TC laboratory is deep cleaned once a month (end of each month) by subcontractor absolutely cleaning services Ltd.
- III. This is a responsibility that is shared between all persons using the TC laboratory.
- IV. Laundry duty.
 - i. Blue Laboratory coats are sent to an external company (Johnsons Apparelmaster) for laundering on a quarterly basis or as when required.
 - ii. The laundry supplies clear laundry bags for the dirty laboratory coats; please contact BioEscalator laboratory technician if you want your blue laboratory coat launder.
- V. Daily and weekly cleaning of microbiological safety cabinet.
 - i. Switch on the microbiological safety cabinet following steps 5 ii and 5 iii.
 - ii. Remove metal work surface.
 - iii. Wipe down all of the surfaces with 70% ethanol.
 - iv. Make sure all surfaces are clean and dry.
 - v. Replace the metal work surface.
 - vi. Switch off microbiological safety cabinet following step 5 iv.
- VI. Monthly cleaning of water bath.
 - i. Before proceeding make sure the water bath is switched off and unplugged from the power socket.
 - ii. Take out the removable metal racks.
 - iii. Empty all water into the tissue culture sink.
 - iv. Wipe down the surfaces of the water bath with 70% ethanol.
 - v. Refill with distilled water and 1 ml of aqua-resit for each litre of water used.
 - vi. Reconnect the water bath to the power socket.
- VII. Monthly cleaning of the CO₂ Incubators.
 - i. Remove all of the cell cultures and carefully place them into a microbiological safety cabinet while the incubator is being cleaned.
 - ii. Remove all of the metal racks from the incubator and empty the water tray.
 - iii. Spray all sides of the incubator, the metal tray and the water tray with 70% ethanol and wipe dry.

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- iv. Clean and refill water pan (outer) with distilled water and 1 ml of aqua-resit for each litre of water used. This can be done on a weekly basis if required.
 - v. Replace the clean metal trays.
 - vi. Transfer all cell cultures from the microbiological cabinet back to the incubator carefully.
 - vii. Check that the incubator returns to 37°C and 5% CO₂.
 - viii. If any signs of infection are observed during the cleaning note down details on the record sheet.
- VIII. Cleaning at the end of a work session. After each work session tissue culture users should make sure the area they have worked in is clean and useable for the next person. This includes:
- i. emptying and cleaning the microbiological safety cabinet.
 - ii. Clean under the metal work tray.
 - iii. BVC vacuum system require emptying i.e. 80% full.
 - iv. wiping down the bench surfaces if used.
 - v. emptying the bins (refer to waste disposal guidelines).
 - vi. disposing of liquid waste (refer to disinfection guidelines).

12) Guidelines for GM work in the tissue culture laboratory

- I. A GM risk assessment must be approved by the BioEscalator Genetic Modification Committee before commencing any work as well as notifying the HSE.
- II. A copy of the CU1 premises notification form (GM Centre number) must be given to the BioEscalator laboratory & facilities manager.
- III. A copy of the CU2 notification form must be given to the BioEscalator laboratory & facilities manager.
- IV. A copy of the signed GM RA must be given to the BioEscalator laboratory manager.

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Appendix 1:

Laboratory Etiquette

DOS	DON'TS
Carry all lab material in enclosed containers outside of the lab ¹	Wear PPE such as lab coats, gloves and safety glasses outside the laboratory ²
Cover cups of coffee/tea/water and food with lids so they are contained ³	Leave drinks on lockers for long periods of time
Always wear PPE (lab coat and safety glasses) when in containment level 2 laboratories ⁴	Leave food/Tupperware on lockers for long periods of time
Use correct exit (goods lift/dumb waiter/ rear fire exit) to transport biological material between floors	Leave coats and bags on lockers ⁵
	Carry food and drink without containment
	Eat or drink in containment level 2 laboratories

¹ All corridors have been classified as clean. Therefore, when carrying lab materials such as glassware and waste they should be contained in a plastic box with a lid. Biological material must be double contained when moving between labs.

² Lab coats and other items of PPE should not be worn in the corridors, even if you are moving to another nearby lab. It is advised to hang lab coats on the doors of all labs you are working in so PPE is readily available when moving between labs.

³ As there are some offices within labs, drinks are permitted to be taken through lab areas, providing they are covered with lids. Drinks can be left on the lockers as the corridors are clean, however ensure that cups and glasses are taken to the kitchen area and washed up.

⁴ It is essential to wear PPE when conducting lab work to prevent cross-contamination when walking through the corridors.

⁵ Do not leave coats and bags on the lockers. If you need a space to put your belongings, place them on the hangers provided or in the lockers and let reception know which locker you are using.

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Use main entrance to reception to bring biological materials between floors

Use passenger lift to transport biological materials between floors

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Appendix 2:

Record of Infected Cultures Sheet:

Please log any infections found in this incubator. Where possible provide details of the type of infection (mould, bacteria, yeast), the cell line affected, the type of plastic-ware used (vented/non-vented flask, plate, petri dish, etc.), the number of infected cultures and details of any antibiotics in the media. Any infected cultures should be decontaminated immediately using a 2% Virkon solution and disposed of. The BioEscalator Laboratory Manager/Apprentice laboratory technician will inform all other tenants currently using the incubator and arrange to clean and decontaminate the incubator refer to section 11 cleaning duties.

Date	User's Initial's	Company	Type of Infection	Cell Line	Plastic-ware	Antibiotic	Other users informed (Y/N)	Incubator cleaned (Y/N)