Microbiological Safety cabinets and Laminar Flow cabinets

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Introduction

Several types of cabinets can be found in laboratories. Some of the cabinets are designed to offer protection to the user - microbiological safety cabinets - while some are designed to primarily protect the items that the person is working on – laminar flow cabinets.

Where operator protection against airborne transmitted organisms is priority, a microbiological safety cabinet should be chosen.

High Efficiency Particulate Air (HEPA) Filter

HEPA filters are generally designed to capture 99.999% of the material that passes through them. HEPA filters are built and certified to the European Standard EN779:2012 and to ISO 29463-1:2017

Types of Microbiological Safety Cabinets (MSC's)

Microbiological Safety Cabinets are currently manufactured as three classes (I, II and III). While all of the cabinets will provide operator protection, it is important to choose the one that is most suitable for the process that is being undertaken. The classes of Microbiological Safety Cabinets are different from the biohazard groups listed in the ACDP approved list of biological agents. See page 6

MSC Class 1

This will protect against organisms in ACDP Biohazard Groups 1 - 3.

The air is drawn in the front of the cabinet and expelled via the roof mounted HEPA filter. Many cabinets will be fitted with two HEPA filters in line as this gives added protection when viruses are handled.

This type of cabinet will generally keep the worker safe but may not keep samples clean if the cabinets are located in an area where there are a large number of airborne particulates

These cabinets are connected to ducts to vent the excess air to the outside.

MSC Class 2

This will protect against organisms in ACDP Biohazard Groups 1 - 3.

Air is drawn into the cabinet via the front grille and is then drawn up through a HEPA filter before being passed downwards over the working surface. The downwards movement of the air produces an air curtain that protects the worker from the material being handled. Waste air is passed through a HEPA filter. In some machines the mixture ratio may be 30% fresh air and 70% HEPA filtered recycled air. As with class 1 cabinets, two HEPA filters may be mounted in line for added protection. The way the air circulates will define a cabinet as type A1, A2 or A3.

These cabinets may be connected to ducts to vent the excess air to external ducts.

It should be noted that class 2 cabinets are more susceptible to the airflow being disturbed than a class 1 microbiological safety cabinet.

This type of cabinet may be the optimum choice between worker protection and ease of handling the samples and protecting the samples.

MSC Class 3

This will protect against organisms in ACDP Biohazard Groups 1 - 4. When using this cabinet all experimental items must be placed into the cabinet prior to work commencing.

These cabinets are completely sealed when in operation and HEPA filtered air is drawn into the cabinet either via fans located on the back or sides of the machines. Samples are manipulated via rubber gloves that are built into the front panel. Air is not generally recycled in these types of cabinets and is expelled via roof mounted HEPA filters. The machine is generally ducted to expel the cabinet air to external ductwork.

This type of cabinet offers the worker the greatest level protection provided that the seals are in place and attached.

Using Microbiological Safety Cabinets'

Daily checks

When using MSC's it is important that daily checks are undertaken. If the transmission route of the organism being handled is via the respiratory route, it is especially important to check that the inlet speeds are within the acceptable limits. Records of the daily checks should be kept.

The acceptable airflow speeds should be between 0.7 and 0.9 M / S for class I MSC's and 0.6 to 0.9 M / S for Class 2 MSC's. If the airflow speeds are above or below this range the machine should not be used and the core facilities staff should be contacted to inform them of the problem.

Cabinet working environment

It is important to minimise the number of items in the cabinet so that the airflow is not excessively disturbed. Class 2 cabinets are particularly sensitive to disturbance of the airflow as the air in the cabinet is a mixture of fresh air taken in via the front grille and recycled HEPA filtered air.

If possible, items that generate their own flow or create turbulent airflows should be avoided e.g. microfuges.

Care should be taken that loose items such as tissues or other lightweight materials are not sucked up into the fans.

Individuals should take care to position themselves correctly at an MSC to avoid back problems and large scale arm movements that could upset the airflow within the cabinet. This is particularly important when used class 2 MSC's.

Operator Protection Factor (OPF)

The level of protection for individuals using MSC's can be calculated following a KI (Potassium Iodide) Discus test (EN12469:2000) where a solution of Potassium Iodide is sprayed into the cabinet while it is operating and the number of particles that emerge in a given period are counted. In most tests an artificial item that represents a human arm is present in the cabinet. The lower the number of particles that escape from the front of the cabinet the higher the operator protection factor. If only 1 out of 100,000 particles was released into the atmosphere this would give an OPF of $10^{5,}$ if only 1 particle out of 1,000,000 is released then the OPF will be 10^{6}.

It is important to note that the level of protection can be reduced if the cabinet is full of objects such as discard pots, racks, bio-bins, sharps bins and the rate of the airflow across the front is lowered.

It is important that a KI test is performed after moving the MSC, or introducing bulky equipment into a MSC as this may affect the airflow. If the lab is being rearranged by the introduction of other large equipment or another MSC, the cabinet should also be retested as these items may affect the airflow. It is normally recommended that if a second MSC will be placed directly opposite an existing machine, a space of at least 2.0M is present to prevent the airflows overlapping and fighting with each other.

Training

Individuals must be trained in how to use the MSC's. While equipment may look similar, there may be variations in the start-up and shut-down cycles and in the meaning of the warning lights. Individuals must be informed of the minimum and maximum airflow rates that will provide adequate worker protection.

The training should be undertaken by the Principal Investigator (PI) prior to the individual starting work and the records must be kept by them. If the PI is not available then a member of the core facilities staff may be approached to be provide training. The Senior Laboratory Manager should be contacted in the first instance.

A prototype training record would discuss

1 Removal of the night door including the switching off of the UV light if present.

2. Meaning of the various switches on the front panel.

3. Starting the cabinet

4. Stabilisation of the airflow and measurement of the airflow.

5. Meaning of the alarms and when it is safe to start work

6. How to work in the cabinet including dealing with waste

7. Dealing with failures in airflow during work

8. Cleaning the cabinet after work

9. Closing the cabinet.

Some of the MSC's in SGUL have powered doors and how to deal with a failure of the mechanism should be discussed with the worker.

Use of cabinets

The cabinet should be chosen on the risk that the organism being handled poses to an individual. People who are pregnant or immunocompromised or under medical treatment may require additional protection.

The use of a microbiological safety cabinet will not make up for poor laboratory practice as it is possible to perturb the airflow of both class 1 and class 2 cabinets.

An MSC is not always required when handling HG1 or HG2 organisms. A risk assessment should identify the need for MSC use e.g. Neisseria gonorrhoeae can be handled on the open bench while wearing the correct PPE and using good aseptic technique and good laboratory practice as it is not transmitted by the respiratory / airborne route. Neisseria meningitidis should be handled in a MSC while using good aseptic technique and good laboratory practice as it is transmitted by the respiratory / airborne route.

It is important that organisms whose primary route of transmission is via droplet / inhalation / respiratory routes e.g. *Neisseria meningitidis, Streptococcus pneumoniae* or *Mycobacterium tuberculosis* are handled in a microbiological safety cabinet.

All HG3 organisms should be in Class I cabinets unless a Risk Assessment argues that it is not necessary e.g. not aerosol transmission route.

Microbiological safety cabinets can also be used to provide protection for work involving tissue culture or preparation of samples for proteomic or genomic analysis that require a sterile environment. Good laboratory practice should always be followed when undertaking any type of work to minimise the chances of contamination.

The cabinet selected for the work and the reason for its use must be mentioned in the project COSHH assessment.

Cabinets must never be used with the front panel raised as this would pose a risk not only to the operator but also to other individuals that are in the area.

Cleaning

The insides of microbiological safety cabinets must always be cleaned at the end of each working session. The cleaning agent selected must not corrode any of the surfaces of the cabinet.

Some cabinets may have a single removable panel or several removable panels as the working surface, the panels must always be lifted up and the base surface cleaned at the end of each session to avoid organisms growing in any spilt media or other fluid.

The cabinet manual should be checked before Virkon or other halide containing agents are used as a cleaning agent as halides may damage the surface finish either by causing the paint to corrode or by causing the bare metal to become pitted. While the damage may not be immediately visible to the eye, it can affect the airflow within the cabinet and lead to a reduction in operator protection.

If 70% Ethanol is to be used as the cleaning agent, it must be confirmed that this will cause a 10^6 reduction in the numbers of the organism that was being handled.

Fumigation

In exceptional circumstances, a MSC may need to be fumigated. If you believe that the cabinet has become contaminated and requires fumigation this should be discussed with the core facilities team.

It is very rare for a cabinet that has only been used for tissue culture or to preserve the cleanliness of proteomic or genomic samples to require fumigation.

Servicing

The servicing of the cabinets will normally be arranged by the core facilities staff. All cabinets that are used to handle biohazard group 3 pathogens or GMO must be serviced every 6 months. Class 2 MSC's are also serviced every six months.

Laminar flow cabinets

These cabinets are designed to protect the sample and not the worker.

It is important to note that many laminar flow cabinets look similar to microbiological safety cabinets and the correct cabinet must be used. These cabinets must never be used to handle micro-organisms that pose an infection risk and whose route of transmission is airborne

In a laminar flow cabinet air is drawn in via a top mounted fan and is blown through a High Efficiency Particulate Air (HEPA) Filter and then down into the cabinet to be expelled out via the front panel. The air flow can be vertical or horizontal until the moment that the air exits the cabinet. The cabinet is run at a positive pressure to the ambient room air to ensure that room air does enter the working surface and contaminate the environment.

It is important to note that the air expelled from the cabinet may enter the workers respiratory zone so correct posture is important.

The associated standard is BS EN ISO 14664-1 for cleanrooms and associated controlled environments.

Biohazard Groups

The ACDP biohazard groups are

1. Bio agents unlikely to cause disease

2. May cause disease but a low hazard with the spread of disease unlikely and with prophylaxis/treatment available. Several lethal infections that are spread by the airborne route are biohazard group 2 e.g. *Legionella pneumophila* or *N. meningitidis*.

3 Possibility of a severe disease with spread of disease possible although prophylaxis/treatment is available.

4. Causes severe disease with serious bio-hazard risk and a high risk of spread of disease. With prophylaxis/treatment not normally available this is the most serious bio-hazard risk group

More information on Biohazard groups is available here

Further information

British Standard BS EN 12469:2000. Biotechnology: Performance criteria for microbiological safety cabinets.

British Standard BS 5726:2005.
Microbiological safety cabinets: Information to be supplied by the purchaser to the vendor and to the installer, and siting and use of cabinets.

The Management, design and operation of microbiological containment laboratories, Health and Safety Commission/ Department of Health, Advisory Committee on Dangerous Pathogens.

Training Videos

A variety of good videos giving information on the use of MSC's is available on YouTube. These videos are not a substitute for being talked through the nuances of the machine you will use.

Microbiological Safety Cabinets are sometimes known as Biological Safety Cabinets (BSC).

Lam Systems Microbiological Safety Cabinets

National Institutes of Health BSC How it works to Protect you.

Monmouth Scientific Microbiological Safety Cabinet Class 2

Working safely in a class 2 cabinet

Use of a Class 1 cabinet