Molecular and Cell Biology

CELL CULTURE WORKING PRACTICES

HWB Cell Culture Areas

March 2017

Before commencing work in any MCB Cell-Culture Areas it is important that you read this document to familiarize yourself with the local rules of use.

The Cell Culture rooms/suites in MCB are multi-user facilities and must be used with consideration for other users in order to ensure their continued smooth running.

CONTACTS

Floor	Key contacts	Contact details	Cell culture lab(s)
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N.B. Departmental Technical staff are listed in **bold** font

DO'S AND DON'TS

- All users must have an induction before starting work in the facilities. **Please contact the relevant member of Department technical staff to arrange.**
- Do not drink/eat/use your mobile phone in the facilities.
- Blood and human tissue should be handled as if infectious.
- Blue lab coats must be worn in the Cell Culture rooms. Remove lab coat when exiting the facility. Please ensure that lab coats are regularly laundered by dropping them in the red container in 0/48 HWB. When a person leaves the Department, the lab coats must be returned to 0/48 for laundering.
- Gloves should be worn at all times in the cell culture area, particularly when accessing hoods or incubators. Gloves should be removed when going out of the cell culture room: when in corridors, wear no gloves, or one glove and open doors with a non-gloved hand.
- Keep the room tidy, clean and waste-free.
- Wash your hands before leaving the facilities.

HAZARDS IN CELL CULTURE FACILITIES

- **Chemicals**: such as disinfectants (Virkon); compounds used to treat cells (transfection reagents, drugs…); fungicidal (copper sulphate); solvents (IMS, Ethanol…). When handling chemicals, read COSHH form before using them for the first time (COSHH folder should be present in every facility), wear nitrile gloves and appropriate PPE.
- **Biological agents:** all cells and tissues should be regarded as potentially infectious. A biological agent is defined as "any microorganism, cell culture or human endoparasite, including any which have been genetically modified, which can cause any infection, allergy or otherwise create a hazard to human health." Before handling cells, check that their use has been risk-assessed according to the Hazardous Biological Agents (HBA) guidance and/or GM registered (check with the Principal Investigator or the Department Safety Officer, DSO).
- **Physical hazards:** such as low O_2 or high CO_2 which can be caused by the presence of liquid nitrogen or a CO_2 leak. In such rooms, there are alarms for this instance: if going off, exit the facility immediately and do not go back until cleared. Before handling liquid nitrogen, obtain training through one of the Cell Culture contacts.

VIRUS WORK

In addition to normal cell culture facility regulations, workers using viruses (adenovirus or lentivirus) need to follow these points:

- Only approved and trained persons can work in the virus facilities.
- **DOUBLE** gloves and specific cell culture lab coats should be worn at all times. The lab coats should not be worn outside the cell culture facility. Use safety goggles if there is a risk of splashes to the eyes.
- Avoid any procedure that results in production of aerosols, such as aspirating. [If aspirating is **absolutely** essential the aspirator should be fitted with a filter that blocks viruses such as a hydrophobic HEPA filter]. The aspirator must be decontaminated with Virkon solution so that the final concentration of Virkon in the waste is at least 1% Virkon and soaked for minimum 30 min.
- Avoid use of sharps (needles, glass, metal etc.) whenever possible. If unavoidable, take particular care with handling and disposal: used needles must not be re-sheathed or removed; needles and syringes should always be disposed of as a complete unit into a sharps container (yellow bin).
- Infectious materials must be transported in sealed primary container inside a sealed and leak-proof secondary containment labelled with a biohazard sticker.
- All areas in which virus work is done should be sprayed with a 1-5% Virkon solution (note: solutions must be coloured to be active), followed by 70% IMS

and then allowed to air dry before and after each use. Note: adenovirus is NOT destroyed by IMS only, therefore Virkon is required.

- Solid waste: all plastic-ware placed inside the cabinet while working with the virus must be decontaminated with Virkon prior to autoclaving in double autoclave bags. This can be done by spraying all plasticware with 1-5% Virkon solution or by soaking in a 1-5% Virkon solution. Especially when working with lentivirus, place an autoclave bag in the Class II cabinet; at the end of the session, the waste bag must be sealed before removal from the cabinet, double-bagged, and autoclaved.
- Liquid waste: treat with 1-5% Virkon solution for at least 2 hrs before disposing down the sink.
- Spillages: the area should be sprayed with 1-5% Virkon solution and 70% IMS. For larger spills, cover spillage with Virkon powder and leave for at least 3 min before placing in double biohazard bag for autoclaving. Then clean whole area with 1% Virkon and 70% IMS.
- When centrifuges are used for biologically hazardous materials, safety caps must be used. Rotors must be disinfected with 1% Virkon solution after each use.

RADIATION WORK

All workers who intend to use radioisotopes must be registered to do so; they must have received appropriate training and understand the working practices relating to the safe use and disposal of radioisotopes. A few key points:

- There are no facilities for storage of radioisotopes in the cell culture suite.
- Any isotope used in the facility must be registered for use in the suite and not exceed the amounts designated on the IRP:1N form, which is on display in the suite.
- Only those areas designated for use of radioisotopes - that clearly display the radioactive hazard warning sign - may be used.
- Any cell cultures in the incubators that are radioactive must display appropriate hazard identification (e.g. tape with the radioactive hazard warning symbol). The type of isotope and the level of activity should be clearly stated in addition to the normal details relating to the cell type etc. Any cultures containing radioactive material in 'unsealed' containers (e.g. dishes and multi-well plates) should be kept in an additional container to contain any spills.
- Any spillages must be cleaned up using Decon solution in the correct manner. Advice should always be sought from the RPS or if not available from another member of technical staff.
- Radiation disposal**: on no account should any radioactive material be disposed of in the cell-culture suite. It is critical that no radioactive material is autoclaved**. There are no designated sinks for disposal of liquid

radioactive waste in the cell culture areas. The aspirators and bottles for liquid waste should also NOT be used for this purpose. Any liquid radioactive waste should be sterilised (e.g. using Virkon) and disposed of via sinks designated for the disposal of radioisotopes that are located within laboratories. Any solid material (e.g. plastic ware, tissues, gloves) contaminated with radioisotope must NOT be disposed of via autoclave bags. This should be disposed of as radioactive dry waste (in yellow 6L cin bins from your lab, which should be then disposed in specified rooms). **Avoid the use of stripettes** when dealing with radiation media as these are difficult to dispose of in yellow bins.

- When using the cell culture room, bring your own yellow bin for tips/gloves/tissue disposal and take it out with you. Ensure that these are labelled appropriately.
- If you are unsure, please contact the Departmental Radiation Protection Officer (DRPO), Dr Olga Makarova (Ext 7103, HWB 3/04, email: om13@le.ac.uk).

ASEPTIC TECHNIQUE & CULTURE CONTAMINATION

Sterile techniques are essential to maintain healthy and contamination-free cells. Contamination by bacteria, yeasts, fungal spores or mycoplasma is a common problem in cell culture; infections are usually introduced by the operator, but may be from work surfaces or non-sterile solutions. Correct aseptic technique provides a barrier between micro-organisms in the environment and the uncontaminated culture in its flask, it is a combination of procedures designed to reduce the possibility of infection. By following these procedures the probability of contamination is minimised.

There are times when cells are more susceptible to infection.

- When cells have been recently stressed after recovery from liquid nitrogen they may be slightly permeable.
- Cultures prepared from live animals are likely to be accompanied by micro-organisms and special care is required in the preparation of primary cultures.
- Splitting cells at too high a dilution can allow micro-organisms enough time to dominate a culture.

Generally, if the culture medium is yellow or turbid or if the cells are growing very slowly, look carefully for signs of infection; this is easier to detect if you regularly split your cells to the same density and know how long they should take to grow to confluence. It is important to get to know the growth characteristics of your cells.

Yeast or mould: yeast - look for cloudy culture medium and floating stringy growths. They are often attached to dead cells. Mould - under microscopy, the mycelia usually appear as thin, wisp-like filaments, and sometimes as denser clumps of spores.

Bacteria: they usually grow quickly. Under the microscope, the bacteria appear as tiny granules between the cells. Look for a white coating on the surface of the cells. Infected cultures usually appear cloudy and pH drops quickly (=more acidic).
Viruses: Viruses are microscopic infectious agents that take over the host cell's 'machinery' to reproduce. Their extremely small size makes them very difficult to detect in culture, and to remove them from reagents used in cell culture labs. They usually do not adversely affect cell cultures. However, using virally infected cell cultures can present a serious health hazard to the laboratory users, especially if human cells are cultured in the laboratory. Viral infection of cell cultures can be detected by electron microscopy, immunostaining with a panel of antibodies, ELISA assays, or PCR with appropriate viral primers.
Mycoplasmas: these are the invisible enemy. They are small prokaryotes that have no rigid cell wall, they can therefore pass through 0.22 µm filters used to sterilise serum and other cell culture components. They alter the metabolism of cells, cause reduced cell proliferation and are capable of changing virtually every property and parameter measured in cell culture. They grow slowly, do not kill the cells and are not visible with a microscope. The only assured way of detecting mycoplasma contamination is by testing the cultures periodically.
Cross-contamination: extensive cross-contamination of many cell lines with HeLa or other fast-growing cell lines is a clearly established problem with serious consequences. Obtaining cell lines from reputable cell banks, periodically authenticating your cell lines and practicing good aseptic technique will help avoid cross-contamination. There are companies that you can send cells to in order to confirm their identity.
Antibiotics: they should never be used routinely in cell culture, because this encourages the development of antibiotic-resistant strains and allows low-level contamination to persist, which can develop into full-scale contamination once the antibiotic is removed from media, and may hide mycoplasma infections or other contaminants. Antibiotics should only be used as a last resort and only for short-term applications, and they should be removed from the culture as soon as possible. If they are used in the long term, antibiotic-free cultures should be maintained in parallel as a control for cryptic infections.

If your cells are contaminated, open the vessel **INSIDE the cabinet** or **OUTSIDE the room**, so as not to spread the contamination to the whole cell culture suite; treat with Virkon for at least 30 min and pour in the liquid waste. Fasten the lid of the contaminated empty flask and discard it into an autoclave bag. Put a notice on the cabinet with the following information: "Not to be used for 15 minutes", date, time, name+lab – contact the relevant member of technical staff for help with this and with cleaning of the incubator.

POOR ASEPTIC TECHNIQUE IS THE MAJOR CAUSE OF INFECTIONS

EQUIPMENT IN THE CELL CULTURE FACILITY

- **Incubators:** they are maintained at the specified temperature (normally 37ºC but can be different) with a humidified atmosphere at 5% CO_2 – note that not all incubators will have the water tray, so always check with the relevant technician/contact.

    - Check the water level in the tray (if applicable) prior to use and push it all the way to the back of the incubator to avoid condensation and water accumulation inside the incubator.
    - Clearly **label your cells** so they are recognisable: include your name, cell type, lab and date.
    - If a spill occurs, clean it up immediately with 70% IMS.

- **Horizontal Laminar Flow Cabinets (HLF):** they provide a sterile environment for cells, but offer no operator protection. Filtered air enters through the back of the cabinet and is directed to the front, directly at the operator. When working in one of these cabinets it is important to remember that the most sterile area is at the back, so work with this in mind at all times. Do not use this hood for human cells!

- **Class II Safety Cabinets (hoods):** they are ventilated cabinets for personnel, product and environmental protection. May be used with low to moderate risk biological agents, minute quantities of toxic chemicals, and trace quantities of radio-nucleotides. Safety advice must be sought to make sure a class II safety cabinet is suitable for the work you are doing. Cabinet servicing is performed twice a year and can be preceded by fumigation if required. This involves the use of formalin and a vaporizer and should only be performed by trained staff. Due to the dangerous nature of the chemical used, the rooms where the hood is being fumigated, will be inaccessible overnight. The responsible person will notify end users well in advance as appropriate.

  Notes for safe and effective use of Class II Biological Safety Cabinets:

    - Check the electronic controls: LEDs should all be **green** and the needle should be in the green/SAFE sector. Any **red** indicators could indicate a problem – **don't** use the hood and seek advice from technical staff.

    - **Before using it**, leave the cabinet running open for 10-15 minutes so the air flow stabilises. Wipe work surface with 70% IMS/Ethanol. Wipe each item you need for your procedures before placing it inside the cabinet.

- Do not disrupt the protective airflow pattern of the cabinet. Avoid rapidly moving your arms in and out of the cabinet. People walking rapidly behind you and open lab doors may also disrupt the airflow pattern and reduce the effectiveness of the cabinet.
- DO NOT place any objects over the front air intake grille. DO NOT block the rear exhaust grille and minimize the storage of materials in and around the cabinet.
- Segregate contaminated and clean items. Work from "clean to dirty." Work centrally within the cabinet, away from the air intake grille.
- **Bunsen burners must not** be used inside the safety cabinet as this can create turbulence in airflow (compromising sterility), heat build-up may damage the HEPA filter and release of gas may result in explosion. A small spirit burner can be used if it is essential to have an ignition source in the hood. Check compliance with the relevant technician first.
- **Clean up spills in the hood immediately**. If you spill anything under the grille this must also be cleaned up. Contact the relevant technician for help with removing the grille for cleaning.
- A waste pot or old culture medium bottle can be used inside the cabinet for collecting waste. It should be emptied into the cell culture waste bottle. Some facilities have aspirators for removal of liquid waste.
- **When work is finished**, remove all apparatus/equipment, materials and wipe all interior surfaces with 70% IMS or any other disinfectant suitable for the agent(s) in use. Remember that Virkon is corrosive, so if you use it in the hood, wipe with 70% IMS afterwards.
- Leave the cabinet running for a minimum of two minutes and then **switch the cabinet off**.

Microscopes: Dust the objectives with appropriate soft (lens) tissues only. Microscopes should have dust covers to protect them when not in use. Ensure that microscopes are **switched off when not in use.**

Centrifuges: Always ensure that tubes placed in the centrifuge are sealed and balanced. Use the screw-caps provided to secure the tubes inside the centrifuge buckets during operation – this is important because any spillage during operation will result in significant aerosol generation – this can easily be prevented using the clear caps. If a tube breaks in the centrifuge, take the whole bucket to a class II cabinet and clean it. **Switch off the centrifuge after use**. Wipe up any spillages immediately and decontaminate using 70% IMS.

WASTE DISPOSAL ROUTE

Where	What
Black-bag-lined waste bins	Non-contaminated, non-sharp waste e.g. paper, plastic wrapping. NO GLOVES!
Autoclave bag	Bio-Hazard waste: flasks, plates, dishes, pipettes, gloves, pipette tips, empty plastic media bottles. NO SHARPS! NO LIQUID! NO CHEMICALS! DO NOT OVERFILL. **When the bag is ¾ full,** seal it with tape, write your initials and lab number and place into the blue bin in the service corridor (HWB).
Glass bin	Broken, or intact clean glass after being rinsed
Liquid waste bottles	Liquid waste coming from cell culture. Before starting liquid collection, add **2 scoops of Virkon** (from the 5kg drum), **or 2 tablets of Virkon** to an empty bottle. **Always** empty the beakers with media waste into the liquid waste bottles after use and rinse the beakers.
Aspirators	Culture media. Aspirators are supplied with 1 **Virkon** tablet each (1 tablet of Virkon=1% solution when diluted in 500ml liquid; if you exceed 500ml of waste, please add another tablet). Leave the liquid waste in contact with Virkon for at least 30 minutes before disposing it down the sink. Pink liquid = Virkon is active (aspirator can still be used); Colourless liquid = Virkon is not active anymore. Please dispose of the waste media (in sink), rinse it and replace the tablet.
Yellow bag	Clinical waste (blood and tissue).
Yellow sharps bin	Any sharps. Small amounts of chemicals. Label bin with type of contents.