Major international standards for Biological Safety Cabinets (BSCs) - Proper Selection, installation and safe use of certified BSCs. Main differences and essential elements for regular servicing, certification and preventive maintenance of Class II BSCs -

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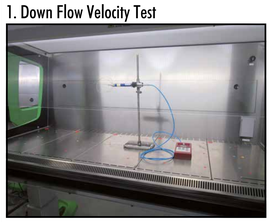
Introduction

Biosafety cabinets (BSCs), also referred to as microbiological safety cabinets or biological safety cabinets, provide a ventilated but enclosed workspace (thus a primary containment measure) for individuals working with biohazardous materials. Based on their design and use, biosafety cabinets are grouped into three classes upon their containment capabilities. The EN12469-2000 is relevant to Class I, II and III BSCs whereas the AS2252.2-2009 and NSF49-2018 only apply to Class II BSCs designed for personnel, environment and product protection (cross contamination). The most significant difference in this area is that the NSF49 defines various Class II cabinet subtypes (i.e. Class II Type A1 and A2; Class II Type B1, B2 and B3; Class II Type C1). This technical paper attempts to highlight and discuss the differences between the main international standards focusing the attention on the physical tests to be conducted on-site to ensure the effectiveness of the BSC. The AS2252.2 Standard states that "*periodic critical performance tests of cabinet shall be conducted at least annually and on any change to cabinet position and function. Carefully sliding a cabinet away from the wall does not constitute a change to cabinet position*". This concept is valid for all the standards (so for EN and NSF too) and even if some tests are performed at the factory before being shipped (they include electrical leakage, light intensity, vibration, noise level and UV light check), the minimum tests to be conducted on-site are basically identical: they are performance-based standards. In other words, the emphasis of the standards is not on construction requirements but on the performance tests criteria, with some exceptions (based on the standard we are referring to), such as: microbiological or chemical tests for operator, product and cross contamination protection, airflow velocity readings, HEPA filter integrity test, cabinet integrity test, airflow smoke patterns test etc.

Common Periodic Critical Performance Tests and acceptance criteria

1. Down Flow Velocity Test



The test measures the velocity of air moving through the cabinet working chamber (volumetric airflow rate) - fig.1.
The purpose is to protect the product from any cross contamination or contaminants generated within the working chamber or coming from the environment. Here below the main parameters to be verified and tested.

Fig.1 - Down Flow velocity test by anemometer with hot wire sensor.

Parameters	AS2252.2 (Class II)	NSF/ANSI 49-2018 (Class II - A2)	EN12469 (Class II)
Down Flow Velocity	0,40 to 0,45 m/s	Based on the biological testing	0,25 to 0,50 m/s
Acceptance criteria (difference of individual points from the mean value)	± 20% of the average	± 20% (or 0.081 m/s) of the average	± 20% of the average
Anemometer Positioning	Measurement plane 150mm downstream of the HEPA down flow filter	100 mm (4") above the top edge of the front aperture	50 mm to 100 mm (2" to 4") above the top edge of the front aperture

2. Inflow (Face) Velocity Test And Air Barrier Containment

A test to determine the calculated or directly measured velocity through the work access opening. On this matter, the EN12469 and AS2252.2 have one distinctive difference for evaluating the retention efficiency at the front aperture of the cabinet, since they recognize an alternative test method (the Potassium Iodide test -KI-Discus- Fig.2- in addition to the conventional microbiological challenge test (recognized by EN and NSF standard) or the aerosol test of the AS 1807.22. The KI-Discus system has been designed to enable aperture/operator protection factors (and where appropriate, product protection and cross contamination factors) to be measured, in the field, for Class I and Class II open-fronted microbiological safety cabinet in accordance with the EN12469 and AS 1807.26. The associated risk is the escape of biological agents into the laboratory and/or the entry of contaminants into the work area.The AS2252.2 does not set any inflow velocity limit given that they evaluate the effectiveness of the air barrier containment by the AS methods above mentioned in addition to the Work Zone Integrity (as per AS1807.5 method).

Fig.2 - KI-Discus Test for the retention efficiency at the front aperture.

Parameters	AS 2252.2	NSF/ANSI 49-2002 (Class II - A2)	EN12469 (Class II)
Velocity	Unspecified	0,51 m/s	≥ 0,4 m/s
Acceptance criteria (difference of individual points from the mean value)	Unspecified	± 0,025 m/s (difference of the average inflow readings from the nominal set point)	± 20%
Method	Aerosol Containment	Balancers flow hood sealed over the front access opening	Measurement device suitable for measuring volumetric rate within the exhaust duct

3. Filter Leak Test (D.O.P. Test)

It will determine the integrity of the supply and exhaust HEPA filters, filter housing, and filter mounting frames while the cabinet is operated at the nominal set point velocities. The test is based on knowing the exact concentration of the poly-dispersed aerosol by an aerosol generator upstream of the HEPA filter and detecting the penetration through the filter, mounting frames and/or filter housing by using a photometer (Fig. 3). The entire face of each HEPA filter should be scanned for leaks by using slightly overlapping strokes of the probe and moving the probe at a rate of not more than 50 mm/s (5cm/s). The probe (Fig.4) should be held approximately 30 mm away from the filter media. The associated risk is the escape of biological agents through the air expulsion filter, cross-contamination of samples and contamination of the operator due to leaks in the downflow filter.

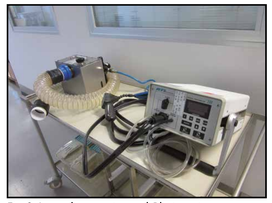


Fig.3 Aerosol generator and Photometer.

Fig.4 Scanning probe of the exhaust HEPA Filter.

Parameters	AS 2252.2 (Class II)	NSF/ANSI 49-2002 (Class II - A2)	EN12469 (Class II)
Instruments	Photometer	Photometer	Photometer or Discrete particle counter
Maximum penetration	0,01%	0,01%	0,01% Photometer 0,05% Particle counter

4. Cabinet Integrity Test (Soap Bubble)

This pressure holding test is performed to determine if exterior surfaces of all plenums, welds, gaskets and plenum penetrations or seals are free from leaks. In the field, based on the international standards, it needs only be performed on Type A1 cabinets (or positive pressure contaminated plenum systems) at the time of initial installation when the BSC is in a free-standing position in the room in which it will be used, after a cabinet has been relocated to a new location, and again after removal of access panels to plenums for repairs or a filter change. The associated risk is that the operator is working in a contaminated environment without being aware of it.

Parameters	AS 2252.2	NSF/ANSI 49-2002 (Class II - A2)	EN12469 (Class II)
Method	Positive pressure	Positive or negative pressure	Positive pressure
Pressure	250 Pa	500 Pa ± 10% for 30 minutes	250 Pa
Visualization method	Soap Bubbles	Soap bubbles or tracer gas (Helium)	Soap bubbles
Where	Factory or field	Factory or field (for positive pressure only)	Factory or field (for positive pressure systems)

5. Alarm System Check

Auditory and visual indicators of alarm shall be checked and in the specific: viewing window, ventilation parameters deviate from those specified by the manufacturer (the threshold limits of the deviation shall be also specified), blower/s fault.
The associated risk is that the operator works in a contaminated environment without being aware of it.

6. Other Tests on request

In addition to the above mentioned critical tests to be performed for routine maintenance testing on the Biosafety cabinets another noteworthy test is the airflow smoke patterns test (which is mandatory for NSF and EN standards - fig. 5) to determine if the airflow along the entire perimeter of the work access opening is inward, if airflow within the work area is downward with no dead spots or refluxing, and if there is refluxing to the outside at the window wiper gasket and side seals.
The secondary tests related to operator comfort and safety are performed at the request of the customer or at the discretion of the certification provider: light intensity, vibration, noise level, UV radiation, electrical safety, gas supply safety.

Fig.5 Smoke test for view screen retention test.

From the operator and service engineer point of view, absolutely to be mentioned is the BSC decontamination process and the installation site (as reported in the two standards (BS5726:2005 and AS 2252.4:2010) as well as the operative procedure to be adopted.

BSC Decontamination

Due to its use with infectious materials, all the regulatory bodies require the manufacturers to provide a process for the user to biologically decontaminate the cabinet. There are, mainly, two different decontamination types. The *surface decontamination* which is a process of applying usually liquid disinfectant to all of the accessible surfaces of the cabinet and this is carried out when work is completed, since residual culture media may provide an opportunity for microbial growth or before the day-to-day activity to avoid any sample contamination. The use of UV irradiation is not recommended as the sole decontaminating agent for the work area. Surface disinfection should be performed before and after every cabinet use. The *total decontamination* (surface + interior parts) where a disinfectant vapor or gas is introduced into the sealed interior of the cabinet and allowed adequate contact time to provide a 6 log reduction of biological agents on the surface as well as in the filter. This is required (as reported in the EN12469, NSF49 and AS2252.4) in the following circumstances:

- before any maintenance work on the cabinet where access to potentially contaminated parts is necessary
- prior to a relocation
- clean-up of a contamination event
- HEPA filter replacements
- where there are any significant changes to the nature of work carried out [e.g. use of significantly different pathogens]
- according to the health and safety program

A risk assessment of the decontamination system shall be performed prior to any decontamination activity.

Decontamination Type & Method	AS 2252.2	NSF/ANSI 49-2002 (Class II - A2)	EN12469 (Class II)
Formaldehyde	Only hazardous level to be monitored are reported (0.3 ppm as specified by the WHO)	0.3 gm/ft3 (11 g/m3) ~8000 ppm Requires relative humidity > 60% Permissible Exposure limit (PEL) 0.75ppm *	60ml of 36% formalin and 60ml of water per cubic meter of cabinet volume RH 65% Permissible Exposure limit 0.75ppm *
Hydrogen Peroxide (Fig.6)	Only hazardous levels to be monitored are reported (PEL 1 ppm)	Is reported as an alternative to the other decontaminants but is to be validated according to the model and size (PEL 1 ppm)	Is reported as an alternative to the other decontaminants but it is to be validated according to the model and size (PEL 1 ppm)
Chlorine Dioxide	Not reported	Fixed amount / Fixed Concentration of CD RH range 60-75 % (PEL 0.3 ppm)	Not reported
*OSHA's Standard on Occupational Exposure to Formaldehyde			

Fig. 6 - H_2O_2 "In loop" Decontamination by Vaporized Hydrogen Peroxide System.

Conclusions

Any of these standards can be used since they validate the biosafety cabinet's performance (mainly to provide protection to worker, environment and product). However, it is important to note that most biosafety cabinets in use today are certified based on the standard each country uses. All preventive maintenance, including certification and repair, must be effected by a qualified technician familiar with the proper maintenance procedures required for the laboratory's BSC. No matter what standard is followed, the effectiveness of the biosafety cabinet to provide protection greatly depends on how the user operates the equipment and takes care of the equipment. The cabinets are not substitutes for good practice and can only complement a careful worker. Good laboratory practices are required when handling materials in the cabinet. The user should be fully instructed in the degree of protection offered by the Biosafety Cabinet, principles of airflow and operator protection test, appropriate and inappropriate use of cabinets, limitations of performance, mode of operation and function of all controls and indicators, how to work at cabinet safely, how and when to decontaminate cabinet after use. Written standard operating procedures shall be prepared and made readily available to the user before any work begins inside the BSC.

References

AS 2252.2-2009 *Biological safety cabinets Class II and referenced documents;* AS 2252.4-2010 *Biological Safety Cabinets Classes I and II - Installation and use- ;* NSF/ANSI 49-2018 *Biosafety Cabinetry: Design, Construction, Performance, and Filed Certification;* EN12469-2000 *Biotechnology - Performance criteria for microbiological safety cabinets - and referenced documents ;* WHO - *Laboratory Biosafety Manual - Third Edition.*

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