University of Reading

ID: TS_LR 303

TITLE: USE OF A MICROBIOLOGICAL SAFETY CABINET (MSC) - CLASS II

		Date
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Purpose

This document outlines the use and maintenance of a Class II Microbiological safety cabinet (MSC). MSCs are designed to protect the user and the environment from biological agents, this may be when deliberately working with hazardous microorganisms, or when working with material which may be potentially infected (e.g. human tissues or primary cell lines) with hazardous microorganisms. In addition, MSCs are often used to maintain the sterility of low risk samples. The effectiveness of a MSC depends on its design, a suitable installation, ongoing maintenance and correct use.

Scope

The standards and practices listed in this document apply to all laboratories using a Class II MSC at the University of Reading.

Risks

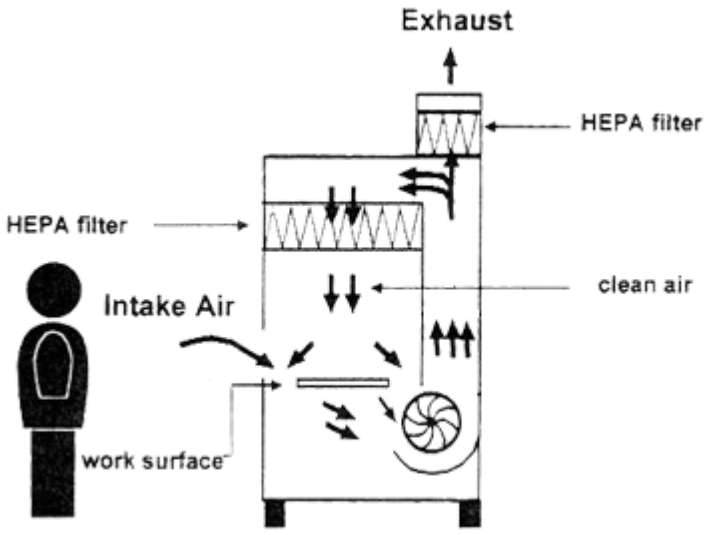
Incorrect use or maintenance can result in exposure of the user to hazardous biological agents or GM organisms. Risk assessments must be written for procedures that are carried out in a MSC.

Associated documents

TS_LR 301 Local Rules for Working in the Laboratory
H&SS Microbiological Safety Cabinets Code of Practice 14- Part 6

How the cabinet works

A MSC is an open fronted safety cabinet with a viewing window. The system is constructed to provide worker protection, both through the intake of air and the movement of sterile air across the front of the cabinet (air curtain). The material is also protected from cross -contamination by the downwards circulation of sterile air in the cabinet. Air is filtered before removing to external exhaust (ducted) or to the laboratory (re-circulating).



Microbiological Safety Cabinet uses

- Users must liaise with Laboratory Co-ordinators to ensure that they select the appropriate cabinet for the work. Appendix 1 contains general guidance on cabinet selection.
  It is important to note that MSCs are not designed to protect the user from all hazards e.g. radioactive or toxic hazards, and HEPA filters are not designed to remove non-biological contaminants. Particular care must be taken when using materials with such additional hazards to ensure these are not discharged into the laboratory environment from cabinets that are not externally ducted, or, as a proportion of the air inside the cabinets is re-used, results in contamination of the inside of the cabinet, equipment and hands.
- The effectiveness of Class II MSCs depends on their correct use; poor technique can compromise the operator protection afforded by the cabinet.
- Bunson burners must not be used in MSCs.
- The UV lamp, if fitted, should never be on while the cabinet is in use.

Procedure

Before Beginning Work

- Remove the night door if used and turn on cabinet fan and lights if necessary. Test the alarm. Check the airflow indicator is in the safe zone, alarms must not be muted. You may also confirm inward airflow by holding a tissue at the middle edge of the opening and ensuring that it is drawn in.
- Disinfect the interior surfaces with a suitable, non-corrosive disinfectant such as 70% ethanol 2% Trigene or Biocleanse.
- Wipe with a clean paper towel.
- Allow cabinet to stabilize for 2-5 minutes.
- Plan ahead. Assemble only the necessary materials required for the procedure and load them into the cabinet. Keep the air grilles clear. Aerosol generating equipment should be placed towards the rear of the cabinet.

During Work

- Wear appropriate PPE (Howie type coat and nitrile gloves). The cuffs of the gloves should be pulled up and over the cuffs of the coat.
- Perform operations as close to the middle of the cabinet as possible.
- Avoid excessive movement of materials and arms through the front of the cabinet; when you do enter or exit the cabinet allow the cabinet to stabilize before resuming work.
- Keep the work area clean and tidy.
- If the alarm sounds during work make the work secure e.g. seal tubes, if the cabinet air flow fails during use seal the front with the night door, switch off and clearly label and contact the technician responsible for the cabinet.
- Keep discarded, contaminated material to the rear of the cabinet.
- If there is a spill during use, surface decontaminate all items in the cabinet and disinfect the working area of the cabinet immediately. Large spills of micro-organisms should be immediately treated with the appropriate disinfectant. Wait at least 10 minutes. Soak up disinfectant and spill with paper towels and transfer to a biohazard bag for disposal.
- Try and limit people passing behind you and the cabinet as disruption in the air flow may alter the cabinet's effectiveness.

Following Completion of Work

- Remove all materials. Close or cover containers before removing them from the cabinet.
- Place all waste into a biohazard bag. Surface disinfect objects in contact with contaminated material before removal from the cabinet. Do not use the cabinet as a storage area.
- Disinfect the interior surfaces of cabinet with an appropriate disinfectant. All parts of the working area of the cabinet should be swabbed with a suitable disinfectant (as recommended by the cabinet manufacturer). Swabbing with 70% ethanol is often recommended for this, but it may present a serious risk of fire if there are any naked flames or sources of ignition in or near the cabinet.
- After using a cabinet with viable biological agents, at the end of the working day, the cabinet should be run for at least 5 minutes, to assist the removal of residual contaminated aerosols.
- Turn off lights and fan. Replace the night door if used.
- Remove contaminated gloves and dispose of them as appropriate; wash hands.

Disinfection

In addition to routine disinfection of cabinets after use, in limited circumstances UV light or fumigation with formaldehyde may be used to decontaminate the cabinet. Technical Services will organise fumigations. These will be carried out by external contractors.

UV Light is not recommended

- UV light used for this purpose must be tested annually to ensure that the appropriate wavelength (254nm) is emitted.
- The skin and particularly the eyes are vulnerable to UV light, therefore, if a UV decontamination cycle is to be carried out, safety interlocks or appropriate UV screens must be in place. Alternatively, personnel must be excluded from the area and a warning sign clearly displayed on the door.
- It is important to note that UV lamps are germicidally active for a very short part of their working life, which is a fraction of the time that they glow purple therefore the efficacy of the germicidal activity of the light should be monitored and the tube should be replaced when the efficacy is reduced.

Fumigation

- Fumigation must be carried out only by a trained responsible person, usually an external contractor.
- Fumigation with formaldehyde vapour is currently the recognised procedure.
- Fumigations of class II MSCs should only be performed in the following circumstances:
- After a major spillage of hazardous microorganisms, or a spillage where inaccessible surfaces have been contaminated
- Before any maintenance work on a cabinet used for medium and high risk activities where access to potentially contaminated parts e.g. HEPA filters is necessary

Maintenance and validation

	Class II		
	Low risk *	Medium risk **	High risk***
Alarms and indicator check	Every use – by user During each maintenance – by contractor		
Face velocity/inflow	Annual-by contractor	Monthly-by local contact (see Appendix 2)	
Inflow/downflow	Annual-by contractor	Monthly-by local contact (see Appendix 2)	
Filter integrity tests	Annual-by contractor	6 monthly-by contractor	
Mechanical and electrical function	Annual-by contractor	6 monthly-by contractor	
Mechanical integrity (including visible ductwork)	Annual-by contractor		
Operator Protection Factor	Annual- by contractor		N/A
In use operator protection factor*	If required	If required	6 monthly

*Low risk – can include MSCs located in containment level 2 laboratories if used only for established culture collections presenting a low-infection risk (e.g. established human cell lines screened by commercial suppliers), or for primary lab animal cell culture;

**Medium risk – HG2 or GM class 2 activities, work with human or wild/farm animal materials including primary cell culture;

***High risk- used with organisms in hazard group 3/class 3 GM or with HG2/Class 2 organisms which present a particular hazard of infection by the aerosol route

Maintenance by contractor

- When work is carried out by a qualified external contractor, a copy of the test certificate must be kept on file and a visible 'Pass' sticker must be displayed on the exterior of the cabinet.
- Records must be kept for five years.
- Cabinets which do not pass these tests must not be used and must be clearly labelled as such.
- For low risk activities, cabinets must be surface decontaminated prior to any work by the engineer. For medium and high risk activities, cabinets must be fumigated by the engineers prior to undertaking any maintenance work/repairs.
- Any HEPA filters for disposal should be bagged in a clinical waste bag and processed through the clinical waste stream (HEPA filters from medium risk MSCs would have been subjected to fumigation by the engineer prior to removal from the MSC).

Maintenance by technical staff

Where required, inflow and downflow velocities must be measured and recorded with an anemometer on a monthly basis. See Appendix 2 for more details.

Training

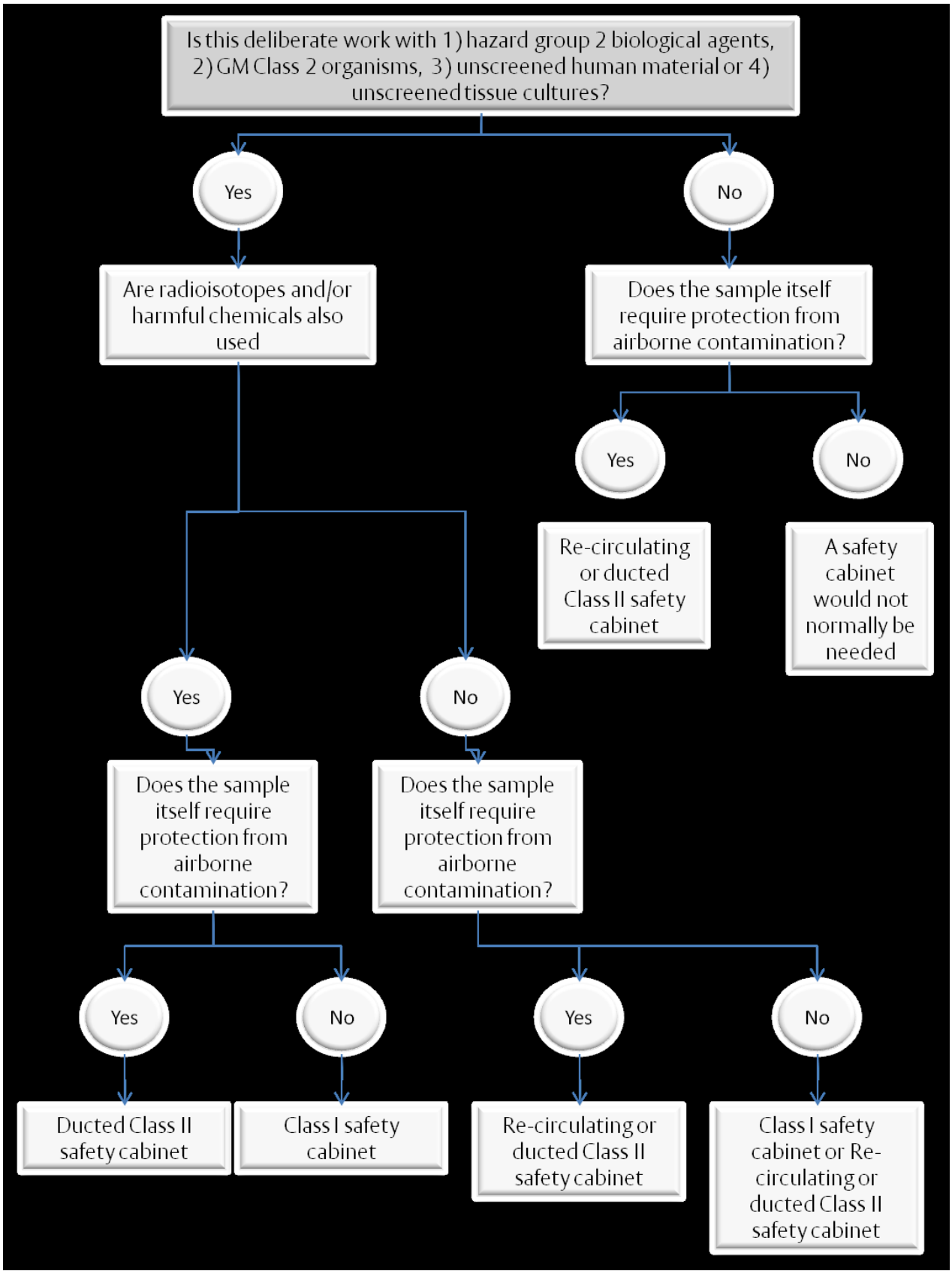
The trainee must have read and understood this document. On having observed the procedure the trainee must carry out the task whilst being assessed by the trainer. This must be repeated and assessed until competency to the satisfaction of the trainer has been achieved. Sign off of the training module can then take place.

Appendices

1. Selection of MSCs.
2. Monitoring and recording airflow velocities
3. Log book cover sheet

Appendix 1

Selection of the correct Microbiological Safety Cabinet



Appendix 2

Monitoring and recording airflow velocities

The airflow velocities of a class II cabinet should be tested on a monthly basis using a calibrated rotating vane anemometer. Inflow air should have a mean inward air velocity of not less than 0.4 m/sec, whilst the downward air velocity should be between 0.25 and 0.5 m/sec. Cabinets should be in 'in use' mode (i.e containing equipment that would normally be present).

Operation of LCA 301 rotating vane anemometer

- Check that the anemometer is within calibration (calibration sticker should be attached to the unit).
- Press the ON/OFF key to turn the anemometer on (left hand button under the display). It should power up in the mode last used (VEL, m2/sec). If it has changed, use the Mode key (right hand button under the display) to select VEL, and then the trigger key to select unit type.
- In order to take a reading, hold the rotating vane (blades) in the airstream, noting the flow direction arrow on the instrument head. Keep the vane in the airstream for the required period before pressing the trigger momentarily to take a reading. This enables the anemometer to reach a steady speed and the reading will be more accurate. The instrument will display the measured value.

Measuring inflow velocities

3 readings must be taken at the intake (front aperture); one at either end and one in the middle of the aperture (refer to record sheet below for more details). The average air velocity must not be less than 0.4m/sec.

- At the intake (front aperture) hold the anemometer in the vertical plane at arm's length to one side of body. Leave in place at one end of the aperture for 15-20 seconds.
- Take and record the reading. Take and record two more readings, one in the middle of the aperture and one at the other end.
- Record the average. If the average reading is less than 0.4m/sec report this to the Technical Manager of Maintenance.

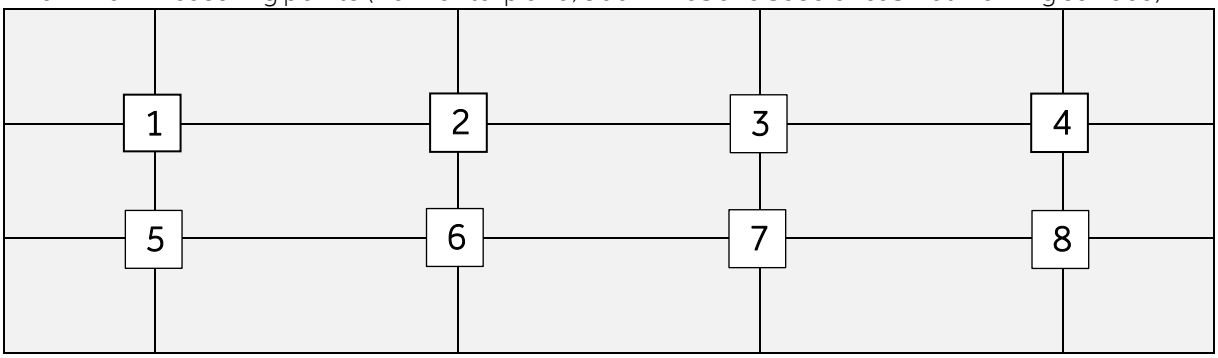
Measuring downflow velocities

8 readings must be taken within the cabinet, four at the front and four at the back across the width (refer to record sheet below for more details). The average air velocity must be between 0.25-0.5m/sec.

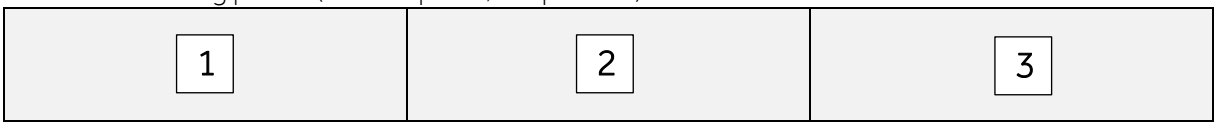
- Across that base of the cabinet, hold the anemometer in the horizontal plane 300mm from the base. Leave in place at one of the reading points for 15-20 seconds.
- Take and record the reading. Take and record seven more readings as depicted in the record sheet.
- Record the average. If the average reading is outside of the specification 0.25-0.5m/sec. report this to the Technical Manager of Maintenance.

Monthly testing of MSC airflows

Downflow measuring points (horizontal plane, 300mm above base of cabinet working surface)



Inflow measuring points (vertical plane, at aperture)



2. m/sec	3. m/sec	4. m/sec	5. m/sec	6. m/sec	7. m/sec	8. m/sec	Av. m/sec*	Date	Name

*If outside 0.25- 0.5m/sec. report this to the Technical Manager of Maintenance.

1. m/sec	2. m/sec	3. m/sec					Av. m/sec**	Date	Name

** If less than 0.4m/sec. report this to the Technical Manager of Infrastructure (Operations)

Appendix 3

Microbiological Safety Cabinet (MSC) Usage and Maintenance Record

Please record the types of biological material that are being used in this MSC. This will be used to ensure that the correct safety testing and maintenance regime is in place for this unit. Each biological material only has to be recorded once and a description of the risks is given below.

MSC ID ..... MSC Location .....

Name	Date material first recorded in MSC	Brief description	Risk* Low/medium/ high	If GM project Class1 / Class2	If GM project include project number

*Low risk – HG1 or GM class 1 activities: established culture collections presenting a low-infection risk (e.g. established human cell lines screened by commercial suppliers), primary lab animal cell culture etc.

Medium risk – HG2 or GM class 2 activities: work with human or wild/farm animal materials including primary cell culture;

High risk- HG3 or GM class 3 activities or HG2/GM class 2 organisms which present a particular hazard of infection by the aerosol route

MSC Repair and Annual Maintenance Record

Name	Date	Details Annual Test/Repair Information	Paperwork held by technical contact Y/N