Chapter 6

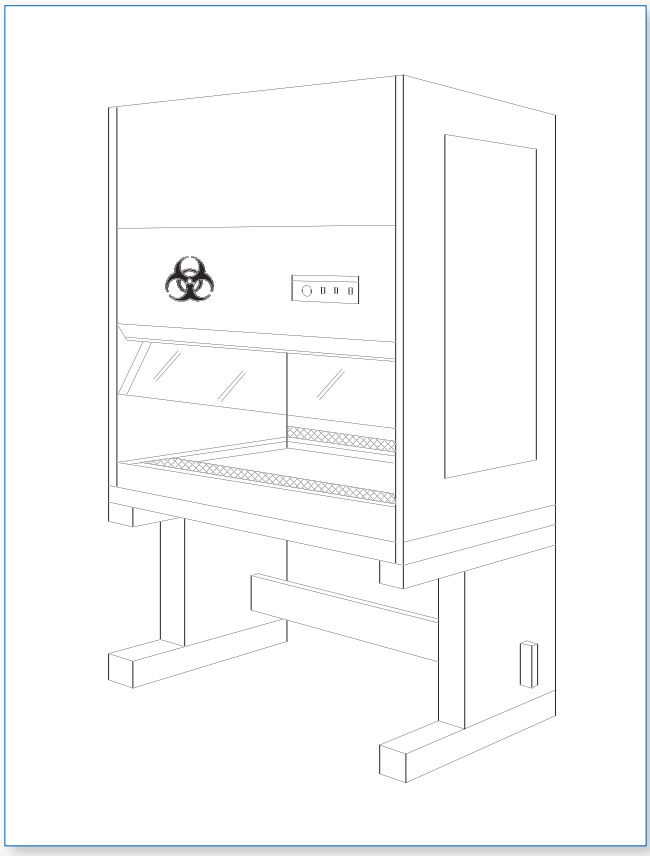
Biological Safety Cabinet

GMDN Code	15698	20652	20653	20654
ECRI Code	15-698	20-652	20-653	20-654
Denomination	Cabinets, biological safety	Cabinets, biological safety, class I	Cabinets, biological safety, class II	Cabinets, biological safety, class III

This equipment is designed for controlling aerosols and microparticles associated with managing potentially toxic or infectious biological material in laboratories in activities such as agitation, centrifugation, pipetting, and opening of pressurized containers. Safety cabinets have been designed to protect the user, the environment and the sample manipulated using appropriate ventilation conditions. They are also known as *laminar flow cabinets* and/or *biosafety cabinets*.

ILLUSTRATION OF A BIOLOGICAL SAFETY CABINET

Figure 19. Biological safety cabinet



PURPOSES OF THE EQUIPMENT

The biological safety cabinet is used for the following:

1. To protect the worker from risks associated with the management of potentially infectious biological material.
2. To protect the sample being analyzed from becoming contaminated.
3. To protect the environment.

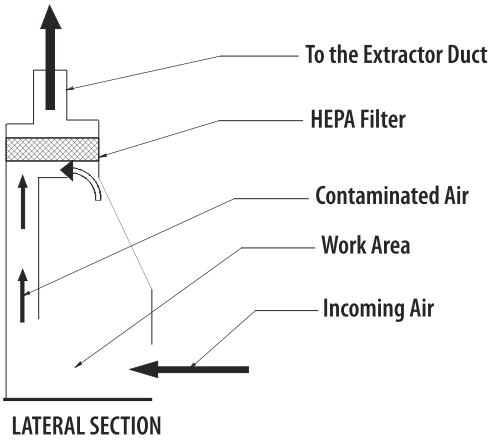
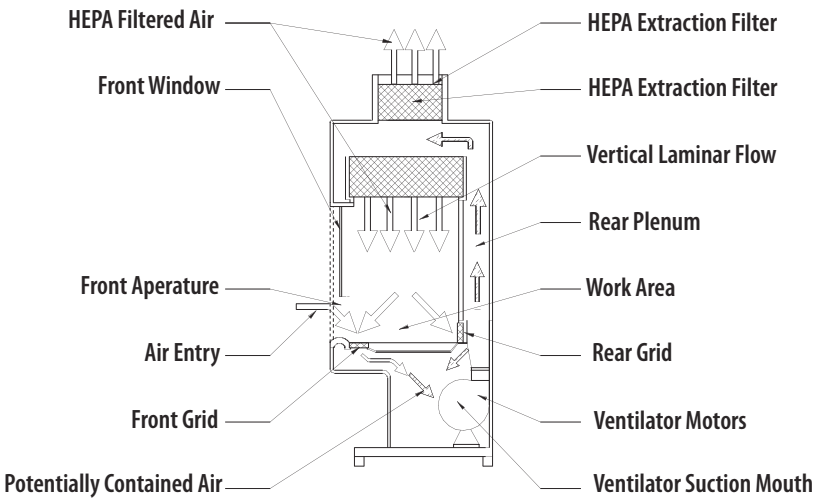
The cabinets are used for routine work related to pathogens (parasites, bacteria, virus, fungus), cell culture and under very precise conditions, the management of toxic agents.

OPERATION PRINCIPLES

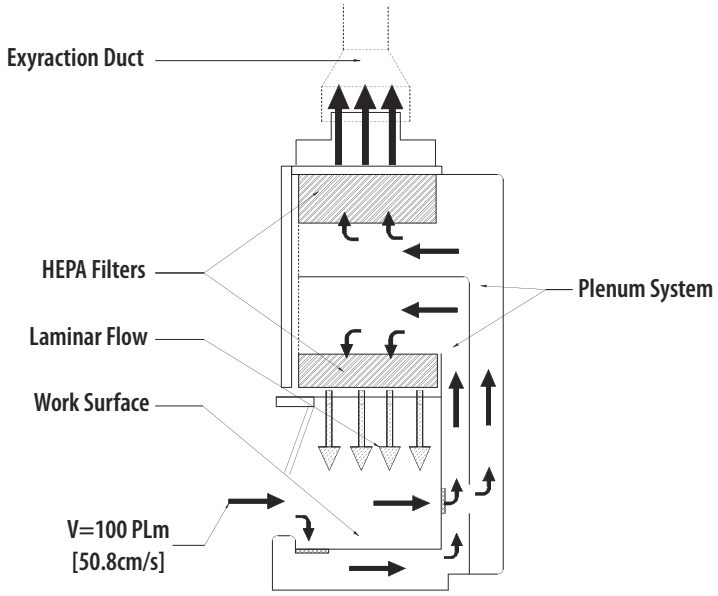
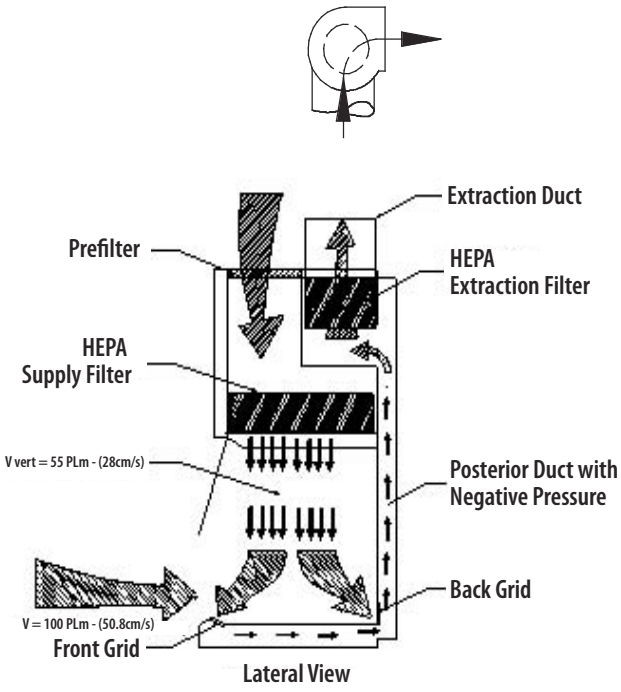
The biological safety cabinet is a chamber generally constructed of steel. It has a front glass window of adjustable height, a ventilation system with an electrical motor, a ventilator and a set of ducts which while functioning, generate a negative pressure condition inside the cabinet. This forces the air to flow from inside the cabinet through the front opening to generate a curtain of air protecting the operator. Internally, the air is conducted through a series of grids and ducts to be finally treated in HEPA[1] filters. Depending on the design of the cabinet, the air is recycled inside the laboratory or extracted and renewed in diverse proportions. The air flow, which in Class II cabinets moves from the filter towards the work surface, is laminar. A summary of the existing type of cabinets and their principal characteristics is presented next.

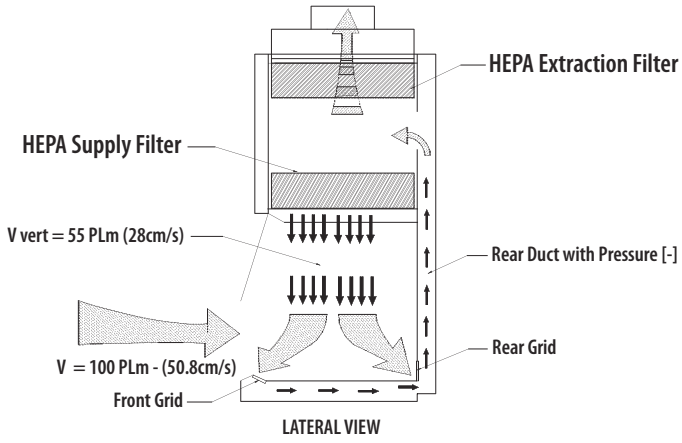
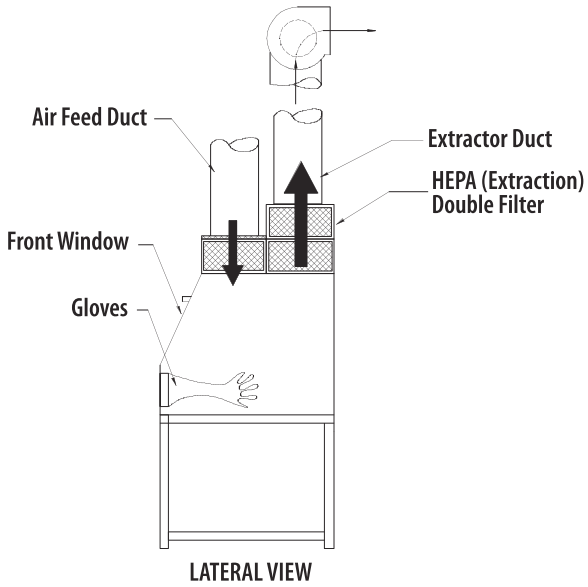
[1] HEPA: High Efficiency Particulate Air.

Summary of biological safety cabinet types

Type of cabinet, with illustration	Characteristics
CLASS I — TYPE A	
	1. Protection provided: to the operator and the environment. 2. Air velocity on entering the cabinet: 38 cm/s. 3. Suitable for working with bio-safety level[1] 1, 2 or 3 agents. 4. Filtration HEPA, located in extraction system which may or may not be connected to the exterior. 5. Disadvantage: Does not protect the sample manipulated in the cabinet.
CLASS II — TYPE A	
	1. Protection offered: To the operator, the product and environment. 2. Air velocity on entering the cabinet: 38 cm/s. 3. Suitable for working with agents with biosafety level 1, 2 or 3. 4. Filtration system: two HEPA filters, one located on the work surface; the second on the extraction system which may or may not be connected to the exterior. If they are connected to the exterior, it utilizes a bell type connection. 5. They recycle approximately 70 % of the air volume and renew 30 % of it.

[1] See biosafety classifications levels of agents in the following section *"Biological safety"*.

Type of cabinet, with illustration	Characteristics
CLASS II — TYPE B1	
	1. Protection provided: to the operator, the product and the environment. 2. Air velocity entering the cabinet: 50.8 cm/s. 3. Suitable for working with agents with biosafety level 1, 2 or 3. 4. Filtration system: Two HEPA filters. It extracts potentially contaminated air (70 %) through a duct and recycles inside of the cabinet, after filtering, air taken from the exterior, through the front grid (30 %). 5. All biologically contaminated ducts have a negative pressure. 6. Allows work with small quantities of toxic and radioactive chemicals.
CLASS II — TYPE B2	
	1. Protection provided: to the operator, the product and the environment. 2. Air velocity on entering the cabinet 50.8 cm/s. 3. Suitable for working with agents of biosafety level 1, 2 or 3. 4. Filtration system: Two HEPA filters. It is known as the total extraction cabinet. It does not have any type of recirculation. 5. All biologically contaminated ducts have a negative pressure. 6. It has an extraction duct which allows work with toxic and radioactive chemicals.

Type of cabinet, with illustration	Characteristics
CLASS II — TYPE B3 OR A/B3	
	1. Protection provided: to the operator, the product and the environment. 2. Air velocity on entering the cabinet: 50.8 cm/s. 3. Suitable for working with agents of biosafety level 1, 2 or 3. 4. Filtration system: Two HEPA filters. 5. All biologically contaminated ducts have a negative pressure. 6. It is known as a combined cabin. It can be connected by means of a duct. It is denominated as Type B3. If the duct is missing, it is a Type A. It recycles 70 % of the air volume inside the cabinet.
CLASS III	
	1. Protection provided: to the operator, the product and the environment. 2. Filtration system: two HEPA filters in series in the extraction; a HEPA filter in the admission. 3. Suitable for working with agents classified biosafety level 4. 4. Totally sealed cabinet. The intake and extraction elements are conducted through a double -door pass-through box. The manipulation of materials is done by using sealed gloves at the front of the cabinet.

BIOLOGICAL SAFETY[1]

Microorganisms have been classified into four categories based on factors such as pathogenicity, infectious doses, transmission modes, and host range, availability of preventive measures and effectiveness of treatment for the disease caused.

1. ***Risk level 1 group*** is composed of biological agents very unlikely to cause sickness in healthy humans or animals. (No individual and community risk).
2. ***Risk level 2 group*** is composed of pathogens which cause sickness in humans or animals but unlikely to be dangerous to laboratory workers, the community, domestic animals or the environment under normal circumstances. Those exposed in the laboratory rarely become seriously ill. There are preventive measures and effective treatment available and the risk of dissemination is limited. (Moderate individual risk, limited community risk).
3. ***Risk level 3 group*** is composed of pathogens which usually cause serious sicknesses to human beings and animals and produce a serious economic impact.

   However, infection by casual contact by one individual to another is not common. The sicknesses these produce are treatable by antimicrobial or anti-parasitic agents. (High individual risk, low community risk).
4. ***Risk level 4 group*** is composed of pathogens which usually produce very serious sicknesses in human beings or animals, frequently without treatments available. These agents are easily spread from one individual to another or from animal to human being or vice versa, directly or indirectly or by casual contact. (High individual risk, high community risk).

INSTALLATION REQUIREMENTS

The following are requirements for a cabinet to function adequately:

1. A laboratory area protected from air currents from windows or air-conditioning systems. The cabinet must also be located far from the laboratory circulation zones in order to avoid air currents that could affect the curtain of air inside the cabinet. It must also be verified that the cabinet is not installed alongside other types of cabinets such as chemical hoods.
2. An electrical connection equipped with the respective control and safety elements; the electrical outlet with a ground pole.
3. A levelled and firm table designed for supporting the weight of the cabinet and allowing the operator to work comfortably. There must be free space for placing the feet and its height must be adequate.
4. The floor on which it is located must be flat and levelled.
5. The free space around the cabinet recommended by the manufacturer must be respected. Likewise, the height of the room must be verified (the ceiling must be of recommended height so that it can function without hindrance).
6. Type B cabinets must have an extraction duct equipped with the following required control devices: regulating valves that allow the flow of air to be isolated and regulated.
7. Gas connections must be in the immediate vicinity of the cabinet in order to facilitate the connection to these service valves.
8. The cabinet must be certified annually to verify that it complies with the established requirements in the NSF 49 Regulation.

USE OF THE SAFETY CABINET

Correct utilization of the biological safety cabinet is achieved by complying with the following instructions:

1. Plan the work to be done in the biological safety cabinet in advance. Determine what procedure and equipment will be used. Coordinate the time of the cabinet's use with the other laboratory professionals in order to avoid interruption or undesired traffic while it is in use.
2. Turn on the cabinet. Turn off the UV lamp if lit. Turn on the fluorescent light lamp and the cabinet's ventilator. Verify that the grids in front and behind are free of obstructions. Prepare the work area. Allow the cabinet to function for at least 15 minutes.
3. Wash hands and forearms with germicidal soap. Put on the personal protective apparel: coat/overall with long sleeves and adjustable cuffs, protective eyeglasses and mask if the work requires it. Prepare the interior surfaces of the cabinet applying 70% ethanol or a suitable disinfectant. After this, let the air flow through.
4. Only load and install the materials and equipment required for the test or manipulation. Distinguish between the clean areas and dirty areas. Place the material in such a way that the clean materials do not mix or cross used or dirty materials or impede the circulation of the internal air through the front and back grids. Place a biosafety bag for disposing waste materials, a container with disinfectant for the pipettes and a container for storing sharps. Avoid locating very large objects near one another. Upon finalizing the placing of the materials, the flow of air must be allowed to sweep through the cabinet for approximately 3 to 5 minutes in order to eliminate any particle produced or freed during the loading of materials and equipment.
5. Initiate activities. Slowly introduce hands into the work area. Carry on the processes and tasks in a methodical and careful manner (from the clean areas to the

[1] *The Laboratory Biosafety Guidelines*, 3rd. Edition-Draft, Health Canada, 2001.

potentially contaminated areas). Keep the materials at least 10 cm behind the front grid. Try to perform the most risky and contaminating activities towards the back of the cabinet's work area. Avoid the use of open flames of lighters since this breaks the laminar flow pattern and may burn the filter. Avoid removing hands from the work area until all procedures are accomplished and the potentially dangerous materials are disposed of in the biosafety bag or in the pipette and sharp containers.

6. Clean the cabinet, allowing the air to flow freely for 3 to 5 minutes upon ending all the procedures.
7. Decontaminate the surfaces of all the materials and equipment in contact with the biologically contaminated material. Apply 70% ethanol or a suitable disinfectant and allow drying. Lift the equipment and materials and disinfect the area underneath. Cover the open containers before removal from the work area. Transfer materials to their appropriate place (incubator, autoclave, etc.).
8. Discard the gloves and remove personal protective elements. Dispose of these following the laboratory's established procedure. Wash hands with a lot of water and soap.
9. Turn off the ventilator, the fluorescent lamp, close the front opening and turn on the ultraviolet light.

Note: In case of a leak or spill inside the cabinet while in use, it must be kept in operation and all the objects or equipment involved must undergo a process of surface decontamination. This will prevent the cabinet from releasing contaminants.

Decontamination of the cabinet

The decontamination of the biological safety cabinet is an activity which must be done before any maintenance work involving opening its surfaces or internal components. Whenever any of the processes indicated next are needed, decontamination of the cabinet must be done previously.

1. Changing of filters.
2. Conducting tests requiring access to the interior surfaces or exposure of the cabinet.
3. Before conducting certification tests when the cabinet has been used with classified agents such as level 2 or 3 biological risk agents.
4. Before moving the cabinet to a different location.
5. After a spill of a material containing high risk agents.

The most suitable decontamination procedure must be defined by the professional responsible for industrial safety and professional risks. In annex G of the NSF 49 Standard, the procedure for decontaminating the cabinet using depolymerised paraformaldehyde is described. Only professionals who have received the relevant training must conduct such procedures.

ROUTINE MAINTENANCE

Warning: The maintenance of internal components must only be done by trained and qualified personnel. In order to carry out maintenance on the internal components, decontamination must be done previously. Personal protection must be worn to perform the routines.

General maintenance required for the biological safety cabinet is for the most part simple to perform. The routines and frequencies are shown below:

Frequency: Weekly

1. Decontaminate the work surface and the interior surfaces of the cabinet with 70% ethanol.
2. Clean the front glass door and the surface of the ultraviolet lamp, using a domestic cleaning solution.
3. Verify the precision of the manometer's reading, indicating any fall in pressure flowing through the HEPA filter. Register the date and the reading in the cabinet's log book.

Frequency: Monthly

1. Clean the exterior surfaces, especially the front and the upper part using a piece of damp cloth in order to remove the dust.
2. Disinfect the surface of the lower compartment with 70% Ethanol or a suitable disinfecting solution.
3. Verify the state of the service valves.
4. Do the tasks due on a weekly basis.

Frequency: Annually

1. Carry out the certification process according to established outlines in the NSF 49 regulation.
2. Check the intensity of the UV lamp[1] with a radiometer. Substitute it if necessary.
3. Test the state of the fluorescent lamp. Substitute it if necessary.
4. Perform the tasks due on a monthly basis.

Removal of the work surface

For the removal of the work surface the following procedure is required:

1. Decontaminate the surface before removing it.
2. Loosen and remove the attachment screws located on the front part of the work surface.
3. Loosen, but do not remove the attachment screws located on the back part.
4. Raise the front end and remove it, pulling it towards the front part of the cabinet.
5. Decontaminate the interior part of the work surface.
6. To assemble it, perform the activities described in steps 2, 3 and 4 in reverse order.

[1] UV lamps have irradiation capacity lasting approximately 7,500 hours. Some manufacturers suggest annual substitution.

Changing of the ultraviolet lamp

In order to change the ultraviolet lamp, the manufacturers' instructions must be followed. In general, the following procedures are done:

1. Turn on the cabinet and leave it working for 5 minutes.
2. Raise the front window to its maximum position.
3. Decontaminate the interior surfaces and the UV lamp.
4. Disconnect the electrical feed to the cabinet.
5. Disconnect the UV tube from its connectors turning it 90 degrees. Next, install a spare part with the same characteristics as the original. Some manufacturers have installed the lamps on a plate located in the front of the cabinet, which is necessary to unscrew and lift so that the assembly of the lamp is kept visible. Once this is done, the lamp can be substituted as indicated above.

Specialized maintenance

Eventually, the cabinet will require specialized maintenance. The following are some procedures to be done according to the manufacturer's technical service manuals by a specialized contractor.

1. Annual certification in accordance with Regulation NSF 49 outlines.
2. Motor change. Generally, it uses maintenance-free sealed rollers and function by induction through frequency control. This motor does not have brushes. (*)[1].
3. Replacing ventilators. (*)
4. Replacing the HEPA filter (*). The replacement frequency depends on the use of the cabinet and the system of environmental control installed in the laboratory. If there is a good control of dust, the filter could last many years.
5. Repair of the electronic control system: flow control alarms, position of the window, velocity controls.
6. Repair/cleaning of the flow regulator valves, bell type adjustment fittings.

Cabinet certification

The certification process of the biological safety cabinets is regulated by Standard NSF 49, which applies to all Class II cabinets. This defines materials, design criteria and construction, operation parameters and tests which allow the cabinet to be guaranteed as safe and suitable for the work performed. The following is a list of tests, in which standards mentioned are included. The standards must be consulted for details. The certification process comprises the following tests:

1. ***Air tightness test.*** This is done on the exterior surfaces. Determine if joints, seals, penetration and solderings are free from leaks.
2. ***HEPA filter leak tests.*** Determines the integrity of the supply and extraction of HEPA filters, their location and mounted frames.
3. ***Temperature increase test.*** Determines the maximum temperature increase in the cabinet when the ventilator and lights are operating.
4. ***Noise test.*** Determines the level of noise produced by the cabinet.
5. ***Luminous intensity test.*** Determines the luminous intensity on the cabinet's work surface.
6. ***Vibrations test.*** Determine how much vibration there is in the cabinet when it is functioning.
7. ***Protection test*** to personnel, to the product and cross contamination biological tests. The test determines if aerosols are contained in the cabinet, if external contaminants reach the work table area and if aerosols are reduced by the cabinet.
8. ***Stability test.*** Determines if the cabinet has structural stability. Analyzes the resistance to shaking, to distortion by means of applied force, to deflection of the work surface subjected to load and resistance to the tilting of the work surface due to heavy loading conditions.
9. ***Vertical flow velocity test.*** Determines the velocity of the air moved vertically towards the work surface.
10. ***Entry flow velocity test.*** Determines the velocity at which the flow enters the cabinet through the front opening and the cabinet's extraction volume.
11. ***Smoke test.*** Determines if the flow of air along the entire perimeter of the front opening advances towards the cabinet, and if the vertical flow moving towards the bottom does not show dead points or flow backs on the work surface.
12. ***Drainage escape test.*** Defines the contention capacity for spills below the work surface.
13. ***Motor/ventilator system functioning test.*** Determines if the system provides the necessary static pressure.
14. ***Electric system test.*** Determines if there are potential risks of electrical discharges. Measures the escaping currents, the polarity, the functioning of the ground defect protection system and the ground circuit resistance.

FUNCTIONAL EVALUATION (ALTERNATIVE)

In case there are biological safety cabinets in the laboratory, but no authorized certification services available, the personnel responsible for maintenance has the option of conducting annual revision procedures based on Standard NSF 49. Duly documented, it should identify with low levels of uncertainty if the cabinet is in good condition and its operation normal[2]. The following are outlines of how these activities must be done.

1. ***Installation evaluation.*** Verify that the cabinet installation conditions are in accordance with the recommendations from the manufacturer.

[1] (*) These require specialized decontamination beforehand.
[2] The functional evaluation is essentially based on the availability (institutional or zonal) of properly trained and experienced technicians and engineers.

2. ***Operational evaluation.*** Test to see if the cabinet is working in accordance with its manufacturing and design characteristics.
3. ***Performance evaluation.*** Verify the cabinet's capacity to provide an adequate work space in normal and critical working conditions.

In the following table are featured the parameters to be taken into account in the functional evaluation. These are generally included in inspection forms[1] designed for this purpose.

[1] Each institution designs its own formats for record keeping of technical maintenance.

Table of functional evaluation of biological safety cabinets

Parameters	Observation
Institutional identification of cabinets	**Brand, model, type, series, location, inventory code, date.**
ELECTRICAL	
Voltage	Voltage measurement. Requires a voltmeter.
Amperage	Amperage measurement. Requires a voltmeter or amperemeter clip.
Motor/ventilator	Verification of operation temperature. Verify noise level and vibration.
Illumination – Fluorescent	Confirmation that the lamp is functional.
– Ultraviolet	Confirmation of the operational hours of the lamps and their light intensity. Requires a radiometer.
Electrical outlet	Integrity revision, quality of the contact and available voltages.
Switches	Control of state and integrity.
Integrity cables and connectors	Visual verification.
Alarms	Testing of state and calibration.
PHYSICAL	
Internal/external finishes	Visual verification.
State of filters and pre-filters	Visual verification. There must be no leaks, neither in the filtering material nor in the seals.
Seals/gaskets	Visual verification. There must be no leaks.
Sliding window	Visual verification. Must be able to be moved smoothly and maintain the selected positions.
OPERATIONAL	
Flow velocity	Control of velocity according to the class and type of cabinet. Requires an anemometer (wind gauge).
Noise level	Requires audiometer.
Pressure differential in the HEPA filter.	Take a manometer reading of the cabinet.
PERFORMANCE	
Counting of particles	Method defined in the Federal Standard 209D, E. Requires DOP generator, photometer and particle counter.
CONDITIONS OF THE INSTALLATION AREA	
Temperature	Requires thermometer: approximately 20–22 °C.
Humidity	Requires hygrometer: approximately 45–55 %.
Cleanliness	Must be adequate.
Air currents	There must be no air currents to affect the working of the cabinet.

TROUBLESHOOTING TABLE[1]		
PROBLEM	**PROBABLE CAUSE**	**SOLUTION**
Neither the light nor the ventilation system in the cabinet works.	The cabinet is disconnected from the electrical outlet.	Verify that the cabinet is connected to an electrical outlet and that the cable is well connected to the cabinet's electrical box.
	There is no electrical feed in the connection.	Confirm that the electrical outlet is energized and that the circuit breaker is not deactivated (thermo magnetic protection). Restart switches.
The cabinet's ventilator is functioning but the light does not.	The lamp is defective.	Replace the lamp. Use one with the same characteristics of the original
	The lamp is badly connected.	Check the lamps connection. Adjust to the correct position.
	The thermo magnetic protection of the service breaker is activated.	Reconnect the circuit breaker.
	The lamp's wire is disconnected.	Check the lamp's wire.
	The lamp's ballast is defective.	Replace the ballast.
The ventilator is not blowing but the light is coming on.	The front window is closed.	Open the window until it reaches the work position.
	The ventilator's motor is defective.	Replace the motor ventilator set.
	The ventilator's motor is disconnected.	Check the motor's connections.
The manometer indicates an increase in the fall of pressure through the filter.	Retention of particles in the HEPA filter has increased.	Normal process during the useful life of the filter.
	There is blockage in the grids or return slots.	Verify that the grids are not obstructed by equipment or material.
	The extraction pipe is obstructed.	Test that there are no existing blockages or restrictions in the extraction pipe.
	There is a blockage or restriction under the work surface.	Verify that the pipe below the work surface is free of obstructions.
There is contamination in the samples manipulated in the cabinet.	Work procedures are inadequate.	Check that the cabinet is being used according to procedures and good practices.
	Restrictions in the return slots or blockage of the extraction duct.	Test the return and extraction system to see if they are free from obstructions.
	The cabinet's external factors affect its flow patterns on the inside and cause contamination.	Verify the installation of the cabinet and the procedures that are being carried out.
	The HEPA filter is defective.	Replace the HEPA filter and certify the cabinet.

[1] Purifier® Delta® Series, *Biological Safety Cabinets, User's Manual*, Kansas City, Labconco Corporation, Part Nº 36960-20, Rev. A ECO B296.

BASIC DEFINITIONS

Aerosol. A suspension of fine solid or liquid particles in the air. Their average diameter ranges between 10^{-4} and 10^{-7} cm.

Air supply. Air which enters the cabinet through the front or work opening and replaces the air extracted from the cabinet.

Biological Safety cabinet. Equipment with appropriate ventilation conditions protecting the user, the environment and the sample from aerosols and microparticles, associated with the management of potentially infectious biological material in laboratories as a result of activities such as agitation, centrifugation, use of pipettes and opening of pressurized containers.

Certification. Procedure establishing that the biological safety cabinet's functioning complies with criteria and minimum requirements to operate safely. Standard NSF 49 applies to the Class II cabins, Type A, B1, B2 and B3.

Decontamination. Removal or destruction of infectious agents; removal or neutralization of toxic agents.

HEPA filter. A filter with the ability to remove particles with average diameters of 0.3 *µm* with 99.97 % efficiency. These filters are constructed of Boron silicate micro fibres bonded together with a water resistant adhesive. The filtering material is folded inside of a frame with the aim of increasing the filtration area.

Laminar flow. Non-turbulent flow of a viscous fluid (e.g. air) in layers near a boundary. It occurs when Reynolds number [Re] is less than 3000.

NSF. An acronym of the *National Sanitation Foundation*, a non-profit organization dedicated to research, education and service, which seeks to resolve problems related to human beings, promote health and enrichment of the quality of life through conservation and improvement of the environment. NSF standards supply the basic criteria for promoting salubrious conditions and public health protection.

Toxic. A substance with a physiologically adverse effect on the biological systems.

Ultraviolet light (UV). This is electromagnetic radiation, the wavelength of which is between 200 and 390 nm. It is used in biological safety cabinets for its germicidal properties.

Work surface. A surface used when performing work, operation or activity inside the biological safety cabinet in this case.