The Oxford Centre for Diabetes, Endocrinology and Metabolism

Radcliffe Department of Medicine

UNIVERSITY OF OXFORD

Head of Department: Prof Fredrik Karpe
DSO: Sandy Humphreys
BSO: Prof Patrik Rorsman

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Handbook for tissue culture workers

Contents

1 General

This handbook is supplementary to OCDEM SOP S0: Local Safety Rules: Clinical and Laboratory Work Containment Level 2, OCDEM SOP S1: Laboratory Rules for the safe Handling of Blood, Body Fluids and other Human Tissues and OCDEM SOP S8: Local Safety Rules: Tissue Culture Rooms; all of which must be adhered to at all times when working in the laboratory.

2 New workers in the laboratory

- Access to the tissue culture room is restricted to authorised tissue culture workers only.
- All new workers in the laboratory must receive an induction to the laboratory by the tissue culture supervisor (Dr Katherine Pinnick) and have received appropriate training in Good Laboratory Practice and Good Aseptic Technique before commencing work.
- Following the laboratory induction new users will be subscribed to the @ocdem_tissueculture emailing list and will receive a username and password for the electronic booking system.

3 Laboratory local rules

- You must adhere to Good Laboratory Practice and Good Aseptic Technique for cell culture at all times.
- Good basic hygiene, including regular handwashing, must be practised at all times.
- Lab coats worn in other parts of the laboratory should be left outside the tissue culture laboratory and a clean green/blue tissue culture lab coat should be worn at all times.
- Wearing of disposable gloves and safety spectacles are mandatory unless a reason for not wearing them is identified by risk assessment.
- Equipment (such as pipettes, etc.) is provided and equipment entering and leaving the laboratory should be kept to a minimum and decontaminated before use.
- Risk assessments and standard operating procedures for the safe conduct of the work must be written and approved, and strictly adhered to.
- 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.
- A microbiological safety cabinet 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.
- The use of glassware and sharps should be avoided.
- The microbiological safety cabinet, bench surface and any equipment used should be decontaminated immediately on completion of a session of work; a 1% Virkon solution should be used for decontaminating benches.
- Equipment must be fully decontaminated prior to maintenance work. A signed statement should be issued to this effect before maintenance work is allowed.

4 Electronic booking system

- To use one of the microbiological safety cabinets a booking must be made using the electronic booking system.
- Bookings can be made on the allocated PC in the tissue culture laboratory or remotely using this address: http://tools.ndm.ox.ac.uk/bookings/ocdem-tissue/day.php?area=4
- To make a booking an active username and password is required.
- Priority bookings: A "PRIORITY" booking system is in place on two of the cabinets to allow equal opportunities for booking. The cabinets are designated as follows:
  - Hood 1 (Oxlip Priority)
  - Hood 2 (DRL Priority)
- The PRIORITY system allows workers in OXLIP to have first choice of bookings for Hood 1 and DRL workers to have first choice of bookings for Hood 2.
- PRIORITY bookings must be made by **5pm the day before the booking**. After this time both Hood 1 and Hood 2 can be booked by any group.
- Hood 3 is designated for viral work and viral work may only be performed in Hood 3. Hood 3 can be used an overflow hood for non-viral work when Hood 1 and Hood 2 are both in use but viral work takes first priority.
- Bookings for all cabinets can be made in 15 minute slots between 8am-8pm Mon-Sun
- All bookings should be made using the electronic booking system as this provides a record of usage of the cabinets.
- Cabinets 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 the electronic system if more or less time is found to be required to complete the work.
- After a booking has been made remember to log out of the system.

5 Use of the microbiological safety cabinets

- All microbiological safety cabinets in the laboratory are operated in the same way.
- Before switching on the cabinet remove the detachable panel to the main work space.
- Press the green button once to switch on, the red button to silence the alarm and the yellow button to turn the light on.
- Allow the air to circulate for a few minutes before using the cabinet and check that the air-flow indicator on the instrument panel is located in the green "safe" zone.
- Do not use the cabinet if the indicator remains in the red zone and report this immediately.
- Before commencing work all accessible surfaces in the cabinet should be wiped down with 70% Industrial Methylated Spirit (IMS).
- All equipment used inside the cabinet should also be sprayed and wiped with 70% IMS before being placed in the cabinet.
- Work in the cabinet should be performed in the centre of the workspace avoiding blocking any of the air vents with equipment.
- 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.

- At the end of a work session all items must be removed from the cabinet and the surfaces wiped down with 70% IMS; 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).
- Any spillages that occur while working in a cabinet should be disinfected immediately according to the disinfection guidelines (see page 7).
- To switch the cabinet off press the green button once. Wait for the light to switch off and the air-flow indicator to return to the red zone. Finally replace the panel to the main workspace.

6 Use of the CO_2 gas incubators

- There are five CO_2 gas incubators in the department which are designated as follows:
  - 1 x small Sanyo/Panasonic incubator: Viral work ONLY
  - 1 x large Galaxy incubator: Primary cultures ONLY
  - 3 x large Sanyo/Panasonic incubators: All other cell lines. (One of these can also function as a multi-gas incubator. Priority usage of the multi-gas incubator is given to experiments that require an adjustment of the gas composition; this incubator can be removed from general usage if this need arises.)
- Each incubator is programmed to run at 5% CO_2 and 37^{o}C and these settings should never be adjusted.
- CO_2: The five incubators are fed by one CO_2 gas cylinder. There should always be a backup cylinder connected to the automatic switchover system to prevent the gas from running out. Workers using the incubators should routinely check there is sufficient gas in both the connected CO_2 cylinder and the backup cylinder. When the system switches on to the backup cylinder the empty cylinder must be replaced with a new one (see instructions for changing the CO_2 cylinder: **Appendix 1**).
- Water tray: All incubators require water to be added to the tray at the bottom of the incubator to maintain correct humidity. Water trays should be filled with autoclaved water and 2-3 drops of SigmaClear water-bath treatment. There is a sensor in the Sanyo/Panasonic incubators which needs to be immersed to monitor the level of the water. A flashing error message on the control panel of *"RH"* indicates the level of water is too low and requires topping up with autoclaved water (or that the sensor has not been placed in the water – check this first).
- 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.
- 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.

- 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

- There are a number of items of equipment that are provided for use by all tissue culture workers. These should never be removed from the tissue culture laboratory.
- Water-bath: The water-bath is set at 37ºC and the temperature settings should never be adjusted. Any tubes or flasks placed in the water-bath should be clean and sealed firmly. The water-bath can be set to shake when mixing is required.
  All items should be removed from the water-bath at the end of the day and the water-bath should be switched off.
- Centrifuge: Tissue culture workers must have received the necessary training and demonstrate that they are competent in the use of centrifuges before using the centrifuge.
  The tissue culture 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 microbiological safety cabinet. 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.
- Microscopes: Tissue culture workers must receive appropriate training before using the microscopes. There are two microscopes in the tissue culture laboratory. Both are fitted with cameras to allow photographs to be taken. One microscope is fitted with fluorescent filters for the detection of GFP, etc. After use the microscopes should be left clean and the light source and cameras should be switched off.
- Cell counter: There is one automated cell counter (a Nexcelom T4 Auto) connected to the PC in the laboratory. Appropriate training must be given before use. Only counting chambers supplied by Nexcelom may be used in the cell counter.
  The cell counter should be switched off at the end of the day.
- Pipettes and pipette guns: A selection of Gilson pipettes are provided. Tissue culture workers will need to provide their own tips; tips with filters are recommended to minimise the risk of liquid being aspirated into the barrel of the pipette. Pipette guns are located next to each microbiological safety cabinet for use with disposable serological pipettes. Care should be taken to avoid aspirating media into the filter of the pipette gun. If this does occur the filer inside the pipette gun should be replaced immediately. When not in use the pipette guns should be plugged into the power to recharge the batteries. All pipettes and pipette guns should be cleaned before and after use.
- Fridges and freezer: There are three fridges which are designated either OXLIP or DRL and one -30ºC freezer for the storage of media and reagents. All items placed in the fridges/freezers must be clearly labelled with the owners 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

- OCDEM SOP S3: Waste Disposal, gives information about the available waste streams within OCDEM.
- All waste generated in the tissue culture rooms must be disposed of in the appropriate bin via the NHS clinical waste stream.
- Serological pipettes and pipette tips must be placed in a yellow burn bin or a Bio bin. These bins should be disposed of regularly; Bio-bins must not be overfilled and burn bins must not be more than ¾ full.
- Non-sharp, non-infectious waste, that will not pierce the bag, must be placed in the orange, clinical waste bags.
- Known infectious waste must be placed either in a yellow bag or a sharps bin as appropriate.
- All sharps (e.g. needles, scalpel blades) must be disposed of in sharps bins.
- Any hazard group 2 material and waste generated from genetic modification work must be autoclaved prior to disposal.

9 General disinfection guidelines

- Disinfectants should be used in accordance with the OCDEM disinfection policy: OCDEM SOP S4: Disinfection in Containment Level 2 Areas.
- All work surfaces (including the microbiological safety cabinets) should be disinfected using 70% IMS before and after each use. Please note that benches should be cleaned using a 1% Virkon solution at the end of each session.
- 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 on the draining board next to the tissue culture sink for the next user.
- 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 plasticware discarded in the orange bins.
- To disinfect blood treat with 2% Virkon (final concentration) for at least 1 hour before disposing down the drain.
- Genetically modified material must be inactivated before being discarded. Solid waste must be autoclaved and liquid waste must be treated with 2% Virkon for at least 1 hour.
- Following a spillage the surfaces should be disinfected immediately according to the following guidelines:
    - Sprinkle powder Virkon onto the spill to cover it completely;
    - wait for the Virkon to absorb the spill;
    - mop up with paper towels and place in the clinical waste bin;
    - clean the surface with a 1% solution of Virkon.

10 Mycoplasma testing

- 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

- All cell lines in use are screened for mycoplasma infections each term. All tissue culture workers in the laboratory are expected to provide samples for testing. Additional screens should be performed when a new cell line arrives in the laboratory or if a mycoplasma infection is suspected.
- Preparation of samples for the MycoAlert assay: One week before mycoplasma testing is due to take place an email reminder will be sent to the @ocdem_tissueculture emailing list with details of when the next test will take place.
- The cells that will be tested need to have been passaged at least twice since being revived from frozen and need to have been **in the same medium for 48 hours** before sampling.
- On the day of the test 1.5ml of medium from a single flask of cells should be transferred to an Eppendorf tube and placed in the rack labelled 'Mycoplasma test' which will be located next to the water-bath in the tissue culture lab.
- Each Eppendorf should be clearly labelled with your initials, the cell line, the passage number and any other info that distinguishes your sample i.e. antibiotic if you have cells under selection, clone number, etc.
- **Samples must be placed in the "Mycoplasma test" rack before 2pm on the day of the test.** If it is not possible to provide medium samples on the day of the test samples can be taken up to 5 days earlier if stored correctly. Medium samples are stable at room temperature for 24h and can be stored in the fridge for up to 5 days provided they are allowed to warm to room temperature before being tested.
- Further guidelines for performing the MycoAlert Assay (Lonza) are provided in Appendix 3
- Results of the mycoplasma testing will be circulated via the @ocdem_tissueculture emailing list on the same day the test is performed.
- Any cell lines found to be positive for mycoplasma should be quarantined immediately and a second sample should be tested within one week of the first test.
- If the second sample is also found to be positive a regime of mycoplasma sensitive antibiotics can be started to eliminate the mycoplasma – this treatment can take up to 3 weeks. Any frozen stocks of the cell line that may also be infected should be tested and then either treated or disposed of.

11 Cleaning duties

The general cleaning staff are not permitted access into the tissue culture laboratory so all cleaning must be undertaken by the tissue culture workers themselves. This is a responsibility that is **shared between all persons using the tissue culture room**.

Any individual who does not participate in the cleaning duties may find their access to the tissue culture room and electronic booking system is revoked.

11.i Cleaning at the end of a work session

After each work session tissue culture workers should make sure the area they have worked in is clean and useable for the next person. This includes:

- emptying and cleaning the microbiological safety cabinet
- tidying away all equipment

- recharging pipette guns
- wiping down the bench surfaces
- emptying the bins (refer to waste disposal guidelines)
- disposing of liquid waste (refer to disinfection guidelines)
- restocking consumables

11.ii Weekly cleaning rota

All tissue culture workers will be allocated to a cleaning team of 3-4 people. Cleaning teams are responsible for cleaning the tissue culture laboratory for one week each month. A quarterly cleaning rota is sent to all tissue culture workers via the @ocdem_tissueculture emailing list. It is each person's own responsibility to note which weeks they are responsible for cleaning and to inform other members of their team if they will be away. Where possible a swap should be made with a member from another group so that the cleaning team is not left short-handed. An example of the cleaning rota and list of duties included in the weekly clean can be found displayed in the tissue culture laboratory and is included in **Appendix 4.** Further instructions for the cleaning duties are below:

11.iii Cleaning the microbiological safety cabinets

- First make sure the cabinet is switched off at the plug socket on the wall
- Lower the front glass panel and remove the metal work surface
- Wipe down all of the surfaces with Distel
- Wipe down all of the surfaces with 70% IMS
- Make sure all surfaces are clean and dry
- Replace the metal work surface
- Close the front glass panel and close the cabinet
- Remember to switch the power back on at the plug socket on the wall

11.iv Cleaning the gas incubators:

- Remove all of the cell cultures and carefully place them into a microbiological safety cabinet while the incubator is being cleaned.
- Remove all of the metal racks from the incubator and empty the water tray.
- **Important: for the primary incubator the CO_2 sensor must not get wet! Make sure it is covered with the black plastic cap before proceeding.**
- Spray all sides of the incubator, the metal tray and the water tray with Distel and wipe dry.
- Repeat this step using 70% IMS.
- Replace the clean water tray and refill with autoclaved water and 2-3 drops of Sigma water bath treatment.
- Replace the clean metal trays.
- Transfer all cell cultures from the microbiological cabinet back to the incubator carefully
- Check that the incubator returns to 37°C and 5% CO_2
- If any signs of infection are observed during the cleaning note down details on the record sheet on the front of the incubator.

11.v Cleaning the water bath:

- **Before proceeding make sure the water bath is switched off and unplugged from the power socket.**
- Take out the removable metal racks.
- Empty all water into the tissue culture sink.

- Wipe down the surfaces of the water bath with 70% IMS
- Re-fill the water bath with de-ionised water and 2-3 drops of Sigma water bath treatment.
- Reconnect the water bath to the power socket.

11.vi Laundry duty:

Lab coats are sent to an external company for laundering; OCDEM uses Hall Laundry Services Ltd T/A The Burford Laundry. The laundry supplies cloth or clear laundry bags for the dirty coats; these can be found in a cupboard in lab F40 (to the right of the door of the lab used for work with radioactive materials). A ticket containing the details of the items and the account number must be included with the bags. Phone the company (01993 822211) on a Monday to arrange collection on the Tuesday; the pink copy of the ticket is kept by the department. Raise a purchase order for the number of coats to be laundered and charge to cost code H73070. The cost per coat in January 2016 was £2.44.

Lab coats contaminated with blood, tissue or biological material must be disinfected prior to laundering by soaking the coat or section of coat in a solution of 1% Virkon for 30 minutes. Rinse out the Virkon and leave to dry before sending to the laundry.

11.vii Refilling the 70% IMS tank:

- The IMS stocks are stored in the external solvents storage locker outside the OCDEM building.
- Collect the key for the storage locker from the key box in the OXLIP freezer room.
- Use a trolley to take the empty 70% IMS tank to the storage locker.
- The IMS and a liquid pump are stored in the right-hand grey cupboard – this should be locked.
- Check the label of the solvent drum; it should read Industrial Methylated Spirits.
- Use the pump to transfer the IMS up to the 70% indicator on the tank.
- Reseal the IMS drum, replace it in the grey cupboard and lock the cupboard.
- Make sure the solvents storage locker is locked before leaving.
- Take the 70% IMS tank to the washing up room and add deionised water up to the water indicator on the tank.
- Mix gently by swirling the tank and then return the tank to the tissue culture lab.
- Remember to return the storage locker key to the key box in the freezer room.

11.viii Changing a CO2 gas cylinder

- There must always be a full back-up cylinder connected to the CO_2 exchange unit.
- The yellow indicator on the exchange unit tells you whether cylinder A or cylinder B is currently supplying the gas.
- Check which cylinder is connected and then check whether the other backup cylinder is full or empty.
- If the cylinder is empty it must be changed for a full cylinder according to the instructions in Appendix 1.
- **Never try to change a gas cylinder on your own. This is a 2 person job!**
- Gas cylinders are stored in the liquid nitrogen room.

- Gas trollies can be found in the GC lab or in the POD.
- When the last full cylinder has been taken from the liquid nitrogen room an order must be placed for 2 new cylinders with BOC.

12 Guidelines for Viral Work in the Tissue Culture Lab

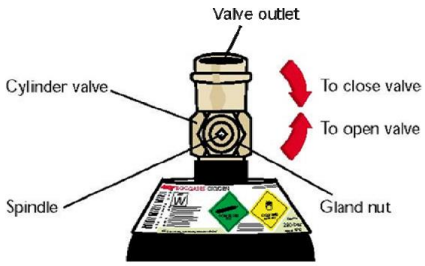
- A GM risk assessment approved by the OCDEM Genetic Modification Committee must be in place before viral work commences and tissue culture GM supervisors (Dr Anne Raimondo for DRL and Dr Katherine Pinnick for Oxlip) should be informed of the proposed work. No orders are to be placed for GM materials until the risk assessment has been accepted and signed by the OCDEM GM Committee.
- Viral work may only be conducted in the designated viral microbiological safety cabinet (MSC) and all viral cell cultures may only be placed in the designated viral incubator.
- When booking the viral MSC it must be clearly stated on the electronic booking system whether the work is **Class 1** or **Class 2.**
- PPE (lab coat, gloves and safety spectacles) must be worn at all times for viral work.
- The use of sharps (scalpels, needles) is not allowed for viral work.
- The risk of aerosols should be minimised e.g. avoid centrifugation of media containing viral particles
- Disposal of contaminated plastic-ware (flasks, plates, pipettes) is always via the autoclave. All plastic-ware should be collected in an autoclave bag. Gilson pipette tips should be collected in a rigid container before being put in the autoclave bag, the lid of the container must be loose to allow steam penetration. Autoclave bags must be sealed loosely with autoclave tape and labelled with *"VIRAL WORK, the users initials and the date".* Autoclave bags should be placed in the red basket next to the TC freezer.
- Viral workers should ensure the autoclave waste basket is emptied twice a week. Waste should be made safe by autoclaving on Program 1 before it enters the normal waste stream.
- Contaminated liquids must be inactivated in a Virkon solution at a final concentration of 2% for a minimum of 30 minutes. Beakers of contaminated liquids that are left unattended should be clearly labelled with *"VIRAL WORK, the users initials and the date"*. After 30 minutes the liquid waste can be flushed down the drain with water.
- The viral MSC hood **must be thoroughly cleaned after each use by the viral worker**. The hood should be dismantled and all surfaces, as well as underneath the metal work surface, should be decontaminated with 1% Virkon followed by distilled water (to reduce the corrosive effects of Virkon) and finally 70% IMS.

APPENDIX

Appendix 1: Quick Guide to Changing the CO_2 Gas Cylinders in the Tissue Culture Room

This task requires **at least 2 people** to do. Before you start, have a gas trolley ready (make sure it is not broken and has back wheels). You can find this by the corridor linking the Oxlip and GC labs.

1. Check that a full cylinder is currently connected to the incubators – look for the yellow mark on the meter. Check that the cylinder to remove is empty – Right-hand side dial points to 0.
2. To disconnect the cylinder, turn off the regulator by turning the gold knob ANTI-CLOCKWISE all the way until you feel it stop. DO NOT OVER TIGHTEN.
3. To switch off gas supply, turn off the cylinder valve by turning the wheel on the top of the cylinder CLOCKWISE.
4. Take off the regulator head by unscrewing the nut in an ANTI-CLOCKWISE direction. You may need the spanner and the plastic mallet to do this at first. Remember to support the regulator with one hand and keep it as level as possible.
5. Loosen the straps surrounding the cylinders and move the empty cylinder onto the trolley by rolling it around carefully on its bottom. You might need to move other cylinders out of the way. Do not lift the cylinder onto the trolley, instead place the trolley right under the bottom of the cylinder.



6. Move the full cylinder back against the wall.
7. Before leaving the room, tie the safety straps back onto the cylinders.
8. Take the empty cylinder down to the Liquid Nitrogen room. Check that there are 2 full CO_2 cylinders of the same kind strapped against the wall behind the door, if not order more!
9. Loosen the pink strap on a full cylinder and unhook. You need to remove the empty cylinder from the trolley and secure it to the wall using the ratchet on the pink strap, and then place the new cylinder on the trolley.
10. When back in the TC room, place the new cylinder in the right position. Remove the plastic dust cap (turn ANTI-CLOCKWISE until it snaps). Check that outlet is free from dust or bits of plastic.
11. Fasten the strap on the new cylinder.
12. Screw the regulator head onto the valve outlet by turning the nut CLOCKWISE. Again, ensure the regulator head is kept level. Tighten with a spanner as tightly as possible by hand and check for leaks using leak detector spray. . Spray at the connection between the regulator and the cylinder; if there's a leak bubbles will form immediately and the nut may need tightening slightly.
13. If further tightening is required use gentle force with the plastic or rubber mallet. Do NOT use excessive force; if the regulator will not seal check the valve outlet is clean and that the threads on both the outlet and the regulator are not damaged.
14. If there is no leak, wipe off excess spray.
15. Open the cylinder valve by turning the wheel on top ANTI-CLOCKWISE, until the right-hand dial goes up to 50.
16. Open the regulator by turning its knob CLOCKWISE until the left-hand dial goes to 1.
17. Before leaving the room, check that all cylinders are secured with the strap.

Refer to **OCDEM RA12 Handling, Storage and use of compressed gas cylinders** for more information

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 owner of the infected cells MUST inform all other people currently using the incubator and MUST arrange to clean and decontaminate the incubator.

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

Appendix 3: Mycoplasma testing

Safety

General lab rules apply. Lab coat, gloves and safety glasses should be worn throughout the procedure.
All waste should be disposed of in the appropriate waste container and sharps bin.

Potential Hazards

Centrifuge

Disodium EDTA dehydrate (contained in the substrate, reagent and buffer solutions)
R22: [Harmful if swallowed. R36/37/38: Irritating to eyes, respiratory system and skin]

Reagents

MycoAlert™ reagent
MycoAlert™ substrate
MycoAlert™ assay buffer
MycoAlert™ Assay Control

Equipment

Centrifuge
Pipettes
White assay plate

Protocol

If first use, reconstitute the kit according to the manufacturer's instructions. Aliquot the MycoAlert Reagent and MycoAlert substrate into 0.5ml eppendorfs at volumes of 250µl and 500µl. Aliquot the Assay control into 50µl aliquots in 0.5ml eppendorfs. Once reconstituted, store at -80°C and defrost just prior to use.

Allow all assay reagents to equilibrate at room temperature for **15 minutes** protected from the light with foil (the optimal working temperature for all reagents is 22°C)

1. Samples should also be at room temperature once removed from the cell culture flask.
2. Spin 2 ml cell sample at **200 x g for 5 minutes**.
3. Remove 50µl of cleared supernatant from each cell line to an empty well on the assay plate.
4. Set up Enspire machine for Reading A
5. Add 50µl of Assay control (positive control) and 50µl of Assay buffer (from the Assay control kit, negative control) to separate wells on the assay plate.
6. Add 50µl MycoAlert™ reagent to each sample and the positive and negative controls. Wait for **5 minutes** then measure luminescence (Reading A).
7. Set up Enspire machine for Reading B
8. Add 50µl MycoAlert™ substrate to each sample and the positive and negative controls Wait for **10 minutes** then measure luminescence (Reading B).

9. Calculate ratio = Reading B/Reading A.
10. The ratio of Reading B to Reading A is used to determine whether a cell culture is contaminated by mycoplasma.

    Ratio Interpretation
    < 0.9 Negative for mycoplasma
    0.9 - 1.2 Borderline: quarantine cells & retest in 24 h
    > 1.2 Mycoplasma contamination

    The interpretation of the different ratios obtained within each experimental situation, may vary according to the cell types and conditions used.

11. Any remaining assay components cannot be re-frozen and should be discarded.

Setting up the Enspire machine for Mycoplasma testing

1. Using the screen pen, tap on the load plate button **A** and insert your plate. Click again for your plate to be taken into the machine.
2. Tap the Edit Protocol button **B** the select the MycotestA Protocol **C** by tapping once on the protocol in the list. To open the file click on the Edit Protocol button **B** which should now have the name of you file listed underneath the button.
3. Tap the Settings tab **D** and tap on the box containing the name of the file you have selected to run **E**.
4. This opens a new box **F**. Click on the file name and change it. Make sure the file name reads Mycotest**A**-Date-Initials. (i.e. Mycotest**A**-29APR14-CJB). Click ok.
5. Select Plate **G** then select 'Undefined-Measured' from the drop down list **H**.
6. Using the screen pen highlight all the wells you have used for samples/positve and negative controls and also one blank well **I**.
7. Tap on the Save changes button **J**.
8. Tap on the Run Protocol button **K**.
9. Select the file name you have just created **L** and tap the run protocol button **M**
10. You can manually record the data but the machine will also store the data.
11. Using the screen pen, tap on the load plate button **N** and insert your plate. Click again for your plate to be taken into the machine.
12. Repeat the process from **1.** For the **B** measurement but remember to change the file name at **4.** to Mycotest**B**-Date-Initials

Appendix 4: Cleaning groups and rota

Week beginning	Group	Extras	Hood	Incubator
1st June	1	Laundry duty	1	2
8th June	2	Clean water bath	2	4
15th June	3		3	1
22nd June	4	Laundry duty	1	2
29th June	1	Clean water bath	2	4
6th July	2	Viral incubator	3	1
13th July	3	Laundry duty	1	2
20th July	4	Clean water bath	2	3
27th July	1	Viral incubator	3	4
3rd August	2	Laundry duty	1	1
10th August	3	Clean water bath	2	2
17th August	4	Viral incubator	3	3
24th August	1	Laundry duty	1	4
31st August	2	Clean water bath	2	1
7th September	3	Viral incubator	3	2
14th September	4	Laundry duty	1	3
21st September	1	Clean water bath	2	4
28th September	2	Viral incubator	3	1
5th October	3	Laundry duty	1	2
12th October	4	Clean water bath	2	3
19th October	1	Viral incubator	3	4
26th October	2	Laundry duty	1	1

Weekly cleaning duties checklist

1. Clean one microbiological safety cabinet*

2. Clean one incubator*

3. Clean the water-bath or do the laundry if scheduled*

4. Check gas cylinders and replace the backup cylinder if it is empty

5. Refill 70% IMS spray bottles and refill the IMS tank if empty

6. Refill sterile water bottles and autoclave them

7. Clean and tidy the bench surfaces and the sink

8. Empty bins and take waste to ground floor waste room for collection

9. Sweep and mop the floor

10. Run the eyewash shower for 15 minutes

* Refer to the cleaning rota to see which incubator and MSC hood are due to be cleaned and when the water-bath/laundry need to be done.

Update history

Version	Date	Reason for update	Updated/reviewed by :	Date next review due
1	Feb 2016	New Document	Author: KP	Sept 2017