Procedures for the Effective Use of Biological Safety Cabinets

APPROVAL DATE BY BIOHAZARDS SUBCOMMITTEE: August 10, 2012
SUPERSEDES: March 1, 2012

This corporate guideline/procedure is intended as a minimum requirement to be applied by individual departments in collaboration with Occupational Health and Safety (OH&S) and Facilities Management.

1.0 DEFINITIONS

CLEAN AIR BENCHES

In the case of clean air benches, there is reverse HEPA filtered laminar airflow towards the worker. Clean air benches provide product protection only. A clean air bench is NOT a biological safety cabinet. The worker is directly exposed to aerosols and particulates from the work. Clean air benches are not to be used for Containment Level 2, 3 or 4 agents or radioisotopes. They are to be used where only a flow of clean air is required.

BIOLOGICAL SAFETY CABINETS

A biological safety cabinet is a ventilated cabinet which uses a variety of combinations of HEPA filtration, laminar air flow and containment to provide personnel, product and/or environmental protection against biohazardous agents or nanoparticles. It is distinguished from a chemical fume hood by the presence of HEPA filtration and the laminar nature of the airflow. For fume hood use please see "Laboratory Health and Safety Manual", OH&S, Section 10.3.

Biological safety cabinets must NOT be used for procedures with hazardous chemicals unless verified by Occupational Health and Safety.

Nanoparticles are defined as 1 – 100 nanometers in any plane. Nanoparticles must be handled in an appropriate biological safety cabinet.

For more information on the nanotechnology program see:
http://www.Western.ca/humanresources/facultystaff/h_and_s/lab_safety/nanotech.htm

There are three classes of biological safety cabinets -

Class I:

These cabinets have unrecirculated airflow directed away from the user that is discharged through a HEPA filter. Class 1 cabinets provide protection to the user and protection of the environment but no protection to the work.

Class 1 cabinets are suitable for some work procedures at Containment level 1 and 2 if no protection of the work is required.

Class II types:

Class II cabinets provide a high degree of protection to the worker, the work and the environment. They are suitable for work at Containment Level 1, 2, 3 and 4 and are divided into two types (A and B) on the basis of construction type, airflow velocities and patterns and exhaust systems.

Class II, Type A1

Cabinet air may be recirculated back into the laboratory or ducted out of the building. It is able to maintain a minimum average face velocity of 0.38 m/s (75 ft/min) and may have positive pressure contaminated ducts and air flow. This type is not suitable for work with low levels of volatile toxic chemicals and volatile radioisotopes.

Class II, Type A2

Cabinet air may be recirculated back into the laboratory or ducted out of the building by thimble connection. It is able to maintain a minimum average face velocity of 0.5 m/s (100 ft/min) and has ducts and air flows under negative pressure. This type is not suitable for work with low levels of volatile toxic chemicals and volatile radionuclide.

Class II, Type B1

The cabinet is hard-ducted through a dedicated duct exhausted to the atmosphere after passage through a HEPA filter. It contains negative pressure air flow and 30% of the air is recirculated within the cabinet. It is able to maintain a minimum average face velocity of 0.5 m/s (100 ft/min). This type is suitable for work with low levels of volatile toxic chemicals and trace amounts of radionuclide.

Class II, Type B2

The cabinet is hard-ducted through a dedicated duct exhausted to the atmosphere to which 100% of the cabinet air travels to after passage through a HEPA filter. The cabinet contains negative pressure air flow and maintains a minimum average face velocity of 0.5 m/s (100 ft/min). It is suitable for work with volatile toxic chemicals and radioisotopes.

An alarm should be provided that is audible at the cabinet to indicate loss of exhaust flow from the building exhaust system. The cabinet internal fan should also be interlocked to shut down when the building exhaust system fan fails, to prevent pressurization of the cabinet.

Class III:

These are totally enclosed, gas-tight cabinets with HEPA filtered supply and exhaust air. The cabinet is kept under negative pressure of at least 120Pa and airflow is maintained by an exterior exhaust system. The work surface is accessed only through glove ports or sealed air locks. These cabinets provide a totally contained area to protect the worker, the work and the environment and are suitable for work at Containment Level 4. Removal of material from the cabinet must be through a dunk tank, double door autoclave or air-lock pass-through for decontamination. Interlock or protocols must be used for the autoclave and pass-through doors to prevent both doors from being open at the same time.

2.0 USE OF CLEAN AIR BENCHES

Notify the Biosafety Officer if a clean air bench is to be installed, moved or relocated from another institution. Benches acquired from another institution or from another laboratory on campus must be decontaminated before being moved to Western laboratories. Documentation will be required.

3.0 USE OF BIOLOGICAL SAFETY CABINETS

For procedures on starting-up, working-in and cleaning-up Biological Safety Cabinets see Appendix 1.

Notify the Biosafety Officer if a biological safety cabinet is to be ordered, installed, moved or relocated from another institution. The proposed location for the cabinet must be known. Cabinets acquired from another institution or from another laboratory on campus, must be decontaminated before being moved to Western laboratories. Documentation will be required.

New cabinets or cabinets which have been moved must be recertified after they are installed in their new location. All Class II biological safety cabinets must be recertified annually by an approved testing service (See *sections 4 & 5* below).

A University Biological Agents Registry Form must be completed for all of the agents that will be used in the cabinet. Physical Plant and Capital Planning Services must be consulted for installation requirements.

1. Purchase of biological safety cabinets:

The class of cabinet selected will depend on:

a) the Risk Level of the agent to be used

b) the degree of protection required for the work

c) whether volatile chemicals, radioisotopes, or nanoparticles will be used in the cabinet.

d) the type of procedures to be carried out in the cabinet

Other factors:

a) cost

b) the other uses of the room into which the cabinet will be installed

c) the amount of space available for installation of the cabinet

d) size of doorways which cabinet must pass through to the final point of use

e) adequate headspace for installation and for the maintenance of the filter boxes, exhaust ducts, alarm systems and fans. There should be, preferably, a 30 cm clearance from the walls and 40 cm from the ceiling

f) availability of service connections, such as electricity

g) if the biological safety cabinet exhausts outdoors, a Certificate of Approval – Air is as required under Ontario Regulation 346. Facilities Management can assist in this application process.

h) Heat generated by equipment in the room

i) Electrical requirements of the Biosafety cabinet.

2. Installation and location of cabinet

The installation of the cabinet in its final location is not always included in the purchase price. This has to be arranged separately and will incur an extra cost. Cabinets are very heavy and awkward and it is recommended that professional movers or certifiers be hired to do this. If the cabinet is damaged during the move the repairs may result in more expense.

The correct location of the cabinet in the room in which it is to be operated will improve the efficiency of cabinet operation. The cabinet should be situated away from doors and windows because air currents, which can disrupt the laminar flow characteristics inside the cabinet, may be created. Cabinets should also be installed away from high traffic areas in the lab and as far away from the exit(s) as possible. The cabinet must not be situated directly under or adjacent to the room air supply as the exhaust air from the cabinet will disrupt the air supply. If possible, continuous operation of the cabinets helps to control dust levels and other airborne particulates.

The cabinet should be installed with at least 30 cm of space on either side and behind the cabinet. A minimum of 40 cm should be available between the top exhaust filter and the ceiling to allow access for certification. (Public Health Agency of Canada, Laboratory Biosafety Guidelines, 3rd edition, 2004). For ducted cabinets, blowers on the exhaust system should be located at the end of the ductwork. An alarm should signal if failure of exhaust flow occurs. To prevent pressurization of the cabinet an interlock system should be installed to prevent the cabinet blower from operating when the exhaust flow is insufficient. Also note that these cabinets generate heat and situating two or more in a small room may require specialized ventilation and heat control.

2A. Relocation of cabinets

The Biosafety Officer must be informed before a biological safety cabinet is relocated. Generally the relocation will involve decontamination with formaldehyde before the cabinet is moved and recertification after relocation as required in NSF Standard 49 and/or CSA Standard Z316.3. If a cabinet is to be relocated to a laboratory at Western from a laboratory at another institution, documentation will be required by the Biosafety Officer to confirm decontamination at the previous location before the cabinet can be moved to a laboratory at Western. Recertification will then be required at Western after the cabinet is installed.

3. Maintenance and cleaning

The stainless steel surfaces inside the cabinet should be wiped with 70% ethanol or isopropanol on completion of work or after any spill. Wescodyne solution may also be used but it may stain the surfaces. A small amount of detergent may be added to the ethanol to assist in the removal of soil. Bleach is not recommended for this use as it may lead to corrosion of the steel surfaces unless they are thoroughly rinsed after the bleach has been used. Periodically the work surface should be lifted (with the cabinet running) and the spill area underneath should be cleaned and disinfected. Spilled medium in this area will allow microbial growth and can be a source of contamination.

3A. Changes of filters

Filters MUST be decontaminated with formaldehyde gas before they are changed. Once decontaminated, they can be disposed of as non-hazardous waste. This measure will protect workers and the environment from biohazards.

4. Certification

New biological safety cabinets **MUST** be certified by an approved certification company on installation, and before use. Please consult the Biosafety Officer to obtain the names of the approved certification companies. Documentation of this certification must be posted in the lab near or on the hood and kept on file by the Biosafety Officer. A label must be affixed to the cabinet stating the certification date, the date of the next required certification, the standard used to test the cabinet and the name of the certifier.

5. Recertification

All Class II biological safety cabinets **MUST** be recertified annually and after any repairs. (Laboratory Biosafety Guidelines: Health Canada, 9.3., 3rd ed. 2004. Annex F of NSF 49, CSA Standard Z316.3-95.). These cabinets **MUST** also be recertified if they are moved to a new location. The recertification of Class I hoods is optional, and will be done as requested to the Biosafety Officer.

Recertification can be preceded by cabinet decontamination by formaldehyde gas for 6 hours.

HEPA filtered infected animal enclosures **MUST** also be recertified annually.
The Biosafety Officer and Facilities Management organize the recertification of Class II cabinets in use on campus at Western annually. Recertification can be arranged at other times by the researcher provided that an approved company is used. Documentation of this certification must be posted in the lab near or on the hood and kept on file by the Biosafety Officer. A label must be affixed to the cabinet stating the certification date, the date of the next required certification, standard used to perform the tests and the name of the certifier.

Annual recertification is also recommended for biological safety cabinets which are being used to maintain sterile work conditions for agents at Level 1, but is not mandatory. Leaks in or damage to the HEPA filters may result in increased microbial contamination in the work area. Over time the HEPA filters become loaded with dust and contaminants and although this will not compromise the sterility of the air, it may result in low airflow problems that may compromise the sterility of the work area and lead to a shortening of the life of the electric motor.

Clean air enclosures for Specific Pathogen Free or immune compromised animals should also be recertified annually.

6. The use of gas burners inside the cabinet

Use of natural gas and propane is **NOT PERMITTED** inside Biological Safety Cabinets. Open flames in the BSC create turbulence, disrupt airflow patterns and can damage the HEPA filter. When suitable alternatives are not possible, touch-plate microburners that have a pilot light to provide a flame on demand may be used.

Alcohol burners may be used with caution inside the cabinet but the volume of alcohol used must be kept to a minimum. The alcohol should be in a metal (not glass) container. Alternative techniques for sterile work include using sterile Pasteur pipettes as an aspirator and using electronic bacterial loop incinerators. Sufficient equipment should be available so that a fresh supply of sterile equipment replaces the need to flame items.

Where gas lines have been permanently or temporarily connected to a cabinet, these must be disconnected.

7. The use of Ultraviolet light inside the cabinet

UV irradiation produced by germicidal lamps can be used to decontaminate the work surfaces inside a cabinet since UV irradiation is effective in killing many microorganisms. There are, however, several factors to take into consideration before using a germicidal lamp as an aid to maintaining sterile conditions inside the cabinet.

a) The 253.7 nm wavelength emitted by the germicidal lamp has limited penetrating power and will not penetrate soil or dust. It is thus only effective against microbes in the air in the cabinet or exposed on the work surface. As soon as the cabinet is turned on the clean air produced in the cabinet by the UV is replaced by sterile air which has passed through the HEPA filter and therefore the gain is brief.

b) The intensity or destructive power of the radiation decreases as the square of the distance from the lamp. Thus, exposure time is related to the distance from the lamp.

c) The intensity of the lamp also decreases with time. Lamp intensity must be checked with a meter if the sterilizing capacity of the UV is to be verified.

d) The intensity of the lamp is drastically affected by an accumulation of dust and dirt on the lamp. Therefore the lamp must be cleaned at least weekly.

The lamp must never be on while an operator is working at the cabinet. The UV light will reflect off the steel surfaces and some will reflect out into the room through the work opening, the lamp must be turned off if a worker is in the same room.

SOURCES OF INFORMATION:

Public Health Agency of Canada, Laboratory Biosafety Guidelines, 3rd edition, 2004.

Canadian Food Inspection Agency, Containment Standards for Veterinary Facilities, 1996.

Canadian Standards Association, Biological Containment Cabinets (Class I and II): Installation and Field Testing, 2002.

Appendix 1

Starting-up Procedures:

1. Turn off UV lights if in use and ensure that the sash is in the appropriate position.
2. Turn on fluorescent light and cabinet blower.
3. Check the air intake and exhaust grilles for obstructions.
4. If the cabinet is equipped with an alarm, test the alarm and turn it 'on'.
5. Confirm inward airflow by holding a tissue at the middle of the edge of the viewing panel and ensuring that it is drawn in.
6. Disinfect the interior surfaces with a suitable noncorrosive disinfectant.
7. Assemble all materials required for the procedure and load them into the cabinet without obstructing the air grilles. Segregate clean items from those that may be contaminated.
8. Wait 5 minutes to remove airborne contaminants from the work area.

Working-in Procedure:

1. Wear protective clothing and gloves as appropriate.
2. Perform procedures as far to the back of the work area as possible.
3. Avoid movement of materials or excessive movement of hands and arms through the front access opening during use. When you enter or exit the cabinet do so in a straight on fashion. After entering allow the cabinet to stabilize before resuming work.
4. Keep discarded, contaminated material to the back of the cabinet and do not discard materials in containers outside the cabinet.
5. Do not work with open flames inside the cabinet.
6. If there is a spill, decontaminate the surfaces of all objects within the cabinet and the working area while the cabinet is still in operation.

Cleaning-up Procedure:

1. Allow the cabinet to run for 5 minutes with no activity.
2. Close or cover open containers before removing them from the cabinet.
3. Surface disinfect objects in contact with contaminated material before removal from the cabinet.
4. Remove contaminated gloves and dispose of them as appropriate; wash hands.
5. Use clean gloves and ensure all materials are placed into biohazard bags within the cabinet.
6. Using a noncorrosive disinfectant, disinfect the interior surfaces of the cabinet.
7. Turn off the fluorescent light and cabinet blower when appropriate.
8. Turn on the UV light if appropriate.