Performance Verification and validation of Biological Safety Cabinets or Small Clean Areas

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Abstract

The information that is provided in this report is of the utmost significance for end-users, manufacturers, and certifiers of Biological Safety Cabinets (BSCs), and it is fundamentally vital for them to have a complete comprehension of the subject matter. The purpose of this study is to provide a comprehensive analysis of a substantial number of significant topics that are linked with BSCs. This section covers a variety of subjects, including their significance, the standard validation methods that are applied, the many types of BSCs, the calibration procedures that are utilized, and their special relevance in medical laboratories, particularly those that are participating in COVID-19 research and testing. There is a full discussion on the evaluation of the performance of BSCs that is presented in the manuscript. Procedures for cleaning and disinfection, roles and responsibilities, testing for leaks in HEPA filters, inflow and downflow velocities, airflow patterns, considerations regarding lighting and noise, safety guidelines, and operational and performance qualification checklists are some of the important aspects that are addressed in this document.

Keywords: ***Biological safety cabinets; Performance; Verification; Validation; Calibration; COVID 19; NSF 49; HEPA filter.***

1 Introduction

Labs and indoors should have safety features like emergency showers, eyewash stations, and fire extinguishers. Familiarize yourself with lab layout and exits, regularly check safety features, and ensure bench work, as some operations require cabinets to mitigate exposure to particulate matter, pollutants and airborne viruses [1-3]. Biological samples, including blood, swabs, and tissue, can contain pathogenic viruses, bacteria, parasites, prions, or fungi. Labs and cabinets are classified based on microbe type and risk of contamination. Repetitive training, hands-on learning, and detailed protocols are essential for safety. Personal protective measures and full follow-up are also crucial [4].

Healthcare professionals are responsible for removing biohazardous materials and correcting chemical and physical hazards to prevent injuries. OSHA regulates workplace equipment,

machinery, first aid, and materials, ensuring a safe working environment free from dangerous machinery, toxic chemicals, noise, extreme temperatures, and unsanitary conditions [5]. The American Industrial Hygiene Association (AIHA) and the American National Standards Institute (ANSI) are developing a national standard titled "Testing and performance verification methodologies for ventilation systems for Biological Safety Level 3(BSL-3) and Animal Biological Safety Level 3 (ABSL-3) laboratories" known as ANSI/AIHA Z9.14. The ANSI Z9.14 standard will focus on performance verification of engineering controls related specifically to ventilation system features of BSL-3/ABSL-3 facilities [6].

Muta [7] studied the safety performance of biological safety cabinets (BSC) used in laboratory experiments as prescribed in test method (EN 12,649, National Sanitation Foundation and American National Standards Institute NSF/ANSI 49) based on the numerical reproducibility visualization, simulation model, the impact of human body and inserted arm on the BSC performance, and leak testing as well as the possibility of replacing the tracer gas method with bacterial testing. Many authors also worked on the performance of the filtration tools towards different aerosols [8], Energy consumption performance of PTFE HEPA filter media during dust loading through compositing them with the efficient filter medium [9], Effects of dust loading on the long-term performance of portable HEPA air cleaner to woodsmoke – A laboratory investigation, Indoor Environments [10], fabrication of high performance filters based on multilayer cellulose filters as replacement of HEPA filters [11]. Biosafety and biosecurity are crucial concepts in laboratory practice, addressing issues like bioterrorism, biocrimes, and biological warfare, as well as challenges related to aerosolization and air dispersal [12].

1.1.Importance of biological safety cabinets

When handling biological materials, a biological safety cabinet (BSC) is essential for maintaining a controlled and secure environment. BSCs are crucial when working with potentially hazardous biological agents, as they use HEPA filters to create a barrier between laboratory personnel and these dangerous contaminants. For research involving sensitive materials or sample preservation, a sterile environment is vital, and Class II BSCs provide this level of protection. They ensure the safe containment and handling of potentially lethal biological agents, preventing accidental exposure. These cabinets are also necessary for laboratories to comply with safety and regulatory standards set by organizations such as the Centres for Disease Control and Prevention (CDC) and world health organization (WHO). By promoting safe laboratory practices and minimizing exposure risks, BSCs help reduce the likelihood of laboratory-acquired infections (CDC 2020) [13]

1.2. Standard methods used for instrument validation

Instrument validation is a crucial step in ensuring measurement accuracy, reliability, and quality, assessing the suitability of an instrument or measurement method for its intended purpose. The precision, accuracy, linearity, sensitivity, selectivity, robustness, repeatability, and stability of the equipment are all essential factors that must be evaluated and validated to ensure reliable performance. Calibration is necessary to confirm that the equipment is operating in compliance with established standards. Linearity testing ensures that the observed values are directly proportional to the concentration or quantity being measured. Sensitivity tests

determine the smallest detectable change or concentration that the device can measure with adequate precision. The selectivity of the instrument is assessed by measuring the target analyte in the presence of potential interferences. Robustness testing evaluates the instrument's performance across a wide range of conditions. Repeatability and reproducibility tests assess the instrument's consistency and accuracy over time. Stability testing ensures long-term precision and reliability by monitoring the instrument's performance over extended periods [14].

1.3. validation of biological safety cabinets

The validation of biological safety cabinets is a critical process to ensure their proper functioning and compliance with safety standards. It involves several steps: Pre-Installation Qualification (PIQ), Installation Qualification (IQ), Operational Qualification (OQ), and Performance Qualification (PQ). The process ensures the cabinet meets required specifications, is suitable for its intended use, and performs under normal operating conditions. The final step is Performance Qualification (PQ), where the cabinet is tested with simulated or actual work processes to maintain containment and protect the operator, samples, and environment [9].

2. Types of Biological Safety Cabinets

Biological safety cabinets (BSCs) are categorized into three classes—Class I, Class II, and Class III—based on their design and airflow configurations. Class I BSCs provide protection for personnel and the environment by using negative pressure to prevent airborne contaminants from escaping. Class II BSCs offer protection for both personnel and the environment, and are further subdivided into four types: Class II Type A1, Class II Type A2, Class II Type B1, and Class II Type B2. These cabinets maintain a sterile environment through inward and downward airflow. Class III BSCs, often referred to as gloveboxes, provide the highest level of protection, offering complete containment for both the operator and the environment, making them ideal for handling highly hazardous biological agents [15].Table 1 illustrates the classification of biological sterilization cabins, the proportion of air entering (feet per minute), their composition, the kind of air movement inside, and the forbidden and permissible poisonous and volatile compounds inside. This information is required to achieve the best feasible protection with the highest performance efficiency [15].

3. Calibration of biological safety cabinets

The importance of regular calibration for biological safety cabinets is underscored by the need to maintain their optimal performance and functionality. Calibration ensures that the cabinet continues to operate in accordance with established safety standards and industry guidelines. Key parameters such as air velocity, HEPA filter efficiency, pressure differential, alarm systems, and electrical systems should be checked and, if necessary, adjusted to ensure that the cabinet maintains its intended operational capacity. By conducting thorough calibration, the system will ensure that the required pressure differential is maintained, alarms are properly functioning, and the HEPA filter continues to protect both the operator and the environment from potential contamination. It is crucial that these calibration processes are carried out by

professionals with expertise in international standards, ensuring compliance and safeguarding health and safety at all times [16].

Table 1: *Definitions of Class II biological safety cabinet types*

Class II BSC Type	Minimum intake velocity (fpm)	Construction	Airflow pattern	Volatile toxic chemicals (gases or vapours) permitted
A1	75	May have biologically contaminated ducts and a common, plenums under positive pressure to the room exhausted	Downflow and inflow air mix in plenum approximately 70 percent recirculated as downflow, 30 percent	No
A2	100	Biologically Contaminated ducts and plenums must be under negative pressure in the room, or surrounded by negative-pressure ducts and plenums	Downflow and inflow air mix in a common plenum, approximately 70 percent recirculated as downflow, 30 percent exhausted	No—when exhaust air is vented back into the room Yes—minute quantities allowed when canopy connected and exhausted to the outdoors
B1	100	Biologically Contaminated ducts and plenums must be under negative pressure in the room, or surrounded by negative-pressure ducts and plenums	Approximately 60 percent of downflow air is exhausted through a dedicated duct; the remainder of downflow air (approximately 40 percent) mixes with intake air and is recirculated	Yes—minute quantities allowed
B2	100	Contaminated ducts and plenums must be under negative pressure in the room, or surrounded by negative-pressure ducts and plenums	Downflow air drawn from the laboratory; inflow and downflow air exhausted with no recirculation in the cabinet or return to the laboratory (100 percent exhausted)	Yes—as an adjunct to microbiological work

4. Biological safety cabinets and COVID

Biological safety cabinets (BSCs) play a critical role in preventing the spread of infectious diseases like COVID-19 and maintaining laboratory cleanliness. By utilizing ULPA/HEPA filters, BSCs create both incoming and exhaust airflow that protects laboratory personnel from contamination and prevents the transmission of airborne pathogens. The World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) recommend the use of Class II BSCs for handling COVID-19 samples, particularly in procedures that generate aerosols. Proper decontamination and sanitization of BSCs using appropriate chemical agents are essential for ensuring biosafety. Regular monitoring of airflow rates and

sash heights is crucial to maintain optimal containment conditions and airflow patterns within the cabinet [17].

5. Methodology of performance verification:

5.1.Cleaning and disinfection:

- Check the electrical requirements on the nameplate before use.
- Turn the UV lamp at least 30 minutes before use. Be sure to turn it off during the operation.
- For worktables and sides, use sterile or non-use cloths, which do not shed particles or fibers.
- To disinfect use cloths damped with a disinfecting solution that does not damage or affect the paintwork, stainless steel, or glass. A previous cleaning to disinfecting can also be done with water and soap.

5.2. Responsibilities:

End-User Employer – It is the employer's responsibility to provide sufficient resources and training to enable the validation. Prior to using the BSC, the organization must additionally guarantee that the validation procedure has been completed in its entirety. Documents must to be kept in a location that is both secure and accessible. The organization's quality system should include information on the BSC, preventative maintenance, and certification schedule locations.

Cabinet Certifier – Before they can be used, all BSCs must be approved. Calibrated instruments must be used by a trained certifying technician to do this process. The cabinet needs to be approved when it is first put together, once a year, and every time it is moved to a new spot. The most important thing that this system needs is certification [18]

Manufacturer/supplier – It is recommended that the manufacturer obtain ISO-9001 certification and subject their products to thorough testing in accordance with NSF 49 [18] standards before to distribution. Additionally, their personnel should offer support in the areas of purchasing, delivery, and installation.

The cabinet should be certified according to NSF 49 or EN 12469 standards, with preference given to NSF 49. The certifier should be NSF certified for field certification and provide training details. The final report should follow the standard, and all necessary tools and instruments should be calibrated and certified.

5.3.HEPA filter leak test (LT) Purpose:

This test is conducted to check the HEPA impulsion/downflow and exhaust filters, the filter housing, and the mounting frames for possible leakage.

5.3.1 Tools:

a) Aerosol generator (cool or hot) (DEHS, DOB, or PAO are recommended)
b) Aerosol Photometer with extended logarithmic scale
c) Accuracy: particle counter for individual particles with a dilution range capable of detecting penetration of 0.01% or less of particles exceeding 0.3 µm (HEPA filter of purity class 100).

5.3.2. Test conditions

The sample chamber of the safety cabinet must be empty windows and doors of the operating room must be closed (no draft).

5.3.3. Methodology:

a) Turn on the photometer and adjust it for the measurement to the manufacturer's instruction
b) Introduce aerosol to the airflow before the filter (entrance of the BSC)
c) With the probe nozzle approximately 2.5 cm from the surface, scan the downstream side of the HEPA filter, including the perimeter of the filter passing the photometer probe over the entire surface.
d) Scan the entire periphery of the filter and the junction between the filter and the frame at a speed not exceeding 5cm/s.

5.3.4. Recommended criteria:

The leak (impulsion/downflow or exhaustion) should be not more than 0.01% of the specific before the filter (escaping volume of the aerosol). This criterion may be adjusted in accordance with the manufacturer's specifications, the risk, and the workload.

5.3.5. Troubleshooting:

Replace the downflow filter/ replace the exhaust filter

5.4. Inflow velocity test

5.4.1. Purpose:

This test is conducted to determine the nominal value of the inflow velocity (air entered into the BSC)

5.4.2. Value to be tested:

inflow velocity V1 (m/s or ft/min)

5.4.3. Test method:

The inflow velocity (V1) is calculated using the values of the exhaust volume and the sample chamber inflow surface.
If the BSC is connected to technical ventilation, inflow velocity can also be determined at the working aperture.

5.4.4. Test Conditions:

For all test methods, the sample chamber of the safety cabinet must be empty, and the windows and doors of the operating room must be closed (no draft).

5.4.5. Testing equipment:

The suitable anemometer measures the velocity (m/s or ft/min). Accuracy of ± 0.01 m/s or maximal deviation of 3% from the indicated airflow velocity.

5.4.6. Calibration of test equipment:

The equipment must be calibrated in an accredited laboratory or national metrological institute by the requirement of ISO/IEC 17025:2017.

5.4.7. Methodology:

The inflow velocity is measured at various points in the working aperture, with the anemometer installed directly to the inside of the front window, 3.8 cm from the lower edge and 10 cm from the edges. Troubleshooting

a) Raise exhaust bower speed
b) Check exhaust blower control voltage
c) Replace exhaust filter
d) Check the installed exhaust system for correct function.

5.5. Downflow velocity test

This test is conducted to check the nominal value of the downflow velocity (displacement airflow) in the work area of the safety cabinet.

5.5.1. Value to be tested:

Downflow velocity (m/s or ft/min)

5.5.2. Reference:

ISO/IEC 17025, NSF 49, and EU EN 12469

5.5.3. Test Conditions:

For all test methods, the sample chamber of the safety cabinet must be empty, and the windows and doors of the operating room must be closed (no draft).

5.5.4. Testing equipment:

a) Suitable anemometer measures the velocity (m/s or ft/min)
b) The anemometer measuring head must be secured tightly at the measuring point to allow accurate measurements.
c) The distance between the measuring points must be correct; the grid must not be distorted.

5.5.5. Accuracy of testing equipment:

Accuracy of ± 0.01 m/s or maximal deviation of 3% from the indicated airflow velocity.

5.5.6. Calibration of test equipment :

- The equipment must be calibrated in an accredited laboratory or national metrological institute by the requirement of ISO/IEC 17025.

5.5.7. Methodology:

a) Move the door to the working position.
b) Install the measuring head at 10 cm 1bove the door edge
c) For BSC of 1200 mm, leave 1/8x120 from all sides. Draw the grid and define the measuring point as the following grid.
d) Calculate the average value for the downflow velocity

5.5.8. Recommended criteria

a) The average value must be within ±10% of the nominal value (0.36 m/s).
b) The difference between measured values at the individual measuring points must not exceed 20%.

5.5.9. Troubleshooting:

a) Raise downflow blower speed
b) Check exhaust blower control
c) Replace downflow and or exhaust filters
d) Check the installed exhaust system for correct function.

5.6. Airflow pattern test

The purpose of this test is to see how the airflows in the sample box behave. Make sure the vertical passage is right and that the displacement airflow goes along the whole work area. Also, check to see if air is escaping through the front window and the housing's joints or seals.

5.6.1 References:

ISO/IEC 17025, NSF 49, and EU EN 12469

5.6.2. Test Conditions:

- For all test methods, the sample chamber of the safety cabinet must be empty, and the windows and doors of the operating room must be closed (no draft).
- Inflow and downflow should be adjusted to the nominal accepted values.

5.6.3. Testing equipment:

- Smoke tube or any safe smoke producer.

5.6.4. Methodology:

- Move the door to the working position.
- Using a smoke tube, scan the work area along its centreline from one end to the other. Hold the smoke tube so that its tip is at a distance of approximately =15cm above the work surface.

5.6.5. Accepting criteria:

The smoke shows a smooth downward flow with no dead spots or reflux. No smoke escapes through the work aperture. This criterion may be adjusted in accordance with the manufacturer's specifications, the risk, and the workload.

5.7. Light Test

This test is to check whether the level and uniformity of the lighting system is the correct one to guarantee a safe working area inside the cabinet.

5.7.1. Reference:

ISO/IEC 17025:2017 and/or NSF 49

5.7.2. Test Conditions:

- For all test methods, the sample chamber of the safety cabinet must be empty, and the windows and doors of the operating room must be closed (no draft).

5.7.3. Testing equipment:

- Light meter

5.7.4. Calibration of test equipment

- The equipment must be calibrated in an accredited laboratory or national metrological institute by the requirement of ISO/IEC 17025.

5.7.5. Methodology:

d) An imaginary division of the working surface will be done in equal parts (not more than 8 and less than 6)
e) The illuminating level will be measured in each of these parts, placing the light meter over the working area.
f) The average of the measures carried out will be calculated as well as the safety cabinet illumination level.

5.7.6. Recommended criteria:

The illumination level average is not less than 750 Lux
Any Individual reading cannot be less than 70% of the average illumination level. This criterion may be adjusted in accordance with the manufacturer's specifications, the risk, and the workload.

5.8. Noise Test

This test is to verify the noise level of the cabinet is within the allowed limits.

5.8.1. Reference:
ISO/IEC 17025:2005 NSF 49
5.8.2. Test Conditions:

- For all test methods, the sample chamber of the safety cabinet must be empty, and the windows and doors of the operating room must be closed (no draft).

5.8.3. Testing equipment:

- sound meter (dBA)

5.8.4. Calibration of test equipment

- The equipment must be calibrated in an accredited laboratory or national metrological institute by the requirement of ISO/IEC 17025:2005.

5.8.5. Methodology*:*

- The sound meter is placed 1m from the front of the cabinet and 1.5 m high. With the cabinet running, 2,3 readings will be taken for different durations (10 and 30 sec).

5.8.6. Accepting criteria

Less than 60 dBA. This criterion may be adjusted in accordance with the manufacturer's specifications, the risk, and the workload. This criterion may be adjusted in accordance with the manufacturer's specifications, the risk, and the workload

6. Safety Instruction and General recommendation

When working to verify the effectiveness of biological safety cabinets, all safety and security precautions must be followed to ensure that their users are not exposed to any potential hazards. These may be summarized as follows:

a) The bench working area should not be used as a laboratory equipment store, as this could lead to unnecessary dust accumulation, posing a risk to sterility.
b) The working area must be clean and sterile, but the bench surroundings may be contaminated, making it crucial to detect and prevent such issues.
c) All the material necessary for the work must be particle-free and previously cleaned.
d) Do not put inside the working area materials such as paper, wood, cardboard, pencils, erasers, etc. since they give off a lot of particles.
e) It is recommended to wash arms, hands, and fingernails with a germicidal soap before and after working. The users must avoid touching their mouths and eyes.
f) It is recommended to use long-sleeved overalls with tight cuffs, and, with special work, protecting gloves. (Alternatively, use mittens). Both the overalls and the mittens must be made of a material that gives off as few fibers and particles as possible.
g) Start the bench 10 minutes before beginning your work with it. Thus, the working area and the material introduced are scavenged and the particles are removed.
h) Whenever pipettes are used, they must be the mechanical suction type. Never use them with mouth suction since you are very likely to inhale the aerosol originated by the suction.
i) Whenever platinum handles must be used, we suggest the use of electrical incinerators and even disposable ones, if possible.
j) If the work to be done requires the use of a Bunsen burner or similar gas flame, we recommend those with a constant pressure button. Remember that an excessively long flame might burn the absolute filters and bend the light diffuser.
k) The shadows and whirls caused by the objects, equipment, and material surrounding the working area must be examined before starting the operation, to determine their possible effects. Remember that the airflow does not go back to its laminar condition possible effects. Remember that the airflow does not go back to its laminar condition before a distance of 2.5 times the diameter of the object causing the obstruction.
l) When vials and tubes are used, we recommend the use of screw caps instead of cotton ones, the latter giving off a lot of particles.
m) After the operation, disposable products, culture medium, samples, tubes, and vials will be removed from the bench using impermeable and sterilizable bags, if necessary.
n) During bench cleaning, it is crucial to avoid damaging HEPA filters by hitting them, pouring liquids, or splashing.
o) Adjusting the seat position when using the biological safety cabinet ensures safety and prevents potential physical damage, such as sight, cervical, and back injuries.
p) Avoid locating the biological safety cabinet in a current that can produce turbulences in the laminar flow.

7. Operational and Performance Qualifications

When verifying the efficiency of the performance of biological sterilization cabins and ensuring their continuous operation and efficient work, the points mentioned in Table 2 must be taken into account, as the room must be tested and a clear label must be placed indicating that it has been tested (according to the American or European system or the instructions of the mandatory drug and health authorities in each country), and its users must determine the periodic Workers must also be instructed on it and how to use the most appropriate procedures for operating it and guaranteeing its cleanliness and sterilization, as outlined in the manufacturer's operating instructions manuals.

Table 2: *Operational Qualification*

Step	Description	Specification or Acceptance Criteria	Result	
			yes	no
1	**Certification**	Before use, has a qualified certifier tested the cabinet before the NSF 49 or European standards the cabinet was listed? Has the certifier labelled the BSC with the successful certification date?		
		Certification should be done annually. Has the next required certification been added to your quality system's preventive maintenance or certification schedule?		
2	**Training**			
	User Training	Have all users been properly trained on the safety, theory of operation, and limitations of the BSC?		
		Do all users understand techniques for: • Cleaning & disinfection of the cabinet's interior • Loading supplies and equipment, • Avoiding cross-contamination, • Not disturbing the laminar flow, Spill control and clean up, • Shutting down the cabinet?		
		Are users aware of ergonomic factors that can cause unnecessary fatigue or personal discomfort? (Refer to the Safety and Comfort section of the User's Manual.)		
3	**Cleaning**			
	Exterior Cleaning	Has the exterior of the cabinet been cleaned of dust that accumulated throughout the installation		
	Interior Cleaning	Have the BSC's interior surfaces been cleaned and disinfected appropriately for the work that is about to be performed in it?		
		Has the towel catch screen located in the rear under the work surface been checked for any foreign debris requiring removal?		
4	**Others**	Defined by the end users		

All these steps should be checked with a yes (100% for acceptance)

Table 3 provides the performance requirements for biological sterilization chambers. Some examples include periodic preventive maintenance intervals, performance tests by calibration or performance evaluation bodies, the life and operation periods of ultraviolet lamps and other

lamps used in lighting, the life periods of filters, and some other precautions used in pharmaceutical and medical laboratories.

Table 3: *Performance Qualification*

Step	Description	Suggested Criteria
1	**Periodic Certification**	
	Cabinet Performance	• Certification should be done at a minimum annually. An experienced certifier can verify the cabinet's performance. • Is the BSC's current certification within the acceptable timeframe set by your organization? • Has there been a procedure established if a cabinet is found to have exceeded its certification due date?
		Is the next required certification noted in your quality system's preventive maintenance or certification schedule?
2	**Maintenance**	
	U. V. Lamp	If equipped, the U.V. Lamp should be replaced at least annually to remain effective.
	Towel Catch	The towel catch screen located in the rear under the work surface should be checked for any foreign debris when the cabinet is cleaned.
	Fluorescent Lamp or D65	Regular maintenance should ensure that the Fluorescent Lamp is operating properly.
3	**Others**	Defined by the end users

NOTE: This Performance Qualification section is only a recommendation of some basic items to consider for your protocol. Your protocol should include tests and inspections that are pertinent to the applications performed within the equipment.

8. Conclusions

Biological safety cabinets (BSCs) are crucial in laboratory settings for protecting personnel, preventing contamination, maintaining sample integrity, and complying with regulations. They are a part of biosafety measures and promote a safe working environment for research, healthcare, and other activities involving biological materials. Validation of BSCs involves pre-installation, installation, operational, and performance qualifications. Class I, Class II, and Class III cabinets offer varying levels of protection based on airflow patterns. BSCs are essential in handling COVID-19 and ensuring safety during the pandemic. This report is A guide has been developed to clarify the importance and complete explanation that must be followed when utilizing biological sterilization cabins in order to serve as a reference and guidance for all workers and users, as well as to ensure their continued operation with efficiency and safety. The most significant safety and security criteria have also been provided to ensure the maximum level of protection for the operations carried out within them, as the parameters mentioned in this report are extremely difficult to obtain from a single scientific source. The information in this report was gathered from a variety of sources, including manufacturer instructions, instructions from international medical authorities, standard standards, and air quality specifications for evaluating biological sterilizing cabins and clean rooms.

9. Declarations

Study Limitations

Competing Interests

The authors have no financial or proprietary interests in any material discussed in this article.

Ethical Approval

Not Required

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