Conventions for work in the Cell Culture Laboratory

Including storage areas:

Fridge 1A : Opened / prepared media

Fridge 2A : Unopened media

Freezer 2B : Frozen culturing solutions (inc. aliquots)

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Introduction

All of the measures laid out within this document are to ensure that the running of culturing and work within the Cell Culture Laboratory (CCL) is carried out with the maximum of efficiency and good working practice. Leading to the production of quality cells, quality work and thus a high standard of science.

Contents

Individual day-to-day responsibilities when working within the Cell Culture Laboratory (CCL)

The following are worth reading carefully as failure to adhere to them will either ruin your work, the work of others or potentially lead to a withdrawal of personal use of the CCL. The key to good practice within the CCL, is the conscientious and professional use of the facility. For example, making the time to think about asceptic technique, the cleanliness of equipment and state of consumable stocks for the next user, ultimately saves time. There have been occasions where weeks of preparative culturing were lost, no specific blame can be placed, but conscientious working could have prevented the incident, saving the researchers time and effort.

1 Access to the CCL

1.1. Entry to the CCL is specifically **restricted** to those individuals working in that area. Equipment within the CCL is there specifically and only for use therein, and thus must not be removed unless prior agreement has been reached.

1.2. Only those cleared to work within the CCL can do so, as a part of this all individuals must have an up to date set of appropriate vaccinations, including Hepatitis B (copies of certificates must be kept with Dr. M. Gumbleton).

1.3. The door is fitted with a door closer ensuring that it is kept shut, quite apart from being awkward when carrying items into the CCL it is required to limit disturbance to work and air-flow within the CCL. Thus, the door should **not** normally be propped or held open.

2 CCL laboratory coats

1.1. All work carried out within the CCL must be undertaken wearing Microbiological Laboratory coats and gloves. Microbiological coats are **not** to be worn in general laboratory areas outside the CCL, general lab coats **must not** be taken into the CCL. On entry to the CCL to work in the

Class II cabinets normal laboratory coats **must** be exchanged for a CCL (microbiological) coat.

1.2. Coats were not supplied for specific individuals, please **do not** leave pens, *etc.* in the CCL coats.

3 Use of Apparatus

1.1. Gilson pipettes (marked **'Cell'**) and Bibby controllers are provided specifically for use in CCL, and should **not** be used elsewhere. Similarly pipettes from general labs (marked **'MG'**) **should not** be taken into the CCL, a full range are kept in the cabinet for culture work. Only in very limited cases, which do not involve cell operations, such as producing sterile buffer's, *etc.* should other pipettes be used in the CCL.

1.1.1. Cleaning Gilson Barrels - they should be cleaned weekly. By detaching the barrel and placing the barrel into 2% Virkon up to 3 inches for 30 minutes, followed by transfer to 70% alcohol and thoroughly washed. The white barrel of the pipette may also be detached and autoclaved if desired.

1.2. Preparation of solutions should be undertaken in CCL only when they are for use in cell culture activities.

1.3. Incubator sterility :-

1.3.1. When moving flasks, etc. into and out of incubators always wear gloves sprayed with 70% alcohol.

1.3.2. Before replacing a flask or plate in an incubator spray the outside of the vessel with 70% alcohol.

1.4. Use of Class II cabinets :-

1.4.1. Clean **before and after** usage for whatever purpose, spray and wipe with 2% Virkon, followed by spray and wipe with 70% alcohol. (*NB.* be sure that you are familiar with, or are shown the procedure for starting up and shutting the flow cabinets). Do not spray Virkon on the plastic front screen, as it will destroy the transparent nature of the screen.

1.4.2. Spray all items to go into the cabinet with 70% alcohol (allowing time for them to dry before use).

1.4.3. If the cabinet is not to be used soon (>4hrs) after your work finishing, close the cabinet down and turn on the UV lamp (which will further sterilise the cabinet). If you are the last to use the cabinet that day be sure to leave the UV lamp on over night.

1.5. Cleaning of Bench work areas - Wipe with 2% Virkon, followed by 70% alcohol wipe.

1.6. Plastic waste pots - 12× 250ml pots are supplied for use within Class II cabinets as waste pots for liquid waste. The liquid waste to be taken and placed in a Winchester bottle. The pots should be washed with virkon and 70% alcohol, and from time to time covered with foil prior to autoclaving in the media kitchen and then returned to CCL for re-use. These plastic pots do not exist for other purposes and should not be removed to any other laboratory, ideally they should be cleaned and returned to the CCL.

4 Storage

1.1. Fridge / freezer space is limited to 1A, 2A and 2B. Medium in current use should be labelled (name and date of opening) and stored in the CCL fridge 1A. All stocks of serum stored in the freezer or fridge compartments must be labelled with name and date of opening. The use of media and serum between individuals is discouraged and should only occur after agreement between those individuals concerned. For the purposes of clarity 'Media' will be used to define serum containing culture solution. The individual nutrient / salt solutions will be defined by their name i.e. 'DMEM', 'RPMI', 'FK12'.

5 Radiation work

1.1. Strict guidelines accompany radiation work, those working with such materials will be aware of this but should clearly mark off (using tape and signs as necessary their working area when working in the CCL. Specific compartments of the fridge and freezer may well be allocated for the storage of radiochemicals, **only** radiochemicals should be stored in these areas.

6 Maintaining a clean area

1.1. When all individuals initially begin working in the CCL they will receive preliminary training in all aspects of CCL work, including an over view of cleaning procedures. The nature of the experimental work undertaken must be detailed to Dr. M. Gumbleton.

1.2. Individuals **must** undertake correct working practice in their work activities within the CCL, including cleaning procedures prior to, and on completion of activities involving, for example, use of a Class II cabinet or bench areas, (including cleaning prior to and upon completion of microscope activities).

1.3. Any infection found in your cells must be recorded in the *'Cell culture infection incident report book',* and reported to the group (at meetings or via e-mail). Thus, steps can be taken to trace and possibly eliminate any cause, a failure to do so may ruin the work of others.

1.4. Along side an individuals daily working practices (such as those set out in section 3), all individuals must take part in the CCL Good Practice rota system for laboratory clean up. A rota exists for cleaning of CCL, Class II cabinet and CO_2 incubators, and will be updated when additional workers join the lab. Note, however, this rota system is in **addition** to individual responsibilities.

7 Media / Consumables

1.1. Areas within the CCL are for the storage of consumable items, their condition and stock levels are down to the individual who last used them to monitor, i.e. if an item is running low take steps to correct it do not simply ignore and hope that more will just appear. Orders are placed through Dr. M. Gumbleton.

Group responsibilities for work within the CCL

The group are those who use the CCL frequently and thus it is in our best interests to ensure individuals are following the agreed polices and that the lab continues to be productive in culture work. On Page 13 are listed some protocols for lab cleaning. These tasks are undertaken using a rota system and are in addition to individual day-to-day responsibilities on pages 4-7.

8 Responsibilities for specified tasks

1.1. Within the group individuals are assigned to various routine tasks, such as maintenance of the liquid nitrogen levels, CO_2 cylinders, aliquoting of certain regularly used culture solutions (FBS & Trpsin/EDTA) and water levels in the incubator. A list of all such assignments will be available within the CCL.

1.2. Respect these individual roles and inform MG and your colleagues if any problems are encountered, alterations to practices, or indeed if you require any help.

9 Cleaning

1.1. From time to time the cabinets are serviced or fumigated, this is done through arrangements between Dr. M. Gumbleton and Mr. J. Menia-Purnell. During which the CCL is closed for normal working on most occasions prior notice will have been given and closure is for a 24 hour period.

Safe disposal of CCL waste

UNDER ANY CIRCUMSTANCES DO NOT OVER FILL ANY WASTE RECEPTACLES / BINS.

NONE OF THE BELOW BINS ARE TO BE USED FOR RADIOACTIVE DISPOSALS.

Yellows Bins - 'Clinical Waste' - Yellow plastic bin liner.

Items **TO BE DISPOSED** in the yellow 'clinical waste' bins include :

- Paper waste - **contaminated only**
- Disposable gloves

Items **NOT TO BE DISPOSED** in yellow 'clinical waste bins include :

Plastics such as Plastic multi-well cell culture dishes or Plastic centrifuge tubes

These latter items must be placed in the yellow Sharps bins provided.

Yellow 'Sharps' bin

Items **TO BE DISPOSED** of in the 'Sharps' bin include :

- Plastic multi-well cell culture dishes
- Plastic centrifuge tubes
- Plastic pipette tips
- Graduated serological pipettes
- Syringes
- Hypodermic needles
- Broken **contaminated** glass

Uncontaminated broken glass to be placed in cardboard glass disposal containers.

Cardboard glass disposal box

The only items placed in these boxes should be **uncontaminated** broken glass and empty aerosol cans, not merely any item that does not fit or appear to belong in another receptacle.

Waste Liquid Media

No open container placed in a waste bin should contain excessive waste liquid, e.g. sealed centrifuge tubes with liquid waste inside are okay, but do not dispose of liquid in unsealed centrifuge tubes.

- Remember if **waste pots** are used to receive liquid waste media during work(see 3.6), they are to be washed out, afterwards covered with foil using autoclave tape and autoclaved for re-use.
- Place liquid media waste in the **'Winchester' bottles** are supplied under the general fume hood, where upon it will be autoclaved and disposed of safely by the media kitchen.

Other waste fluid / solutions **must be disposed of correctly**, consult with either Mr. J. Menia-Purnell or Dr. M. Gumbleton **prior** to commencing the work.

Use of the CO_2 Incubators

The incubators are connected to a CO_2 cylinder and are humidified using a water tray. Note, a small amount of copper sulphate is required in the water tray to reduce microbial growth.

Open flasks, dishes, etc. may be placed directly onto the shelves.

If you are culturing using media that is buffered with Hepes for pH stability, you do not strictly need atmosphere of CO_2 for buffering purposes. However, CO_2 is considered by some an important nutrient in the media, nevertheless a sandwich box may protect your cells if an infection enters the CO_2 cabinet.

You may place your cultures in a sandwich box and close prior to placing in the incubator. However, if you are culturing in open plastic flasks, i.e. vessels which allow loss of media through evaporation petri dish, culture wells then you may need to place some water in the bottom of the sandwich box to prevent media loss by evaporation.

T-flasks closed tight and that have filters do not allow the media to evaporate, you will not need to humidify the atmosphere in the sandwich box.

'Sandwich boxes' (four of them will be kept in the lab at all times) have been specifically purchased for use in the CO_2 incubators. They must be cleaned out every time you have to change media or subculture.

Tissue culture plastics

Spore contamination, during storage of sterile T-flasks, centrifuge tubes, freezing vials.

Before opening a sterile plastic film-wrap of T-flasks, centrifuge tubes, or freezing vials ensure that all screw tops are completely closed.

This can be done by checking and tightening tops on flasks through the sterile plastic film-wrap.

Alternatively if this proves difficult, then spray the sterile plastic film-wrap with ethanol (70%), place in a class II cabinet and open the wrap under asceptic conditions and then to secure any ill fitting tops.

Casual / excessive use of plastics.

- At all stages of culture work consider the amount and nature of plastic consumables such as pipettes and plates that you will consume during a single use in the class II cabinet. No one is asking you to compromise safe experimental design or technique, simply to plan and employ common sense in limiting the use of plastics which may often be unnecessary. Consider for instance setting down a serological pipette carefully, preventing tip contamination by re-sheathing the pipette carefully into the plastic wrap.

Protocols for CCL Good Practice Rota Cleaning

Cleaning out the CO_2 incubators

(Clean every two weeks).

- Take out the shelves and wipe clean with 2% Virkon (NB. Virkon solution becomes ineffective after 7 days evident by a loss of pink colour). If given permission it may be preferable to perform this task in the media kitchen sink. Follow Virkon with 70% alcohol wipe, this is to prevent Virkon deposits on the metal shelving.
- Wipe out incubator with 2% Virkon followed by 70% alcohol, etc.
- Please do not forget the water in each incubator needs to be changed every 2 days and Copper sulphate added to inhibit microbial growth.

Cleaning Bench areas

(Clean as necessary)

- Wipe with 2% Virkon, followed by 70% alcohol wipe.

Cleaning of type II cabinets

(Clean every two weeks, in addition to individual day to day clean up procedures)

- Remove the grid plates which form the working base to the cabinet
- Clean the bottom of the cabinet with 2% Virkon followed by 70% alcohol
- Clean the grid plates the with 2% Virkon and 70% alcohol prior to replacing them in the cabinet

All the above should be done with the cabinet airflow on and at the end of a day so that the UV lamp can be left on over night, before work recommences next day.

Sweeping and moping the floor

(Cleaning carried out by technical staff)

From time to time the technical staff will clean the floor with disinfectant, notification will be given to the group before it is carried out. If at anytime the floor looks in need of cleaning notify technical staff in the Media kitchen.

Movement of cell lines in & out of Liquid Nitrogen frozen storage

Before removing (or storing) cells, consult with Dr. M. Gumbleton as to the nature of cell work undertaken. The stocks are recorded on a set of sheets found in the blue folder kept with the Cyroplus 1 cell storage system, check the numbers of the cell lines, and passage numbers required for use prior to commencing any thawing. Ensure that the record sheets are updated and new sheets are produced when required through Dr. M. Gumbleton. Remember it will be expensive to replace cell lines whose stock has been allowed to run-out. Grow cells up to replenish used vials and consult with cell line co-ordinator Dr. M. Gumbleton.

- Protocols for freezing and thawing cell lines are available within the lab manual, be familiar with them before starting work.
- Gloves and protective facemask are kept with the system and should be used each time the system is opened. The system is kept locked, keys are kept in the lab, if you haven't been shown where ask a member of the group.
- Cells frozen in the Cyroplus system are located by their canister (A-F), the box number (1-10). Thus a cell line may for example be located in A2, in other words canister A, box 2. Each cell line generally has a box to itself so that confusion is kept to a minimum and levels of stocks can be checked at a glance.
- When amending the records note that care should be taken to write on the stock sheets in such a way that other users have room to amend at a later date and read the information that you record.
- On freezing down cells ensure that all information is on the vials :-
  - → Cell line (MDCK, A549, etc.)
  - → The freezing down date (month and year)
  - → Cell density (i.e. 1×10^6 = 1 million cells / ml)
  - → The cryopreservative used (i.e. 10% glycerol)
  - → Passage number (such as PN+5)
  - → Name of the person who froze the cells

  This same information is required in the stock sheets in the folder.

Maintenance of Asceptic Technique

The following points are for the benefit of your working practice and should be considered before and during any cell work undertaken in the CCL. These points are taken from 'Culture of Animal Cells, a manual of basic technique', by R. Ian Freshney (Second edition, 1987 Published by Wiley-Liss), which is available in Dr. Gumbletons office and contains detailed information on good practice in all areas of cell culture.

1. Check cells visually (microscopically), every time that they are handled.
2. Check reagents, tips, *etc.* are sterilised before commencing any work.
3. Do not share bottles of media, etc. with other workers or use the same stock with a variety of cell lines.
4. Do not over fill tubes and bottles with media as during handling small amounts may seep around the screw cap, interacting the environment allowing possible contamination. Especially a problem if container tops get wet in the waterbath.
5. Correct sterile technique should be used at all times
    - 5.1. Have in the cabinet only the items you need to use.
    - 5.2. Arrange the apparatus to make working easier and reduce the chances of spillage or brushing sterile apparatus with unsterile sleeves, etc.
    - 5.3. Work within your range of vision, e.g. place pipettes with the tip pointing away from you so that it is in your line of sight continuously and not hidden by your arm.
    - 5.4. Mop up any spillage immediately and swab with 70% alcohol.
    - 5.5. On completion remove everything and clean the cabinet.

CCL work is best summed up by a quote from page 49 of 'Culture of Animal Cells' by R. Ian Freshney. (Second edition, 1987 Published by Wiley-Liss)

"The essence of good sterile technique is similar to good laboratory technique anywhere. Keep a clean clear space to work and have on it only what you require at one time. Prepare as much as possible in advance so that cultures are out of the incubator for the shortest possible time and various manipulations can be carried out quickly, easily, and smoothly. Keep everything in direct line of sight and develop an awareness of accidental contacts between sterile and non-sterile surfaces. Leave the area clean and tidy when you finish."