CURTIN HEALTH INNOVATION RESEARCH INSTITUTE | Curtin University

Tissue Culture Rooms

Room 238 Tissue Culture 1*: Designated for "quarantine" work i.e. primary human cultures which have not been tested for any known human pathogens, viral work, parasite work and *Mycoplasma* status positive cell lines undergoing treatment.

Room 240 Tissue Culture 2*: Designated for "dirty" work.

Please follow the signs on the incubators and hoods and work only in the designated area.

MYCOPLASMA NEGATIVE hood and incubator: cell lines with at least one *Mycoplasma* negative result

PRIMARY CULTURE *MYCOPLASMA* UNTESTED hood and incubator: primary cultures negative for known human pathogens (most animal-based work from the SPF facility), new stock of cell lines that tested previously positive for *Mycoplasma*, cell lines which are *Mycoplasma* status unknown, cell lines new to the facility

Room 241 Tissue Culture 3: Designated for "clean" cell lines which have been regularly tested for *Mycoplasma* and have two negative results minimum.

Room 239 Tissue Culture 4: Designated for "ultra clean" cell lines which have been regularly tested for *Mycoplasma* and have three negative results minimum. Cultures in this room are required to be cultured without antibiotics.

*** Please be aware that work in the designated quarantine rooms carry risk of *Mycoplasma* contamination to your cell lines.**

Tissue Culture Rules

1. Users must notify cell culture staff of their intention to work in the cell culture room/s in order to receive specific instructions and to have assigned spaces (fridge and freezer)
2. All cell lines have to be tested for *Mycoplasma* and re-tested every 3 months
3. No *Mycoplasma* positive cell line is allowed in the Tissue Culture facility with exception of very rare and valuable cell lines that are undergoing appropriate *Mycoplasma* treatment
4. All Tissue Culture rooms have to be thoroughly clean every 3 months
5. Fridge and freezers are only for storing items for tissue culture
6. Everything stored in allocated space/incubators must be labelled with your Name, Date and Contents
7. Labelled items/cultures should not be removed from incubators/fridge/freezers without permission of the labelled user
8. Gloves and a dedicated lab coat must be worn at all times
9. If a user does not turn up after 15 minutes of the booking, their booking is forfeited
10. If a user finishes earlier than expected please let the next user know
11. Users must use the dedicated equipment within each room
    - DO NOT remove pipettes from allocated rooms or go to another room to centrifuge etc.
12. Incubator settings are not to be altered without permission
13. Hoods are intended only for sterile work.
    - For a solution to stay sterile, it can only be opened in an operating biosafety hood.
14. Users will be regularly monitored for correct aseptic technique
15. The hood is not to be used as a storage area
16. Hoods are to be cleaned with 70% ethanol before and after use
17. Clean spills of culture or media immediately and report it to the high end user of that TC room
18. Put ALL equipment and reagents away after use
19. Ensure plastic and liquid waste is disposed of appropriately
    - DO NOT put any waste into the general bins
20. Report ALL contaminations to cell culture staff
21. Report incidents and accidents immediately, irrespective of their seriousness
22. Report any problems with equipment immediately

23. No mobile phones are allowed

24. Failure to abide by any of these rules will result in restricted access/termination of access
    - First warning: verbal communication to the user from tissue culture facility staff.
    - Second warning: written communication to the user, supervisor/s and the Head of School.
    - Third warning: a second written communication from the Head of School.
    - Failure to respect these rules will result in eviction from this facility.

The following protocols apply:

OPERATING INSTRUCTIONS FOR CLASS II BIOLOGICAL SAFETY CABINETS

AIM and SCOPE OF THIS DOCUMENT: To outline the procedures for the correct use and maintenance of Class II Biological Safety Cabinets, including treatment of biological wastes prior to disposal.

START-UP

● Turn on light (this automatically switches UV off).

● Raise window to working position [blue ▲]; or remove front guard.

● Turn on mains switch [I/O], confirm by pressing "set".

● After ~40 sec, control panel will indicate normal operating mode. ALLOW TO THEN EQUILIBRATE FOR AT LEAST 5 MINUTES before commencing work.

SHUT-DOWN

● Remove all cultures to storage or incubators.

● Remove all waste for sterilization and disposal. See separate instructions for treating biological waste for disposal.

● Wipe any equipment with 70% ethanol prior to removing from hood. Do not store equipment in hoods.

● Wipe work surfaces with 70% ethanol.

● Remove gloves if worn and dispose; wash hands.

If there is another booking in less than 1 hour => leave the hood working, otherwise:

● Turn off light.

● Allow hood to run a further 5 mins to effectively purge air.

●Turn off mains [I/O], toggle blue arrows to change Yes/No, press "set"

● Close window fully [blue▼+ RED safety button], or replace front guard.

● Activate UV.

FOLLOWING SPILLS

With cabinet **ON,** lift off work surface and clean the well below with 70% ethanol. Wipe surrounding areas and wash work surfaces.

DO NOT USE ACIDS, OTHER CORROSIVES, OR ABRASIVES.

PERIODIC MAINTENANCE

Every three months, the cabinet should be thoroughly cleaned.

● With cabinet ON, remove work surfaces and any other removable components.

● Wipe down all surfaces thoroughly with F10 detergent (diluted 1:1000) followed by 70% ethanol.

● Dry any residual moisture from work surfaces and other removable components and re-assemble the cabinet.

● Turn cabinet off, close window, or replace front guard and expose to UV at least 15 mins. Place a notice on the cabinet informing users of appropriate times for recommencing work in the hood.

BREAKDOWN (CABINET GOES INTO ALARM)

● Stop all work; secure all biological samples and remove from the hood.

● Turn off services inside the hood (e.g. suction, power point, light).

● Remove gloves (if worn). Wash hands.

● Switch off power on cabinet [I/O], toggle blue arrows to change Yes/No, press “set”.

● Close window fully [blue▼+ RED safety button], or secure front guard.

● Switch off wall socket.

● Notify tissue culture facility staff.

● Attach a sign indicating “Out of Order”.

STERILISATION AND DISPOSAL OF BIOLOGICAL WASTE

All used plastics must be sterilised prior to disposal by autoclaving.

To autoclave wastes: Waste should be collected in an autoclavable plastic bag – DO NOT overfill bags. The bag should be labelled with your name and which tissue culture laboratory the work has been carried out in. Bags are then taken to washup room 2 and placed into the available containers that will contain any leaks, should they occur. Sterilization is then carried out at 121^{o}C for 16 mins. Once treated, the waste can be sent for incineration.

All liquid waste must be sterilised prior to disposal by Virkon solution or bleach.

To sterilise liquid waste solutions are placed in the available containers with 0.5-1% Virkon solution (diluted 1:10 from stock solution). Liquids are soaked overnight (minimum) in 0.5-1% Virkon and then can be disposed of by the general sink.

PREPARATION OF VIRKON

- Note: in powder form Virkon is an irritant. Virkon is safe to use in liquid form.
- Need to use a fresh bottle each time
    - Virkon will expire rapidly if it is prepared in the same bottle as expired stock
- Add RO water to your bottle and then take to a hood with a balance in the hood.
- Startup the hood as per the instructions above.
- The maximum solubility is Virkon is 4-5% made up in RO water.
- E.g. if you have 500 ml water then add 20-25g of Virkon powder.
- Remove the Virkon and turn off the hood

TISSUE CULTURE CO_2 INCUBATORS: MAINTENANCE

The following describes routine maintenance procedures, and protocols to be followed in the event of microbial contamination of tissue culture incubators.

NB: REMEMBER TO TURN CO_2 OFF BEFORE BEGINNING CLEANING PROCEDURES

ROUTINE MAINTENANCE

Every three months:

- Remove shelves and water pan, empty and clean thoroughly
- Wipe down all surfaces thoroughly with F10 detergent (diluted 1:1000) followed by 70% ethanol.
- Re-assemble shelves. Replace water pan and fill with 1L RO H_2O. A small amount of anti-bacterial agent (F10 detergent diluted 1:1000) should be added to pan to inhibit bacterial growth.
- Allow incubator to stabilise at the correct temperature and CO_2 levels before using.

NB: SHELVES, BRACKET SUPPORTS AND THE WATER PAN ARE ALL FULLY AUTOCLAVABLE.

HEAT DECONTAMINATION (NUAIRE incubators)

There are two possible temperatures - 145°C dry or 95°C humid.

PREPARATION

Empty the incubator and thoroughly clean all surfaces to remove all visible spills, etc. (otherwise these may be baked on!). Manufacturer's recommended cleaning agent is 70% isopropanol.

Ensure the hole in the access port plug at the back of the incubator is open.

OPTIONS MENU – press "SEL" to dEc (95/145)

NB: The 145°C dry cycle runs for 10 hours; the 95°C humid runs ~14 hours

145°C dry is the default option. If using 95°C humid use "up/down" arrows to toggle to this option.

145°C dry

Press the 95/145 button and hold 3sec until display stabilises and the 95/145 LED flashes.

Open incubator doors, remove and empty the water pan and replace it in the chamber (empty). Close doors.

Press the 95/145 button again to start decontamination cycle.

95°C humid

Press the 95/145 button and hold 3sec until display stabilises and the 95/145 LED flashes.

Open incubator doors, remove and empty water pan and disinfect it with 70% alcohol.

Refill pan with 300mL pure distilled water and replace in the incubator ON THE HIGHEST SHELF! Close doors.

Press the 95/145 button again to start decontamination cycle.

NB: DO NOT OPEN INCUBATOR DOORS WHILE CYCLE IS IN PROGRESS!

BURN RISK FROM HOT SURFACES INSIDE INCUBATOR IMMEDIATELY AFTER COMPLETION OF CYCLES.

CYCLE COMPLETE

Observe temperature read-out before opening doors due to possible high temperatures inside the cabinet.

Refill water pan with 1L dH_2O and add F10 detergent and replace pan in the bottom of the incubator.

Press 95/145 button again to resume normal operations.

FORTNIGHTLY MAINTENANCE

Incubators

- Remove pans from incubators, empty and clean thoroughly
- Replace water pan and fill with 1L RO H_2O with F10 detergent (diluted 1:1000).

Water baths

- Empty water bath and clean thoroughly
- Fill water bath with approximately 5L RO H_2O with F10 detergent (diluted 1:1000).

PROCEDURE FOR DEALING WITH CONTAMINATED CULTURES

- Cultures must be treated with 1% bleach for at least 30 minutes and disposed of via the sink
- Any solutions in contact with the contaminated culture must be treated with either bleach or Virkon and disposed of (i.e. media, PBS, trypsin etc).
- Clean the water bath :-
    - Empty water and clean thoroughly with 70% ethanol
    - Fill water bath with approximately 5L RO H_2O with F10 detergent (diluted 1:1000).
- Clean the incubator :-
    - Remove shelves and water pan, empty and clean thoroughly
    - Wipe down all surfaces with F10 detergent (diluted 1:1000) followed by 70% ethanol.
    - Re-assemble shelves. Replace water pan and fill with 1L RO H_2O with F10 detergent (diluted 1:1000).
    - Allow incubator to stabilise at the correct temperature and CO_2 levels before using.
- If problems persist then incubators can be decontaminated with heat procedure as per above.

Nikon TS-100 and L3 Camera – Short Form Instructions

Imaging

Insert USB thumb drive into camera

Turn camera on

Set illumination on microscope to highest intensity

Turn knob on right hand side to direct light path to camera

Wait for image to appear, then focus image on screen

Select camera button with mouse, brings up main menu

Check scene mode is DIC/Ph – Phase Contrast mode

Perform white balance directly on the sample. If not satisfied with result remove sample and place some sheets of white paper on stage and perform white balance again. Determine if colour balance is now representative.

Hit main menu button at top and select Menu 3 Shot/Rec

Select record mode

Select image format and resolution setting – default for image format is TIF

Arrow back to main menu

Pull down arrow on main menu at bottom

Select Exposure mode – Prog for auto exposure, or Man for manual exposure

In Prog auto exposure mode you can adjust auto exposure compensation to make image on screen brighter/darker

In Man manual exposure mode you can set the exposure time to make image on screen brighter/darker

If happy with image hit Capture on main menu

To view captured image select main menu and then View window

Click thumbnails and you will see all the images in the USB drive

Select image with mouse that you want to view, then hit Play

To exit out of saved image and return to live image, hit the X at top right hand corner

Measurement

Hit main menu

Select tools option at bottom

Select calibrate

Select calibration value to correspond with zoom setting on microscope

Calibration Setting	Objective selection on Microscope
M1	4x
M2	10x
M3	20x
M4	40x
M5	blank
M6	blank
M7	blank

Check that the calibration value you select is confirmed at bottom left – for example if you select M3 it should display 20x at bottom left

You can now commence measurements – or if you just want to display a scale select the scale button

On Screen Measurements

You must do above first

Select T Bar on bottom of main screen

Select appropriate colour

Select straight line measurement

Make measurements on screen

Capture

Pull down arrow on T Bar toolbar to see Erase Icon – select this to erase all measurements

If you want to save measurements for display in Excel, select CSV after measurements are made and prior to hitting Capture

Alternatively if you want CSV data to save globally on all occasions

Main menu

Tools

Tool Set

Main

Tick CSV data

Arrow back to main menu

End of Session

Turn of LCD Panel

Remove thumb drive

Check and reset eyepieces on microscope to zero

Return knob at top of microscope to forward position so optical path is reset for us