Cavendish Laboratory, BSS Physics of Medicine, Biolab Cell Culture Facility:
Induction, Safe Usage and Local Rules Document

Index

1. Welcome to the Biolab

General Information

- The Biolab Manager and Biological Safety Officer is currently Tim Fitzmaurice (x48914, tjf11@cam.ac.uk).
- The main Biolab area physically comprises 3 rooms. The Biolab tissue culture rooms themselves are 1.18 Cell Culture Lab, 1.16 Cell Culture Prep room, and 1.22 Autoclave Room, within the Physics of Medicine (PoM) building.
- The fourth room within in the physical area is Room 1.20. This and Room 1.24 just outside the door are Bacteria laboratories and considered part of Biolab for most access purposes.
- **USE THE BIOLAB TISSUE CULTURE FACILITY IS LIMITED TO AUTHORISED USERS**.
- The Biolab rooms are all Containment Level 2 designated biological laboratories.
- The Biolab is a user-maintained facility and therefore everyone needs to contribute to its successful operation by following these Guidelines.
- **The following Guidelines are the minimum standard of work within the Biolab and will neither override any Risk Assessment requiring higher standards of work, nor be a substitute for the proper training required to carry out any experimental work safely.**

Access and Induction

- Access is contingent upon being a registered user of the Physics of Medicine building
- Each potential new User **must attend an induction** by the Biolab Manager or their designated person; sign the induction form **agreeing to follow these guidelines** and get the form counter-signed by their PI before using the Biolab facility.
- Following induction, users will be added to the list of authorized users by the Biolab manager and they must subscribe to the **bssphy-biolab** mailing list to receive important notifications and to communicate with other users.
- **Do not be afraid to challenge persons unknown to you to confirm their access is authorized or to ask the Biolab Manager.**

- Names and contact details of the current Biolab Manager and Biological Safety Officer are posted inside the Biolab; please contact them if you have any questions or concerns
- Following induction, the user's **supervisor/group leader is responsible** for ensuring that each user is familiar with the risk assessments for their work, is properly trained, monitored, and is following these Guidelines for use.
    - Users in breach of these Guidelines **may have their access to Biolab denied** until they can demonstrate compliance and competence, at the discretion of the Departmental Safety Officer and/or Biolab Manager.

Guest Access

- Temporary guest access to the Biolab can be arranged, if appropriate, for short term visits.
- Once Hub Admin procedures have been completed for visitor access to PoM, the Biolab Manager must meet the Guest and give permission for their access to the Biolab.
- The Guest must then be accompanied by an authorised user at all times
- The authorised user has full responsibility for the Guest complying with Biolab Rules and Guidelines.
- This Guest access to Biolab will be considered on a case by case basis.
- Repeat Guest access will lead to that guest needing to be inducted as a regular user.

General User Working Requirements and Responsibilities

- **Do not cross-contaminate areas by moving between the Bacterial Labs, other non-Biolab areas, and the Biolab culture rooms without changes of PPE. Bacteria ,in particular, are a serious contaminant in eukaryotic cell cultures.**
- All users will operate to the University's Safe Biological Practise Policy as a minimum standard: University Safe Biological Practice HSD028B Policy

    In particular:

    - You MUST wear a Howie type lab coat whenever working in the Biolab.
    - DO NOT eat, drink, chew gum or apply cosmetics.
    - DO NOT wear gloves or lab coats from other areas into the Biolab T/C.
    - DO NOT bring in personal items (e.g. outdoor jackets and coats)
    - DO NOT remove lab coats from the Cell Culture Facility.
    - DO NOT use mobile phones in the Biolab work rooms.
    - DO NOT wear open toed footwear while working in the Biolab.
    - Avoid moving items between non-Biolab and Biolab rooms.
- Each authorized user carrying out regular work, will be added to the Biolab's weekly **Maintenance Rota**. The responsibilities of being 'on Rota' are posted in the Biolab.

- BEFORE STARTING WORK, each user is responsible for ensuring that their work is conducted to comply with relevant **risk assessment(s) (RA) and COSHH forms** that have been signed off by each user plus their supervisor and/or the Biological Safety Officer/ Biolab Manager, and properly filed.
- RAs must be updated/reviewed annually by the responsible person in the laboratory. This is a requirement under University of Cambridge Policy linked to legislation.
- Out of hours working in the Biolab must be carried out in accordance with Departmental Policy (see: https://www.phy.cam.ac.uk/intranet/hands/Policies2/afterhr). Any such work must logged in to the Out of Hours system noting which rooms you are working in. This is for your own safety and so that you can be accounted for should an evacuation of the building occur.
- As Containment Level 2 biological laboratories, by definition, these rooms are not Low Level Risk working areas for the purposes of the Department's Out of Hours working policy.
- Lone working out of hours in the Biolab is by definition above Low Level Risk and so not permitted by default.

Alarms and equipment failure

- In the event of alarms going off or equipment failing, please contact the listed name on the equipment, or the Biolab Manager.
- If it is an emergency and you need someone to attend (fridge/freezer/incubator failure) please consult the emergency contact information on the equipment or in Room 1.16.

Leaving the BSS/PoM/Biolab

- Inform the Biolab Manager if you are leaving for any significant period, so they can take you off the Users list.
- Unsubscribe from the email list.
- Properly dispose any of your unused/unwanted reagents or pass them to another lab member.
- Discuss with your supervisor if you will need to leave vials of cells in cryogenic storage.
- Discard any unnecessary vials in cryogenic storage.
- Arrange for a group member/your supervisor to take responsibility for the remaining stored cell stocks.

2. General Working Guidelines

Health and safety

- All risk assessments (RAs) for your project need to be in place for all procedures carried out by all users **before you start your project**.
- Each group leader should ensure that all work done by their group is covered by suitable and sufficient risk assessments – the level of detail of each risk assessment must match the complexity/level of risk of the project.
- Prior to starting work in the Biolab cell culture facilities, you need to provide the Biological Safety Officer with your RA forms, or read and sign any existing relevant RA forms. If anything is not clear, please discuss with your supervisor.
- The red folder located on the shelves beside the autoclave in room 1.22 contains the printed copies of Biolab RA forms.
- A blank copy for your use in creating new RAs is stored on the BSS Internal Pages website under the Safety link “Biological Risk Assessments”.

Lab coats

- Lab coats MUST be worn whenever working in the Biolab.
- Only Biolab-designated lab coats, which are to be of the Howie coat style, stored on the hooks outside of room 1.16, are to be worn when working within the Biolab.
- Other lab coats **must not** be worn in the Biolab Tissue Culture rooms under any circumstances.
- Lab coats should be washed **at least once every 3 months**.
- As the Biolab conducts CL2 work, lab coats must be autoclaved before sending to laundry.

Storage of Consumable Stocks/Solutions in the Biolab

- Each user or group will be provided with designated space to store their reagents, on an as-needed basis, by the Biolab Manager. Remember to update their presence in Cheminventory.
- **DO NOT use reagents that don’t belong to you** and which are not common consumables; if in doubt, ask first.
- **Some reagents need to be kept sterile/aseptic** so if you open that tube outside of a cell culture hood without the owner being aware of this, you are risking contaminating someone else’s experiment!
- Please ensure that **all user solutions/reagents are labelled** at least with a user ID (e.g. initials or name), contents, and either a preparation or an expiration date.
- Any opened reagents not properly labelled are **at risk of disposal** without prior notice, and pose a safety risk for others, as we won’t know what the contents are.

Transport of Biological Material out of the Biolab

- All biological material removed from the Biolab Cell Culture Facility to other areas of PoM, must be transported in secondary containment e.g. plastic Tupperware box, the outside of which must be wiped down with 70% ethanol.
- Cell culture dishes must be sealed with Parafilm before transportation.
- Biological material leaving PoM entirely must be contained appropriately for its destination. University Transport of Biological Material Policy

New Cell Lines, Sterility and Mycoplasma Testing

- Please inform the Biolab Manager of any new cell line(s) you intend to receive and provide the appropriate risk assessments **BEFORE starting work**
- New cell lines during the testing period must be placed in the lower incubator on the lowest shelf, to minimize risk of spreading any contamination to other users' cultures.
- Test all new cell lines for sterility and mycoplasma contamination **AS SOON AS POSSIBLE AFTER YOU BRING THEM INTO THE BIOLAB** if they have not been tested before arrival.
- Ideally cells should be confirmed as mycoplasma negative BEFORE entering the Biolab**.** Results may not be trustworthy in the presence of a mycoplasma infection as this negatively affects a variety of cellular functions.
- Test your cells for mycoplasma regularly. A mycoplasma infection can spread rapidly, and wipe out or invalidate all work in the Biolab. Ask your PI which test system your lab uses.
- Use sterility broth for testing media and cell culture supernatants for bacterial contamination. This won't test for fungal contamination. However, this is readily confirmed visually.
- Routine use of antibiotic in cell culture media can mask the presence of low level bacterial contamination. Also, standard tissue culture antibiotics do not kill mycoplasma.
- With good sterile technique, equipment and reagents, you should not need to use antibiotics. However, this is a shared facility and users do not have perfect control of the working environment. Therefore, it is up to each user to decide on antibiotic use.
- For further information on testing options or if unsure about any suspected contamination, please consult with the Biolab Manager.
- Use of UV is not considered a suitable routine sterility control method.

3. Tissue Culture in the Biolab

Conducting Eukaryotic Cell Culture

- Eukaryotic cell culture requires the use of sterile technique, sterile reagents and equipment, within a Microbiological Safety Cabinet (MSC). Failing to follow proper precautions means your results may be invalid.
- The Biolab contains Class II Microbiological Safety Cabinets.
- You must receive **proper training** from an experienced researcher in the techniques that you will use.
- You must work with steady hands and attention to detail at all times.
- **If in doubt, throw it out** – don't use anything you don't have full confidence in.

Microbiological Safety Cabinets – MSCs (General)

- All users should attend the University Training on Containment Facilities and MSCs
- The Class II cabinets used in the Biolab filter the air and generate air curtains in order to prevent contaminants from entering your cell cultures and reagents. They must be used properly to ensure aseptic working conditions are met.
- MSCs are NOT chemical fume hoods, so do not use them as one. They provide **no chemical protection and in fact ours recirculate hazardous chemicals** used directly into the room.
- Cabinets must be switched on **at least 15 minutes prior to use** to allow a complete air change of the non-sterile air present through the HEPA filters and to allow air flows in the room to equilibrate.
- When not in use, the cabinet door should be shut to minimize entry of contaminated air.
- Do not leave items in the hood after working, unless necessary.
- Ultraviolet light is not to be used as a routine surface decontamination method.
- Items left inside the hood, after it has been switched off, must be treated as non-sterile and their outer surfaces should be cleaned prior to use.
- MSCs have booking sheets above the working area. It is recommended that you book your timeslots to avoid clashes and loss of work. Please try not to overbook the amount of time that you need, but do include time for the MSC to turn over before you start.

Annual MSC Servicing

- MSCs are serviced annually by a specialist engineer, who administers a 'KI Test' in order to ensure that they are working to protect your samples from contamination and to protect you from any biological hazard that those samples represent.
  This is arranged by the Biolab Manager. Prior notice will be given of servicing dates.

- Please schedule your experiments around the service visit, as the cabinets will not be available for use during the servicing period.
- Please be aware of the date that the service and 'KI test' expires (this is noted on the side of the cabinet). MSCs become non-validated after that date. This means that the MSCs are not proven to protect you or the environment at that time and therefore by law they MUST NOT be used for those purposes.

Starting a Work Session in the Biolab

- You should wash your hands in the sink outside the Cell Culture Facility and dry them.
- Watches and/or bracelets should be taken off, as they can be both a source of contamination and be damaged by the disinfectant sprays.
- Put on fresh gloves, spray with 70% ethanol and rub hands/fingers together.
- Put on your cell culture designated lab coat (on hooks in hallway).

Subsequently Starting Work in one of the MSCs

- Switch on power to MSC.
- Raise hood to safe working level; air flow will commence automatically.
- Check the aspirator waste level if you need to use it (below the hood). If it is getting full then empty before use, as described below (change your gloves after).
- If aspirator is ready, switch on vacuum pump.
- Spray your gloves with 70% ethanol and rub hands and fingers together to clean all surfaces of your hands.
- Wipe down interior hood surfaces and any objects left inside hood with 70% ethanol and paper towel.
- Do not spray directly onto any sensitive equipment or pipettes – spray onto a paper towel first, then wipe.
- Spray 70% ethanol into the vacuum tubing until clear (avoid backspray getting into eyes by keeping the screen between you and the tubing).
- Leave all to settle for 15 minutes for MSC to turn over enough air for safety of your work and to equilibrate airflows in the room.
- During this time, you may pre-warm your media, as needed, in the 37°C waterbath in room 1.18. You can also check on your cultures under the microscope, and read your experimental plan in your lab notebook.
- If you need to leave the room during this period, either leave a note or make sure you are booked on the booking sheets attached to the MSCs.

Routine MSC use

- Have only the **necessary** equipment in the hood to minimize any negative effect on the air flow – put any equipment, tip boxes etc ideally at the sides of the hood.
- Do not block any of the grills/flow vents at the back nor the low level laminar flow of air across the baseplate of the MSC.
- Spray what you take into the hood with 70% ethanol including your gloved hands.
- Always replace lids on bottles and flasks as quickly as possible.
- Avoid passing anything including yourself over the top of open reagents or cell plates, as particulates can drop down leading to contamination.
- Work across the MSC from a clean area to a dirty/end point area.
- Clean spillages immediately with 1% Distel then 70% ethanol and paper towel. Distel corrodes metal, so you must spray ethanol immediately to rinse off excess Distel after use.
- Change gloves at regular intervals. This will depend upon the work and individual comfort.

TO REPEAT: MSCs in the Biolab are of the recirculating air type. They do not protect from chemical hazards and actually pump any such hazard back into the room.
Use a fume cabinet for any hazardous chemical, as the first choice.
If a hazardous chemical needs to be used in the MSC to keep the experiment clean, then its use and control measures MUST be risk assessed to manage the hazard.

Completing a Work Session in the MSCs

- Spray 70% ethanol down the aspirator tubing until it runs clear and clean (protect your eyes from backspray).
- Remove all items from the hood and store them in a drawer to keep the contents sterile and the outer surfaces dust-free.
- Spray the hood with 70% ethanol and wipe with fresh paper towel.
- Close hood and switch off.
- Turn off vacuum pump for aspirator; empty liquid waste (as below) if full.
- Dispose of solid biohazardous waste in double bagged autoclave bags.
- Dispose of serological pipettes and Gilson pipette tips into the white biobins.
- If any autoclave bags or biobins are full, close them, add autoclave tape and place in the grey 'Un-autoclaved Waste' bin. Steam must penetrate into autoclave bags so DO NOT seal them in a manner that prevents this.
- If there are more than 4 bags/biobins in the 'Un-autoclaved Waste' bins, autoclave them!
- Turn off microscope lamp if used.
- Clean up and store items away properly.

UV Light Treatment and Exposure

- UV light as a sterilisation method is a practice in relatively common use in tissue culture laboratories. In practice it is dubious that any perceived benefits are achieved in most laboratories.
- Most laboratories do not conduct any checks on whether a pathogen is susceptible to UV crosslinking nor what energy levels required to crosslink targets sufficiently. Bulbs are rarely tested to determine if output energies are as expected. Frequently units used to provide UV emission are unsuitable for the planned use.
- Therefore UV is not to be used in the Biolab for decontamination purposes without a fully written justification, a suitably safe design of the emission unit, a regular maintenance program ad a management system for use written into a formal risk assessment.

4. Waste Handling and Waste Streams

Treating Biohazardous Waste (General)

Different disinfectants work against different organisms so you will need to select an appropriate one for your work. The Biolab keeps a number on hand:

- **Distel and Trigene** kill a wide range of microorganisms and cells and so can be used generally to maintain a clean working environment. However, they are expensive. Use for spot cleaning following spillages of cell cultures.
  Note that both Distel and Trigene **corrode metal**, so you must spray ethanol immediately afterwards, to rinse, and wipe off the excess.
- **70% ethanol** is also good for killing certain microorganisms though not as wide a range as Distel or Trigene. It should be used routinely for surface cleaning.
- **Virkon** should be routinely used only to treat aspirator waste. It is an irritant to the eyes and when inhaled, so please minimize exposure. The tablet form of the disinfectant is preferable to the powder for this reason. Use a fume cabinet or dust cabinet to handle the powder and make the solution. Virkon also will corrode metal like Distel and Trigene. High protein content liquids rapidly deactivate Virkon and so a higher concentration (5%) is needed for such liquid waste.
  Note that **Virkon loses its decontamination activity over time.** As a rule, it is only effective when it is **pink**. Therefore, the powder/tablets should be added shortly before you intend to treat and discard waste.
- When the 70% ethanol or 1% Distel spray bottles are empty, **refill them**. Even though the person on the weekly Biolab rota will refill the bottles, they can run out in between rota visits.
- 100% Trigene solution, 100% Distel solution, 1% diluted Distel spray, 96% ethanol bottles, 70% ethanol stock jug, and Virkon powder stocks are located beside or under the sink in room 1.16.

Solid Biohazardous Waste

- Plastic graduated serological pipettes, scraper, plastic pasteurs, inoculation loops and pipette tips should be placed in the white Biobin by the MSC
- Once full, close the Biobin according to the instructions on the top and add some autoclave tape to the top.
- Place the Biobin in one of the grey bins labelled 'Un-autoclaved Waste' in room 1.22.
- Replace the Biobin from stock (Room 1.22).
- Other solid biohazardous waste goes into double bagged autoclave bags in support frames. Do not overfill the bags as they are likely to split in handling or the autoclave..
- No liquid waste should be disposed of via this route. The exception is small quantities (as a guide - 5ml or less) of liquid in sealed plasticware.
- When an autoclave bag is ¾ full at most, close the neck loosely with autoclave tape and place it in one of the grey bins labelled 'Un-autoclaved Waste' in room 1.22.
- **DO NOT SEAL** autoclave bags to be airtight. The autoclave is validated with steam penetration of bags. Sealing the neck with knots, tight fold-overs or excessive tape prevents steam entry and so may not inactivate the waste. The bag is also at risk of bursting when the pressure releases.
- Replace with two new bags on the wire frame.
- Solid waste is then autoclaved, placed into the 'safe to dispose' bins in Room 1.22 and the cleaners will remove it.
- Autoclaving waste is everyone's responsibility. The person on Rota will deal with the small, regular volumes of waste that build up with basic levels of work, with a weekly run. However, if you generate large amounts or overfill the storage then you must autoclave it yourself. The bins in the Autoclave room can hold 4 items (bag/biobin). If you have material to dispose of and you exceed that storage limit you must run the autoclave or inform the Laboratory Manager of the excess and the reason that you have not put an autoclave run on in person or by email.

Aspirator/liquid biohazardous waste disinfection

- Liquid biohazardous waste in the MSC should be drawn up into the aspirator bottle.
- If you do not use the aspirator a waste container that can either be sealed or contains disinfectant **MUST** be used.
- **Check the level of waste in your aspirator bottle before starting, and during your work**: It must be below the end of the suction pipe and emptied before reaching this pipe.
- If a bottle is over-full when you start work please report it to the Biolab Manager, as improper use risks both contamination and destruction of the vacuum pump,
- Remove liquid from tubes/plates/flasks by aspiration before placing any you are finished with, empty plasticware into the autoclave bags.

- **To disinfect the aspirator waste:**
    - Put on eye protection (safety glasses, goggles etc) – splashes in the eye (even with normal prescription glasses) have caused serious damage in the University.
    - Take the aspirator bottle to the sink and add Virkon powder to a 2% or 5% w/v solution, as required (see above in the Virkon section).
    - Swirl to mix, holding below eye level, let bottle sit in the sink for at least 10 mins before discarding down the sink.
    - Rinse drain and bottle thoroughly with water, add 0.05 vol 100% Trigene or Distel.
    - Replace aspirator under the MSC.
- You can also treat **other biohazardous liquid waste in other containers** before disposal down the sink. Use disinfectant volumes as outlined above.
- **NEVER tip untreated biohazardous waste from any container down the sink!** You must treat all waste appropriately to disinfect it**.** If drains are contaminated, everyone's work will be affected.
- If the **Virkon runs out**, add a further 0.1 vol 100% Distel or Trigene and allow time for it to treat the waste, **before** tipping down the sink.
- If **all of the disinfectants run out**, then leave the full flask under the hood, disconnect the pump tubing to prevent suction, leave a note on the vacuum pump telling others not to use it, reorder the disinfectants and notify the Biolab Manager.

Liquid Spills

- Any spill in the Biolab – in or out of the MSCs - must be dealt with promptly, whether biohazardous or not. Wear gloves!
- If the spill is large, prevent its spread with absorbent material e.g. tissue paper.
- Apply appropriate disinfectant to the spill and paper directly.
- Mop up the spill in the paper and dispose of as solid waste (autoclave bag).
- Treat the area affected with disinfectant treated tissue paper.
- Clean the area with 70% Ethanol.
- If the spill is inside an MSC, check if any has gone under the working baseplate and then treat it as you handled the main spill. Even plain media split under the baseplate becomes a growth area for bacteria and fungi, contaminating the MSC.

Chemical Waste

- Hazardous chemical waste (e.g. formaldehyde) needs to be disposed of separately.
- The required disposal route will be described in the procedure's Risk Assessment and be subject to Departmental procedures, University requirements, and potentially third party rules.

- If you are not clear about this process, please consult laboratory literature (See:https://www.phy.cam.ac.uk/intranet/hands/hazards/wastedisposal/chemicalwaste) and if necessary consult with the Departmental Chemical Safety Officer.
- For mixed waste, the biohazard must be dealt with before dealing with the chemical disposal route. Both disposal methods must be carried out appropriately.
- **Cytotoxic chemical waste** contaminated sharps require a specific disposal bin. See the Biolab Manager to obtain one.

Glass pipettes and other sharps

- Sterilised glass pipettes can be used for aspirating cells and media. Plastic versions are preferable and available.
- They are in metal containers labelled by tape colour **- don't mix them up!**
    - Green tape: Clean autoclaved (sterile) pipettes
    - Red tape: Dirty
- Take care when handling glass pipettes to avoid breakages; clean up any breakages immediately. If you sustain a cut from broken glass, contact a first aider immediately (see "Emergencies" red/green/white posters in each lab) and report the incident to the Biolab Manager too – in the Biolab Manager's absence, report to the Departmental Safety Officer.
- Plastic aspiration pipettes are provided as the safer and preferred alternative. You must have a defined and valid reason to replace plastic with glass.
- Do not touch the thin end of any clean glass pipette with your hands or other pipettes, to avoid cross-contamination.
- Place used glass pipettes in a metal container with **red tape** on it next to or in the MSC.
- Tape shut full containers of dirty pipettes with autoclave tape and leave in the space for items for autoclaving on the shelf in room 1.22 (do as part of the weekly cleaning rota), before disposing in the glass bin.
- A **yellow sharps bin** is used to dispose of any other biohazardous sharps waste (needles, glass coverslips, broken glass) used in the Biolab. .

Non-hazardous Waste

- Non-hazardous waste can go into the general waste bin. This includes pipette wrappers and similar plasticware wrappings.
- Try not to add excessive amounts of non-hazardous waste to hazardous disposal routes but if you are unsure of contamination, err on the side of caution and treat items as hazardous.
- The departmental cleaners will dispose of regular non-biohazardous rubbish.

5. Biolab Equipment, Facilities and Consumables

Routine Incubator Use and Maintenance

The air circulating in an incubator and particulate matter that it carries will come into contact with your cells/media unless you have a filter or physical barrier. It is important that this air is clean, otherwise you can contaminate your cells.

Therefore, keep incubators as clean as possible by doing the following:

- Wipe shelves down with a tissue sprayed with 70% ethanol when taking cells in and out.
- If any media has been spilt on the shelves or there are marks on a shelf, spray Distel™ on to tissue and wipe the shelf, then spray tissue with 70% ethanol and wipe it over.
- Make sure your plates/flasks have no media on the outer surfaces: use 70% ethanol to clean any spills/drips.
- Open the incubator for as short a period as possible, and check the door is shut properly.
- When you are finished with your culture experiments, discard your plates right away. Do not just leave old cultures in the incubator as this can be a major source of contamination.
- Before opening incubator, put on clean gloves where practicable.
- Pharmacidal™ spray can be used to replace both cleaning agents above and is non-toxic to most cells in culture, allowing routine cleaning to be carried out without moving cells.
- If you notice any growth on the shelves, clean it immediately and inform the Biolab Manager.

Incubator Water Pans

The incubators are humidified to limit evaporation of media from flasks and particularly dishes. This is achieved by adding distilled water and a water treatment agent to the incubator.

- Check the water in the tray or bottom of the incubator regularly for any signs of microbial turbidity, sludge or fungus. This will be done by the person on rota but all users should check If any notices a problem, notify the Biolab Manager immediately
- If there are any microbial contamination issues in the incubator, the water tray will need to be cleaned and fresh water added immediately.
- This immediate response is the responsibility of the person who finds the contamination. People on rota change the water on a regular basis to limit the need for reactive cleaning.

Response to Microbial Contamination

- The surfaces in the incubator should look clean and shiny. If any new patches or spots appear, or white fuzzy growths, that could indicate contamination.
- If you have contamination, clean the incubator with Pharmacidal spray immediately.
- This will limit the spread of the infection to other cell cultures and the water in the incubator.
- If you find contamination, inform the Biolab Manager immediately, and clean the incubator.
- If the problem persists, then the Biolab Manager will arrange to deep clean the incubator.

- Until this is done, cells should be moved to an alternative incubator or boxed.

Cleaning Incubator after Single Contamination

- Remove all contaminated cells from the incubator, aspirate off the media, add Distel™ to the flask/plate, leave to soak for 5 mins, aspirate off the Distel, and discard plate in the autoclave bin
- Wipe down the shelves that the cells were on, and any spillages, with Distel spray. Wipe with paper towel, and then immediately after with 70% ethanol spray. Wipe again with paper towel.
- Replace the water with fresh clean water by doing the following:
    - For the top incubator:
        - Remove the water using the pump from the autoclave room.
        - Replace with 2L deionised water containing incubator disinfectant.
    - For the bottom incubator:
        - Remove the water tray and discard water down the sink
        - Wipe tray with Distel™
        - Wipe tray with 70% ethanol
        - Return tray to incubator
        - Add 1L DI water with incubator disinfectant to the outer ring of the tray in the incubator.

      DO NOT add water to the inner central ring of the tray.
- Wipe the inside and outside of the door with Distel and 70% ethanol
- Wipe the outer door and handle with Distel and ethanol
- Inform other users of the incubator of a contamination, by sending a message to the **Biolab users email list**, and immediately informing the Biolab Manager

Rota-based Cleaning of Incubators

- The person responsible for cleaning, will put a notice on the incubator letting you know one week in advance when the cleaning will occur.
- Users should remove cells to another incubator prior to the cleaning where there are concerns for the cells.
- At the point of cleaning, any unlabelled flasks/plates (without name, initials, or CRS-ID, and date) will be discarded without notice.
- Labelled plates less than 2 weeks old will be moved to an alternative incubator if space is available.
- If your cells must not be moved then please put a note explaining the details on the outside of the incubator, so the rota person can contact you.

Deep Clean of Incubator – by Biolab Manager

- The Biolab Manager will place a note on the incubator at least two weeks in advance, to inform you of the time and date of the start of the deep clean. You will need to remove your plates/flasks to an alternative incubator.
- The incubator will be out of use for a day at least.
- Shelves and water tray will be removed, cleaned with Distel™ and 70% ethanol **and then heat sterilised**.
- Inside the incubator will be cleaned with Distel™ and 70% ethanol.
- Shelves will be sprayed with 70% ethanol prior to being placed in the incubator
- Fresh water will be added to the incubator.

Strip clean – only as necessary, if a persistent contamination problem exists

- See the Biolab Manager if you identify that this has become necessary for details. In short;
- All the insides of the bottom incubator will be removed, cleaned with Distel™ and 70% ethanol **and then heat sterilised**.
- The bottom incubator will be reassembled with fresh water.
- For the top incubator, there is a self-cleaning high temperature cycle that will be run, following wiping down of all surfaces with Distel and 70% ethanol; fresh water will be added following the self-clean cycle

Water bath

- The water bath is cleaned and refilled and topped-up by the person on rota.
- Keep the temperature set to 37 °C. Leave the lid slightly ajar when it's on, to prevent excessive condensation on the lid underside.
- Turn it off when not in use and cover lid securely.
- Clean your tubes and bottles with 70% ethanol over the sink, tissue **immediately** after removing from the water bath.

Pipettors, tips and tip boxes

- Biolab supplied pipette tips are **pre-sterilised**; tips ready for use are stored on the shelf in room 1.16. Please replace the tip box in the hood with a full one if you use the last tips.
- Empty tip boxes/tip racks should be taken to room 1.22 and placed in the grey bin labelled 'Contaminated STARLAB Pipette Tip Boxes & Tip Racks'. When full, the bag should be secured with autoclave tape and autoclaved and then emptied into the white cardboard recycling bin underneath the benchtop Priorclave.

- Everyone shares Biolab tips, so please use with care and do not contaminate the tips. If you are concerned about contamination, label a box with your name and store separately.
- Everyone shares the pipettors, so please take care to avoid contamination. If you accidentally aspirate liquid up into a pipettor, clean it out as well as you can using Distel or 70% ethanol sprayed on a paper towel by wiping it down and expelling the excess liquid onto the paper towel, then mark the pipettor with tape labelling it as being "dirty", and notify the Biolab Manager immediately so that proper decontamination measures can be taken.
- The Biolab Manager can arrange for pipettors to be serviced annually to ensure accuracy is maintained.
- If you are concerned about contamination risk from tips or the pipettor, then please consider purchasing filter-plugged sterile tips for your own usage with your supervisor's prior permission (these are not provided by the Biolab). Do not use another group/user's filter tips as they are very expensive.

Restocking – every user's job

- If you empty a cupboard stock, please restock immediately – all users are responsible for restocking when things are emptied entirely.
- Stock is checked and replaced at the weekly rota for general levels of usage.
- Stock Plasticware can be found in the cupboards in the hallway. Details of the range are on the cupboard door.
- Please re-order common consumables as needed through the BSS ordering site or Laboratory Manager.
- If you enter the Biolab and find that it has not been restocked, please report it to the Biolab Manager.

Cryo-Storage (Liquid Nitrogen [LN_2]Dewar)

- There are cryogenic freezing boxes (known as 'Mr. Frosty') available in Room 1.16 and 1.18 for freezing down cells at the ideal rate for good cryopreservation (≈1^{o}C/min).
- Remove cells from the Mr. Frosty **and return it to room temperature** as soon as possible.
- To freeze cells short-term, store in the -80°C upright freezer.
- For long-term, cell lines are stored in the small liquid nitrogen Dewar located in room 1.16
- DO NOT use the cryo-storage/LiqN_2 tank or liquid nitrogen without prior training from the Biolab Manager or a designated person and updating your training record.
- To access the LN_2 storage tank you must wear appropriate PPE (lab coat, face shield/eye protection, and cryo-protective gloves).
- You MUST follow the appropriate procedures especially if the oxygen (O_2) alarm goes off.

- All vials must be clearly labelled with:
  - Cell name; Date; Your name, initials; or CRS-id, Group Leader's initials.
  - Please also include passage number if relevant, and any other relevant information.
  - Vials should be labelled with ethanol resistant pen/marker.
  - **Unlabelled tubes will be discarded**.
- The location of the vial should be recorded with:
  - Stack; Box; Position in box
- Enter details in the database on the BSS site under the link Liquid Nitrogen Cell Stocks Tracker
- Vials not recorded in the database may be removed and discarded without warning.
- Do not remove other users' vials from the cryo-store without their permission.

Liquid Nitrogen Maintenance

- On the weekly cleaning rota, the level of LN_2 is checked, and topped up as required.
- If you notice the level of LN_2 in the dewar has fallen below 25 cm deep then please inform the Biolab Manager or the person on the weekly rota immediately.

If you drop a box of cryovials into the liquid nitrogen dewar, please contact the Biolab Manager immediately. Do not try to rescue the cells yourself – you could have severe cold burns. **Follow this procedure:**

- Put on PPE, as above, to work with liquid nitrogen dewar. Remove all boxes of cryovials from the metal racks in the liquid nitrogen dewar and place in the -80°C freezer. Make sure that all boxes are kept in the same order and segregated by colour.
- Contact the Liquefier Facility on liquid.gases@phy.cam.ac.uk, explain the problem and ask them to pick up the liquid nitrogen dewar from the back door.
- Wheel the liquid nitrogen dewar to the back door, push it into the lift and press the button to transport it downstairs**. Do not travel in the lift with the liquid nitrogen dewar** – use the stairs. If the Dewar leaks in the lift you will not have enough oxygen to breathe.
- Take a container for transporting the box of cells back to the Biolab.
- The Liquefier Facility personnel will empty the dewar in the presence of the Biolab Manager/BSO or an individual nominated by them.
- Take the box of cryovials back to PoM and return to the -80°C freezer.
- Liquefier Facility personnel will refill the liquid nitrogen dewar and drop it off at the back of PoM.
- Wheel the liquid nitrogen dewar back to the Biolab, using the lift to transport the dewar, again NOT travelling in the lift with the dewar.
- Put on PPE for work with liquid nitrogen and return all racks of boxes of cryovials to the liquid nitrogen dewar.

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Biolab Cell Culture Guideline induction form- USER COPY

This form will be kept by the Biolab Manager/Biological Safety Officer for reference and for use in the event of any breach of guidelines.

Current version of guidelines _____

Your name _____ Supervisor's name _____

(Print name clearly)

Your CRS-id/Email address ----------

Position at Cavendish Laboratory (circle as appropriate):

Part II student, Part III student, PhD student, technician, Research Associate, Post Doc, PI/Group Leader, Visitor, Other (specify) _____

Anticipated length of stay in Biolab/PoM, to (date): ……

I have read the Biolab Guidelines and signed the relevant risk assessments to cover my work and have been inducted into the Biolab. I agree to abide by the rules.

_____ _____

Signature of Biolab user Date

_____ _____

Signature of Biolab user's Supervisor Date

Induction done by:

Name _____ Signature _____ Date _____

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Biolab Cell Culture Guideline induction form – BIOLAB MANAGER COPY

This form will be kept by the Biolab Manager/Biological Safety Officer for reference and for use in the event of any breach of guidelines.

Current version of guidelines _____

Your name _____ Supervisor's name _____

(Print name clearly)

Your CRS-id/Email address ----------

Position at Cavendish Laboratory (circle as appropriate):

Part II student, Part III student, PhD student, technician, Research Associate, Post Doc, PI/Group Leader, Visitor, Other (specify) _____

Anticipated length of stay in Biolab/PoM, to (date): ……

I have read the Biolab Guidelines and signed the relevant risk assessments to cover my work and have been inducted into the Biolab. I agree to abide by the rules.

_____ _____

Signature of Biolab user Date

_____ _____

Signature of Biolab user's Supervisor Date

Induction done by:

Name _____ Signature _____ Date _____