



GUIDANCE NOTE 3: SAFE USE OF EQUIPMENT

University Policy requires that:

Equipment and facilities are maintained and tested, where appropriate, to ensure efficient and safe operation;

All equipment used should be assessed to establish the risks of contamination by biological hazards. It should be clearly established if there are any risks to users of equipment and local procedures must be drawn up. Decontamination of equipment must be performed before any outside contractor is allowed access to service or maintain that equipment.

Some guidelines are set out below for the use of certain types of equipment in biological laboratories (this is not an exhaustive list). Other safety issues are raised when working with all electrical equipment and these must be considered when establishing local procedures.

Safety Cabinets

The British Standard on Performance Criteria for microbiological safety cabinets (BS EN 12469:2000) specifies requirements for safety cabinets with respect to safety and hygiene. It sets performance standards for cabinets used for work with micro-organisms, and specifies test procedures for cabinets with respect to protection of the worker and the environment, product protection and cross contamination.

The other standard relevant to microbiological safety cabinets is BS 5726:2005. This provides recommendations and guidance on information to be supplied by the University to safety cabinet manufacturers, vendors and installers on the siting and use of cabinets.

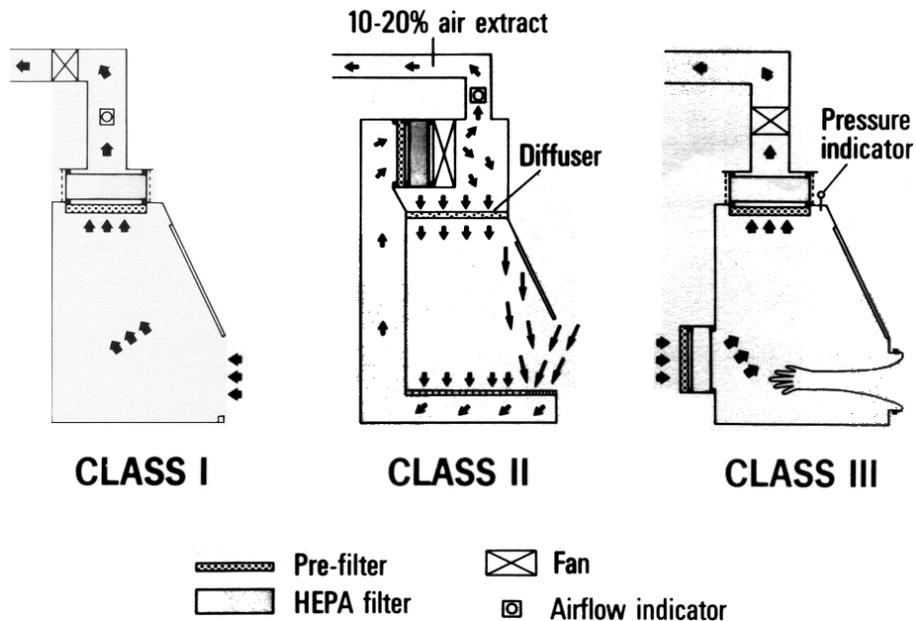
The cabinet manufacturer or supplier must be able to produce a test certificate demonstrating type compliance with BS EN 12469:2000. If cabinets are poorly sited they may be affected by airflows from pedestrian movement or ventilation systems.

There are three classes of safety cabinet:

Class I: An open fronted cabinet through which air is drawn to minimise aerosol escape. The hood is extracted to the outside via a High Efficiency Particulate Air Filter (HEPA). The cabinet protects the operator and not the work and is suitable for work with Hazard Group 2 and most Group 3 agents. An Operator Protection test should be carried out at least once a year. The airflow of Class I cabinets should be checked with an anemometer, weekly or at least monthly dependent on frequency of use.

Class II: An open fronted cabinet where the workspace is protected by a downflow of sterile air. Air is also drawn into the cabinet through grilles at the front, thus providing operator protection. Therefore, the operator and work is protected. The hood is either ducted to the outside via a HEPA filter or is a recirculating hood with a HEPA filter in the exhaust. Some cabinets will be fitted with a double HEPA filter in the exhaust. This type of cabinet is mainly used for Tissue Culture, and can be used for work with Hazard Group 2 agents. Class II cabinets that meet the requirements of the British Standard should be able to afford a degree of protection equivalent to that provided by a Class I cabinet. However, they are much more easily affected by operator movement and movement near the cabinet, caused by traffic through the laboratory or opening and closing of doors. Therefore, the use of a Class II cabinet for work involving Group 3 agents may need to be justified and special "in-use" operator protection tests must be carried out. The hood would generally be extracted to the outside in this case.

Class III: This is a totally enclosed cabinet where the operator is separated from the work by gloves attached to ports. Both incoming and outgoing air is filtered via HEPA filters. The Hood is extracted to the outside. These cabinets provide high level protection for both work and operator and are used for Group 3 and 4 agents. It should be noted that the operator's movement is restricted by the gloves and dexterity may be reduced.



General Safety Points

Maintenance and testing:

Safety cabinets should be serviced at least every six months, and an operator protection test (KI discus test) **must** be carried out at least once a year on Class I and Class II cabinets. More frequent checks will be needed in containment level 3 facilities.



KI Discus test in operation

Airflow of Class I cabinets should be checked regularly (weekly) with an anemometer. Readings should be taken at each of the four corners and the middle of the cabinet front opening and may be considered acceptable if in the range 0.7 - 1.0m/s.

The cabinet **must** be fumigated before repair (involving access to filters or internal cabinet workings) if biohazards have been used in the cabinet. It should also be fumigated if a significant spillage occurs within the cabinet that can not easily be cleaned up. Fumigation of cabinets before service visits is mandatory at containment level 3. Guidance on fumigation can be found elsewhere in this document.

Siting:

Placement of cabinets should be discussed at an early stage with the Health and Safety Unit. The British Standard, BS 5726: 2005, provides guidance on siting.

Whenever a cabinet is moved a KI Discus test must be carried out on the cabinet in its new location to ensure it provides adequate containment.

Other types of Local Exhaust Ventilation

It is important not to confuse laminar flow cabinets with safety cabinets as they do not provide user protection. They simply provide a sterile working environment. The HEPA filtered air is either blown down over the cabinet floor, or is blown horizontally towards the operator, and therefore there is no containment. Fume cupboards should NOT be used for any type of work involving biohazards.

Arrangements for exhaust air

The preferred option is to duct exhaust air to the exterior of the building. This means the cabinet will maintain a negative pressure within the laboratory and fumigant (formaldehyde) can be safely discharged. Providing the risk from the work is only microbiological it is permissible for cabinets to discharge exhaust air into the laboratory at CL2, but this should NOT be permitted at CL3.

UV light

It should be noted that UV light is generally ineffective for the disinfection of safety cabinets. Radiation is unidirectional, which means the cabinet has to be empty for it to exert any effect, and the lamp is only

active microbiocidally for a short period of time. If fitted, there must be an interlock to prevent operator exposure to UV light. UV light should not be relied on as the sole method of decontamination.

Safe Use of cabinets

Whilst working at the cabinet the following procedures should be observed:

- *The cabinet should not be used unless the viewing screen is in place and the airflow is satisfactory. Some cabinets take a few minutes to reach optimum airflow.*
- *The operator should be comfortably seated and able to see clearly through the viewing screen.*
- *Equipment should be kept to a minimum (back of Class I and middle of Class II).*
- *Centrifuges should not be used in cabinets.*
- *Gas burners should be avoided. Ordinary Bunsen Burners must not be used but micro-incinerators are permissible. Solenoid valves that only release gas while the cabinet fan is running should be fitted, but this does not eliminate the risk of explosion. Gas flames must not be left unattended.*
- *Movement around the opening of cabinets should be kept to an absolute minimum when in use.*
- *The operator should work in the middle – back of the cabinet and should not block the grilles with tip boxes, paper or other materials, as this will affect the airflow.*
- *If an alarm sounds, make the work safe, stop using the cabinet and report the fault immediately.*
- *Spillages should be cleaned up immediately and regular cleaning rotas should be established.*
- *After use the cabinet should be left running for 10-15 minutes to allow aerosols to disperse.*
- *Ensure the cabinet is decontaminated when work is complete.*

Further information can be obtained from the British Standards:

BS EN 12469: 2000 Biotechnology – Performance criteria for microbiological safety cabinets

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 – Recommendations and guidance

Centrifuges

Improperly used or poorly maintained centrifuges can present significant hazards to users. Failed mechanical parts can result in release of flying objects, hazardous chemicals and biohazardous aerosols. The high speed spins generated by centrifuges can create large amounts of aerosol if a spill, leak or tube breakage occurs. Centrifuges should conform to BS EN 61010-2-020:2006, and the manufacturer's guidelines should be followed at all times. Local assessments should be made on the use and maintenance of centrifuges in general. In particular the following points should be considered:

- *Centrifuges should be positioned so that operators can see into the bowl.*
- *Users should ensure the rotor and lids are tightened correctly before use*
- *Centrifuges should be properly balanced and the condition of tubes and bottles should be checked.*

- *Biohazards should be centrifuged in sealed buckets or rotors (this is mandatory at containment level 3). The outside of the cups or buckets should be decontaminated before and after centrifugation.*
- *O-rings should be inspected regularly and replaced if cracked or dry.*
- *For ultracentrifuges, maintain a logbook of rotor use for each rotor, recording the speed and length of time for each use.*
- *The centrifuge should be maintained in good condition. Broken door latches and other problems should be repaired before using the centrifuge.*
- *Whenever centrifuging biohazardous material, always load and unload the centrifuge rotor in a biological safety cabinet. Avoid the use of pop-top tubes which can create aerosols upon opening. Use screw capped tubes instead.*
- *If a centrifuge tube has broken the sealed bucket should be opened in the appropriate class of safety cabinet after allowing aerosols to settle.*
- *High strength aluminium corrodes easily. To prevent this, buckets should be kept clean and dry.*
- *Only clean aluminium buckets with a suitable cleaner – do not use Chlorox or Virkon.*

The following procedure for a broken tube should be used, and should be written into the risk assessment for the work:

CENTRIFUGATION RISKS: Note that breakage of a centrifuge tube, improper loading, or potential equipment failures during centrifugation, have the potential to generate aerosols that could pose an infection/contamination risk. Always ensure that buckets are properly seated on the centrifuge rotor, balanced, and that the tubes used will not prevent the bucket from swinging to the horizontal position. Tubes should be capped. Take special care if centrifuging tissue culture plates.

In the event of a suspected accident during centrifugation — e.g. excessive noise and vibration — immediately turn off the centrifuge. **DO NOT OPEN THE LID OF THE CENTRIFUGE FOR AT LEAST 30 MINUTES AFTER THE ROTOR HAS COME TO REST**, to allow time for aerosols to settle. Post prominent warning notices to stop others from opening the centrifuge.

Where possible, use sealed buckets for centrifugation of materials that may pose a significant infection risk. If failure of a centrifuge tube within a sealed bucket is suspected, transfer the entire bucket to a biological safety cabinet before opening.

Always wear protective gloves to clear up after an accident. Transfer fluids to a waste pot for autoclaving or absorb with paper tissues that are subsequently soaked in disinfectant and/or autoclaved. Soak or thoroughly spray the contaminated centrifuge components with a suitable non-corrosive disinfectant, e.g. Tristel, Klercide 5, etc. After at least 10 minutes contact time, thoroughly rinse the centrifuge components with water, and dry. If the centrifuge components may have been damaged, arrange for these to be inspected by a qualified engineer before re-use.

Ultrasonic Disintegrators

These may produce aerosols which may be liberated into the environment, particularly if the probe type is used. Therefore:

- A complete assessment needs to be performed and if necessary it must be used in an appropriate safety cabinet.
- Cleaning after use is essential.

Cryostats / Microtomes

Cryostat work can involve lengthy periods of work manipulating specimens in a cold environment

(-15C to -35C). The movements are repetitive and it is easy to adopt a poor posture. Additional hazards include exposure to very sharp blades and the handling of potentially pathogenic (unfixed) specimens.



- All users must be trained and certified competent to use the cryostat;
- Use an automated cryostat if possible and ensure you have an adjustable chair with a footrest;
- Limit the time spent working at the cryostat and take regular breaks;
- Report any arm or shoulder discomfort to Occupational Health;
- Wear a cotton fingerless glove liner or Kevlar glove if possible to reduce cold and risk of cuts – even if only on one hand;
- Do not place utensils within the cryostat – keep them outside when not in use to maintain them at room temperature;
- Do not carry out cryostat work out of hours;
- Be familiar with the accident reporting arrangements and seek help immediately in the event of a cut and/or exposure to unfixed material;
- Ensure the cryostat is decontaminated after use

Waterbaths

The following should be noted:

- Waterbaths rapidly become contaminated with micro-organisms, including *Legionella sp.*
- Anything put into the water bath may therefore become contaminated. Suitable biocides can be used, but consideration must also be given to the effect on the waterbath material itself.
- Regular cleaning rotas are essential.
- Ensure baths are equipped with redundant heat controls or automatic cutoffs that will turn off the power if the temperature exceeds a preset limit.

Other equipment

The following equipment should also be assessed when considering biohazard contamination and regular cleaning and decontamination procedures should be locally established. The type of equipment includes:

- homogenisers
- filtration equipment
- freeze dryers
- colworth stomachers
- shakers
- large scale culture equipment
- incubators
- refrigeration equipment
- electron microscopes
- pipettes *etc.*