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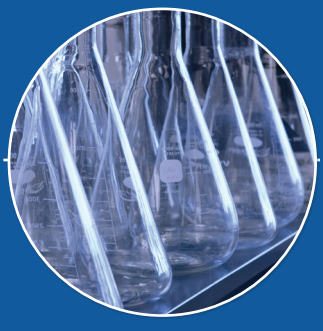
NSF/ANSI 49 - 2014

Biosafety Cabinetry: Design, Construction, Performance, and Field Certification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NSF International Standard/
American National Standard
for Drinking Water Additives —

Biosafety Cabinetry: Design, Construction, Performance, and Field Certification

Standard Developer

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Contents

Foreword[2]

The purpose of this Standard is to establish minimum requirements for materials, design, construction, and performance of Biosafety Cabinetry that are designed to protect personnel, product, and the environment. This Standard details requirements for performance testing as well as field certification testing.

This edition of the Standard (NSF/ANSI 49-2014) includes the following revisions:

Issue 48: This revision adds a motor stability test procedure for motor speed control systems.

Issue 49: This revision updates the Sealant use language in Annex H.

Issue 50: This revision affirms new language regarding the type of fans used in Biosafety Cabinets.

Issue 51: This revision affirms new language regarding the Type Biosafety Cabinet blower startup.

Issue 52: This revision clarifies details surrounding the DOP port location in section 5.22.

Issue 53: This revision adds definitions to clarify biosafety cabinet shell penetrations and cable ports with considerations given to service technicians and cabinet users relating to safety.

Issue 55: This revision updates the instrumentation language.

Issue 60: This revision updates the Airflow Grid language in sections A.8.3.1 and A.8.3.2, and the related figure A15.

Issue 61: This revision updates the language in sections 5.19.4 and 5.25.1 to include a section requiring the use of a sash position too low alarm.

Issue 72: This revision updates multiple figures throughout the Standard to improve clarity.

This Standard was developed by the NSF Joint Committee on Biosafety Cabinetry using the consensus process described by the American National Standards Institute.

Suggestions for improvement of this Standard are welcome. This Standard is maintained on a Continuous Maintenance schedule and can be opened for comment at any time. Comments should be sent to Chair, Joint Committee on Biosafety Cabinetry at standards@nsf.org, or c/o NSF International, Standards Department, PO Box 130140, Ann Arbor, Michigan 48113-0140, USA.

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NSF/ANSI Standard
for Biosafety Cabinetry —

Biosafety Cabinetry: Design, Construction, Performance, and Field Certification

1 General

1.1 Scope

This Standard applies to Class II (laminar flow) biosafety cabinetry designed to minimize hazards inherent in work with agents assigned to biosafety levels 1, 2, 3, or 4. It also defines the tests that shall be passed by such cabinetry to meet this Standard. This Standard includes basic requirements for the design, construction, and performance of biosafety cabinets that are intended to provide personnel, product, and environmental protection; reliable operation; durability and structural stability; cleanability; limitations on noise level; illumination; vibration; and motor/blower performance.

1.2 Minimum requirements

Cabinets qualifying under this Standard shall have passed all of the designated tests. Units with component parts covered under existing NSF standards or criteria shall conform to those applicable requirements.

1.3 Variations in design and construction

Cabinetry varying in design, construction, or installation of accessory equipment may qualify under this Standard, if appropriate tests and investigations indicate that the equipment is durable and reliable, can be cleaned and decontaminated, and performs in conformance to this Standard. Such equipment shall meet the requirements for materials and finishes in this Standard.

Major modifications require appropriate tests for conformance. Major modifications include, but are not limited to, changes in the following: location or capacity or quantity or all three of blower/motor(s); size or design or both of air plenums; position of High Efficiency Particulate Air/Ultra Low Penetrating Air (HEPA/ULPA) filters; position or redesign of work surface; work area intake and exhaust air grilles; window placement or design; access opening size; location and size of exhaust port; and built-in accessory equipment (centrifuges, ultraviolet lighting, supports for intravenous drug container, arm rests, etc.). Relocation of utility service equipment (electrical outlets, petcocks, etc.) is not considered a major modification if other provisions of this Standard are not compromised.

2 Normative references

The following documents contain requirements that, by reference in this text, constitute requirements of this Standard. At the time of publication, the indicated editions were valid. All documents are subject to revision, and parties are encouraged to investigate the possibility of applying the most recent editions of the documents indicated below.

ACGIH, *Industrial Ventilation: A Manual of Recommended Practice*[3]

ANSI 226.1 – *Test No. 17*[4]

ANSI/NFPA 70: *National Electrical Code*, 1999[5]

APHA, *Compendium of Methods for Microbiological Examination of Foods,* 1976 (Spore staining techniques)[6]

APHA, *Standard Methods for the Examination of Water and Wastewater*, Seventeenth Edition (Standard dilution plate methods)[6]

ASHRAE 111-2008: *Practices for Measurement, Testing, Adjusting and Balancing of Building Heating, Ventilation, Air-Conditioning and Refrigeration Systems*[7]

IEST-RP-CC001, *Recommended Practice for HEPA Filters*[8]

IEST-RP-CC007, *Testing ULPA Filters*[8]

IEST-RP-CC013, *Institute of Environmental Sciences Recommended Practice*, Tentative, August, 1986[8]

IEST-RP-CC021, *Testing HEPA and ULPA Filter Media*[8]

MIL-F-51079B, *Filters, Particulate, High Efficiency, Fire Resistant, Biological Use*[9]

NIOSH Pocket Guide: *bis(chloromethyl)ether*[10]

OSHA, CFR 29 1910.100, *Bloodborne Pathogens*[11]

The Lighting Handbook: *Reference and Application*, 10th Edition, 2011[12]

[3] American Conference of Governmental Industrial Hygienists, 1330 Kemper Meadow Dr., Cincinatti, OH 45240 <www.acgih.org>

[4] American Public Health Association, 800 I Street NW, Washington, DC 20001 <www.apha.org>

[5] National Fire Protection Association, 1 Batterymarch Park, Quincy, MA 02269 <www.nfpa.org>

[6] American Public Health Association, 800 I Street NW, Washington, DC 20001 <www.apha.org>

[7] American Society of Heating, Refrigerating, and Air-Conditioning Engineers, 1791 Tullie Circle, N. E. Atlanta, GA 30329 <www.ashrae.org>

[8] Institute of Environmental Sciences and Technology, 5005 Newport Drive, Suite 506, Rolling Meadows, IL 60008-1699 <www.iest.org>

[9] US Department of Defense, Navy Publishing and Printing Service Office, 700 Robins Ave., Philadelphia, PA 19111-5094 <www.defenselink.mil/pubs/>

[10] NIOSH, Department of Health and Human Services (DHHS), Publications Office, 4676 Columbia Pkwy., Cincinnati, OH 45226 <www.cdc.gov/niosh/>

[11] Superintendent of Documents, US Government Printing Office, Washington, DC 20402 <www.gpo.gov>

[12] Illuminating Engineering Society, 120 Wall Street, Floor 17, New York, NY 10005-4001 <www.iesna.org>

UL 94[13]

UL 61010A-1[13]

UL 61010-1[13]

3 Definitions

3.1 accessible: Fabricated to be exposed for cleaning and visual inspection using simple tools (screwdriver, pliers, open-end wrench, etc. [Also see definition of "readily accessible."])

3.2 airflow

3.2.1 downflow velocity: The velocity of HEPA-filtered air as it flows downward through the work area, providing product and cross contamination protection. The velocity is measured in a plane 4 in (10 cm) above the bottom edge of the sash, when it is in its normal operating height.

3.2.2 downflow velocity profile: The individual downflow velocities as measured and averaged, on a predetermined grid pattern. Airflow velocities and the average of the airflow through the work area may be calculated as a whole (Uniform) or may be separated into 2 or more adjoining areas (Zoned) with averages calculated for each zone.

3.2.3 inflow: The velocity or volume of air that flows from the room into the Front Access Opening, providing an air barrier to prevent the escape of aerosols generated in the cabinet's workzone.

3.2.4 unidirectional airflow: Air traveling through an area in a single pass in the same direction at a uniform speed to minimize potential cross contamination from aerosols.

3.2.5 non-uniform (zoned) downflow: A downflow velocity profile comprised of several contiguous zones. The average downflow velocities vary from zone to zone.

3.2.6 uniform downflow: A downflow velocity profile wherein the individual point velocities are all approximately the same.

3.3 biohazard: (a contraction of the words biological and hazard): Infectious agent(s), or part thereof, presenting a real or potential risk to the well-being of man, animals, and/or plants, directly through infection or indirectly through disruption of the environment.

3.4 biosafety cabinet nominal width: The interior sidewall to sidewall width. The cabinet nominal width is expressed in 1 foot increments for cabinets with an interior sidewall to sidewall width greater than 33 in. Cabinets with an interior sidewall to sidewall width of 33 in or less are classified to the nearest half-foot. This definition is provided for the purpose of determining the required downflow velocity grid spacing requirements and personnel protection slit sampler positioning.

3.5 biosafety cabinet shell penetrations/cable ports

3.5.1 sealed service penetration: A structure that seals an adjustment fixture and/or test connection that passes from a contaminated area of the cabinet to the outside environment (e.g. an exhaust damper (choke) adjustment shaft in a Type A BSC) meeting the requirements of Annex A.1. Its installation is durable, not typically requiring service and/or replacement, and its function is to allow the certifier to make the necessary adjustments or test measurements without releasing contaminants.

[13] Underwriters Laboratories, 333 Pfingsten Rd., Northbrook, IL 60062-2096 <www.ul.com>

3.5.2 user-modified pass through: A structure that allows the user to pass wiring, cables, tubing, etc. from the outside environment into the work area of the cabinet. Portions of this pass through structure may be permanently attached to the work area of the cabinet, not typically requiring service and/or replacement, but the retaining element(s) for the various cables, tubes, etc. are readily replaceable by the user. Its functions are to retain the object(s) the user has installed in the pass through, and prevent the escape of contaminants via the pass through. The pass through shall bear cautionary labels both interior and exterior referencing use.

3.5.3 sealed service pass through: A structure that allows wiring, cables, tubing, etc. to pass from the outside environment into a contaminated area of the cabinet (e.g. electrical wires for the fan in a Type A BSC). Its installation is durable, not typically requiring service and/or replacement. Its functions are to immobilize the items passing through it, and to provide a seal meeting the requirements of Annex A.1.

3.6 biosafety cabinet carcass, hull, chassis, shell, body: The outside of the cabinet exposed to the environment after removing any decorative or dress panels, providing a barrier between the inner, potentially contaminated areas and the environment.

3.7 biosafety levels:[14] The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in *Biosafety in Microbiological and Biomedical Laboratories*.[15] The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

3.7.1 biosafety level 1 (BSL1): Biosafety Level 1 is suitable for work involving well-characterized agents not known to consistently cause disease in immunocompetent adult humans, and present minimal potential hazard to laboratory personnel and the environment. BSL-1 laboratories are not necessarily separated from the general traffic patterns in the building. Work is typically conducted on open bench tops using standard microbiological practices. Special containment equipment or facility design is not required, but may be used as determined by appropriate risk assessment. Laboratory personnel must have specific training in the procedures conducted in the laboratory and must be supervised by a scientist with training in microbiology or a related science.

3.7.2 biosafety level 2 (BSL2): Biosafety Level 2 builds upon BSL-1. BSL-2 is suitable for work involving agents that pose moderate hazards to personnel and the environment. It differs from BSL-1 in that:

- laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures;

- access to the laboratory is restricted when work is being conducted; and

- all procedures in which infectious aerosols or splashes may be created are conducted in biosafety cabinets (BSCs) or other physical containment equipment.

3.7.3 biosafety level 3 (BSL3): Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with agents that may cause serious or potentially lethal disease through inhalation route exposure. Laboratory personnel must receive specific training in handling pathogenic and potentially lethal agents, and must be supervised by scientists competent in handling infectious agents and associated procedures. Secondary barriers for this level

[14] Previously referred to as risk levels (low, moderate, and high).

[15] Centers for Disease Control and Prevention, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 5th Edition, December 2009 <http://www.cdc.gov/biosafety/publications/bmbl5/index.htm>

include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

3.7.4 biosafety level 4 (BSL4): Biosafety Level 4 is required for work with agents that pose a high individual risk of life-threatening disease, aerosol transmission, or related agent with unknown risk of transmission. Agents with a close or identical antigenic relationship to agents requiring BSL-4 containment must be handled at this level until sufficient data are obtained either to confirm continued work at this level, or re-designate the level. Laboratory staff must have specific and thorough training in handling extremely hazardous infectious agents. Laboratory staff must understand the primary and secondary containment functions of standard and special practices, containment equipment, and laboratory design characteristics. All laboratory staff and supervisors must be competent in handling agents and procedures requiring BSL-4 containment. Access to the laboratory is controlled by the laboratory supervisor in accordance with institutional policies.

There are two models for BSL-4 laboratories:

- a Cabinet Laboratory where all handling of agents must be performed in a Class III BSC.
- a Suit Laboratory where personnel must wear a positive pressure protective suit.

BSL-4 Cabinet and Suit Laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment.

3.8 cabinet classification: Although this Standard covers only Class II biosafety cabinetry, Class I and Class III cabinets are currently defined and known to be commercially available. Biosafety cabinets can be used for work with biological agents assigned to biosafety levels 1 through 4, depending on the facility design as described in *Biosafety in Microbiological and Biomedical Laboratories*. Special note should be taken that BSL4 agents should only be used in Maximum Containment Laboratories and that Class I and Class II biosafety cabinets are only acceptable in Maximum Containment Laboratories with positive pressure containment suits.

3.8.1 class I: A Class I BSC provides personnel and environmental protection without product protection. Personnel protection is provided as a minimum velocity of 75 lfpm (0.38 m/s)[16] of unfiltered room air is drawn through the front opening and across the work surface. The air is then passed through a HEPA/ULPA filter in the exhaust plenum, providing environmental protection.

3.8.2 class II: Class II (Type A1, A2, B1 and B2) BSCs are partial barrier systems that rely on the movement of air to provide personnel, environmental, and product protection. Personnel and product protection is provided by the combination of inward and downward airflow captured by the front grille of the cabinet.

Side-to-side cross-contamination of product is minimized by the internal downward flow of HEPA/ULPA-filtered air moving towards the work surface and then drawn into the front and rear intake grills. Environmental protection is provided when cabinet exhaust air is passed through a HEPA/ULPA filter. When used as designed, the cabinet exhaust air may be recirculated to the laboratory (Type A1 and A2 BSCs) or discharged from the building via a canopy connection (Type A1 and A2 BSCs). Exhaust air from Types B1 and B2 BSCs must be discharged to the outdoors via a sealed connection.

All Class II cabinets are designed for work involving procedures assigned to biosafety levels 1, 2 and 3. Class II BSCs may be used with procedures requiring BSL-4 containment if used in a BSL-4 suit laboratory by a worker wearing a positive pressure protective suit.

Class II BSCs provide the microbe-free work environment necessary for cell culture propagation and also may be used for the formulation of nonvolatile antineoplastic or chemotherapeutic drugs.

[16] Barbeito MS, Taylor LA. *Containment of Microbial Aerosols in a Microbiological Safety Cabinet*. Appl. Microbiol. 16:1255-29, 1968.

3.8.2.1 class II type A1 cabinets (formerly designated type A): cabinets that

- maintain minimum average inflow velocity of 75 ft/min (0.38 m/s) through the work access opening;

- have HEPA/ULPA filtered downflow air that is a portion of the mixed downflow and inflow air from a common plenum (i.e., a plenum from which a portion of the air is exhausted from the cabinet and the remainder supplied to the work area);

- may exhaust HEPA/ULPA filtered air back into the laboratory or to the environment through an external exhaust system connected to the cabinet with a canopy connection; and

- have all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums.

Type A1 cabinets are not suitable for work with volatile chemicals and radionuclides.

3.8.2.2 class II, type A2 cabinets (when exhausted to the environment were formerly designated type B3): cabinets that

- maintain a minimum average inflow velocity of 100 ft/min (0.51 m/s) through the work access opening;

- have HEPA/ULPA filtered downflow air that is a portion of the mixed downflow and inflow air from a common exhaust plenum;

- may exhaust HEPA/ULPA filtered air back into the laboratory or to the environment through an external exhaust system connected to the cabinet with a canopy connection; and

- have all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums.

Type A2 cabinets used for work with volatile chemicals and radionuclides required as an adjunct to microbiological studies must be exhausted through properly functioning exhaust canopies.

3.8.2.3 class II type B1 cabinets: cabinets that

- maintain a minimum average inflow velocity of 100 ft/min (0.51 m/s) through the work access opening;

- have HEPA/ULPA filtered downflow air composed largely of uncontaminated recirculated inflow air;

- exhaust most of the contaminated downflow air to an external exhaust system through a dedicated duct connected to cabinet with a direct connection and exhausted to the atmosphere after passing through a HEPA/ULPA filter; and

- have all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums.

Type B1 cabinets may be used for work with volatile chemicals and radionuclides required as adjuncts to microbiological studies.

Type B1 cabinets may be used for work treated with volatile chemicals and radionuclides required as an adjunct to microbiological studies if work is done in the direct exhausted portion of the cabinet, or if the chemicals or radionuclides will not interfere with the work when recirculated in the downflow air.

3.8.2.4 class II, type B2 cabinets: cabinets that

- maintain a minimum average inflow velocity of 100 ft/min (0.51 m/s) through the work access opening;

- have HEPA/ULPA filtered downflow air drawn from the laboratory or the outside air (i.e., downflow air is not recirculated from the cabinet exhaust air);

- exhaust all inflow and downflow air to the atmosphere through an external exhaust system connected to the cabinet with a direct connection after filtration through a HEPA/ULPA filter without recirculation in the cabinet or return to the laboratory; and

- have all contaminated ducts and plenums under negative pressure or surrounded by directly exhausted (nonrecirculated through the work area) negative pressure ducts and plenums.

Type B2 cabinets may be used for work with volatile chemicals and radionuclides required as adjuncts to microbiological studies.

3.8.3 class III: The Class III BSC was designed for work with highly infectious microbiological agents and other hazardous operations. It provides maximum protection for the environment and the worker. It is a gas-tight (no leak greater than $1x10^{-7}$ cc/s with 1% test gas at 3 in pressure water gauge[17]) enclosure with a viewing window that is secured with locks and/or requires the use of tools to open. Access for passage of materials into the cabinet may be through any of the following: a dunk tank that is accessible through the cabinet floor, a double-door pass-through box that can be decontaminated between uses, integrated double door autoclaves and portable docking stations with double sealing connecting mechanisms that can be decontaminated between uses. Reversing that process allows materials to be removed from the Class III BSC. Both supply and exhaust air are HEPA/ULPA filtered. Exhaust air must pass through two HEPA/ULPA filters in series, before discharge to the outdoors. Airflow is maintained by an exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure according to manufacturer design pressure criteria. Sometimes because of laboratory conditions an optional exhaust fan may be required. This exhaust fan should generally be kept separate from the exhaust fans of the facility ventilation system. If a cabinet exhaust system is required it should be equipped with an appropriate alarm system which both notifies the cabinet user and shuts down the cabinet exhaust system in the event of a facility exhaust system failure.

Rubber glove/sleeves or equivalent glove material, are sealed to ports in the cabinet and allow direct manipulation of the materials isolated inside. The glove material shall be compatible for use with the materials being used in the cabinet. The exhaust system for the cabinet shall provide inflow to the cabinet arm port in the event of a rubber glove/sleeve breach. The minimum breach velocity shall be measured with a hot wire in the middle of the arm port and shall be no less than 100 ft/min (0.51 m/s). It is not a requirement for the work area to be free of turbulence or cross contamination.

3.9 calibration: Comparison of the measurement of a standard or instrument of unknown accuracy with another standard or instrument of known accuracy to detect, correlate, report, or eliminate by adjustment any variation in the accuracy of the unknown standard or instrument.

3.10 canopy connection: A BSC exhaust connection where there are one or more openings or gaps in the connection between the BSC and the external exhaust system.

[17] National Institutes of Health, *Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant DNA Research* January 1979 <http://orf.od.nih.gov/NR/rdonlyres/6D8AAB74-2FF5-430B-A553-B9410474B1A4/16202/LaboratorySafetyMonograph1979.pdf>

3.11 certification, cabinet design: Cabinet design certification is formal validation by a qualified design testing organization that a designated cabinet model meets all the requirements of Annex A of this standard.

3.12 certification, cabinet field: Cabinet field certification is formal verification by a qualified field-testing certifier that an installed cabinet meets all the requirements of Annex F of this standard.

3.13 chemical resistance: Capability of materials to maintain their original surface characteristics under prolonged contact with cleaning compounds, decontaminating agents, and normal conditions of the use environment.

3.14 closed: Fabricated with no openings exceeding 0.031 in (0.079 cm).

3.15 concurrent balance value: This value is determined using the duct traverse measurement method as specified in ASHRAE 111-2008, a minimum of 7.5 duct diameters downstream of a direct connected BSC. Prior to determining the concurrent balance value, it shall be confirmed that the cabinet is operating at its nominal setpoints for inflow and downflow velocity ± 3 fpm. The primary DIM method shall be used for setting the inflow velocity. The accuracy of the DIM shall be better than or equal to ± 3% and ± 7 cfm. The static pressure is also measured approximately two duct diameters from the cabinet exhaust connection. Appropriate filter load and tolerance values shall be added to the base static pressure value to accommodate filter loading: 0.3 in w.g. shall be added for Type B1 cabinets and 0.7 in w.g. shall be added for Type B2 cabinets. The resulting values may be used for design and balance exhaust/supply HVAC requirements.

3.16 decontamination: Inactivation or destruction of infectious agents or neutralization of toxic agents.

3.17 direct connection: A BSC exhaust connection where the connection between the BSC and the external exhaust system is air tight with no designed gaps or openings.

3.18 direct inflow measuring device (DIM): A volumetric airflow measuring device consisting of a capture hood with a sensing component that provides a readout as a single value for volumetric flow rate and meets the requirements of Annex B.

3.19 high efficiency air filters (for use in class II biosafety cabinets):

3.19.1 high efficiency particulate air (HEPA) filter: A throwaway, extended/pleated medium, dry-type filter with the following:

- rigid casing enclosing the full depth of the pleats;

- minimum particulate removal of 99.99% for thermally generated monodisperse dioctyphthalate (DOP) smoke particles or equivalent with a diameter of 0.3 µm (Type C);

- minimum particulate removal of 99.99% and determination as the lower efficiency when tested for particle size ranges of 0.1 to 0.2 µm or 0.2 to 0.3 µm in accordance with IEST-RP-CC007 (Type J);

- minimum particulate removal of 99.995% and determination as the lower efficiency when tested for particle size ranges of 0.1 to 0.2 µm or 0.2 to 0.3 µm in accordance with IEST-RP-CC007 (Type K);

- maximum pressure drop of 1.0 in w.g. (250 Pa) when clean and operated at rated airflow capacity; and

- no area showing a penetration exceeding 0.01% when scan tested with a polydisperse aerosol having a light scattering median size of 0.7 µm and a geometric standard deviation of 2.4.

These filters conform to all the performance and construction requirements of a Type C, a Type J, or a Type K filter respectively, contained in IEST-RP-CC001.4. Filter media shall be tested in accordance with the methods of IEST-RP-CC021 with performance levels to meet the minimum efficiency requirements as specified above and the pressure drop requirements as required by the specific application.

3.19.2 ultra-low-penetrating air (ULPA) filter: A throw away, extended/pleated medium, dry-type filter with the following:

- rigid frame enclosing the full depth of the pleats;

- minimum particle removal of 99.999% and determination as the lower efficiency when tested for particle size ranges of 0.1 to 0.2 µm or 0.2 to 0.3 µm when tested in accordance with IEST-RP-CC007;

- maximum pressure drop of 1.0 in w.g. (250 Pa) when clean and operated at rated airflow capacity. ULPA filters may have higher airflow resistance than HEPA/ULPA filters for the same rated airflow; therefore, care shall be taken to ensure that the pressure drop is compatible with the cabinet motor/blower capability; and

- no area showing a penetration exceeding 0.01% when scan tested with a polydisperse aerosol having a light scattering median size of 0.7 µm and a geometric standard deviation of 2.4.

This filter conforms to all requirements of a Type F filter contained in IEST-RP-CC001.4, HEPA and ULPA filters.

3.20 leak tight: Free of leaks at 2 in w.g. (500 Pa) of air pressure as described in Annex A.

3.21 nominal set point velocities: The cabinet downflow and inflow velocities that the manufacturer designates as the settings at which the cabinet is intended to operate and the settings at which it passed the tests listed in 6.7 and Annex A, section A.7.

3.22 polydisperse aerosol: Aerosol with a light scattering median size of 0.7 µm and a geometric standard deviation of 2.4.

3.23 readily accessible: Fabricated to be exposed for cleaning and visual inspection without using tools.

3.24 readily removable: Capable of being taken away from the main unit without using tools.

3.25 removable: Capable of being taken away from the main unit using simple tools (screwdriver, pliers, open-end wrench, etc. [also see definition of "readily removable"]).

3.26 sash: A fixed or sliding window located at the front of the biosafety cabinet, that forms a barrier between the operator and the work area.

3.27 sealed: Fabricated with no openings that will permit entry or leakage of air (leak-tight).

3.28 smooth: A surface free of pits and inclusions, with cleanability equal to or exceeding the following.

3.28.1 interior work surfaces and exposed interior surfaces: Number 3 (100 grit) finish on stainless steel.

3.28.2 other interior surfaces and exterior surfaces: Commercial grade cold-rolled, hot-rolled, or combination cold/hot-rolled steel free of visible scale.

3.29 surfaces: (see figure 1)

3.29.1 interior work surfaces: Surfaces used when performing a task, operation, or activity.

3.29.2 exposed interior surfaces: Exposed interior surfaces, other than work surfaces, that are subject to splash, spillage, or airborne contamination during normal use.

3.29.3 other interior surfaces: Interior surfaces not exposed to splash or spillage but exposed to vapor or volatile toxic substances or both.

3.29.4 exterior surfaces: All exposed surfaces not defined as interior.

3.30 toxic: Having an adverse physiological effect on biological systems.

3.31 visible medium: A visible aerosol that is sufficiently neutrally buoyant in air to see air disturbances without influencing them. Examples include chemical ventilation tubes and thermally generated aerosol. The delivery velocity of the visual medium should be slow enough to assure that there is no interference to the air flow under test.

3.32 w.g. (water gauge): Another common name for the inch of water column. The word "gauge" after a pressure reading indicates that the pressure stated is actually the difference between the absolute or total pressure and the air pressure at the time of the reading.

3.33 work area: The horizontal plane inside the cabinet extending from sidewall to sidewall and from back wall to the inside of the sash at a point approximately 2 in (5 cm) above the lower level of the sash.

3.34 work tray: The solid floor of the work area identified by the manufacturer as the location for the user's activity. This is differentiated from work area.

4 Materials

4.1 General

Materials shall withstand normal wear, corrosive action of gases or liquids, cleaning compounds, and decontaminating agents and procedures. Materials shall be structurally sound, dimensionally stable, fire and moisture resistant, and compatible with other materials used in the laboratory.[18]

4.2 Interior work surfaces

Interior work surfaces shall be smooth, 300-series stainless steel.

4.3 Exposed interior surfaces

Exposed interior surfaces shall be smooth and abrasion- and corrosion-resistant or shall be rendered corrosion-resistant with nontoxic material that resists crazing, cracking, and chipping. Recirculated air diffuser materials shall be tested in accordance with UL 94. Nonrigid diffuser materials shall conform to Class 94HBF; rigid diffuser materials shall conform to Class 94HB.

[18] See Annex H for material selection guidance.

4.4 Other interior and exterior surfaces

Other interior and exterior surfaces shall be smooth and abrasion- and corrosion-resistant or shall be rendered corrosion-resistant with nontoxic materials that resist crazing, cracking, and chipping.

4.5 Materials and finishes

4.5.1 Windows/sashes

Windows and sashes shall be optically clear and not adversely affected by accepted cleaning methods and decontaminating agents. Glazing materials shall be laminated glass, tempered glass, safety plastic, or equivalent. Edges shall be ground or provided with protective stripping.

4.5.1.1 Flammability

Safety plastic view screens shall be tested in accordance with UL 94 and conform to Class 94HB.

4.5.1.2 Abrasion resistance

Windows shall be abrasion-resistant and show no more than 5% change in haze when tested in accordance with 5.17, Test No. 17 of ANSI 226.1.

4.5.2 Protective coatings

4.5.2.1 Chemical resistance

Protective coatings shall be resistant to prolonged contact to liquids, cleaning compounds, and procedures. Specifically, the protective coatings used shall be resistant to the following chemicals, when tested in accordance with Annex D:

- 1N hydrochloric acid;
- 1N sodium hydroxide;
- 1% quaternary ammonium compound;
- 5% formaldehyde;
- 5000 ppm hypochlorite;
- 2% iodophor;
- 5% phenol; and
- 70% ethyl alcohol (ethanol).

When a coating is exposed to these chemicals following the test methods in Annex D, there shall be no visible effect on the finish other than a slight change of gloss, discoloration, and/or temporary softening of the finish, with no loss of adhesion or film protection.

> NOTE – When special chemical solutions are intended to be used, the resistance of the material thereto shall also be evaluated.

4.5.2.2 Abrasion resistance

Protective coatings for exposed interior, other interior, and exterior surfaces shall meet the following requirements when tested in accordance with Annex D:

- maximum weight loss – 100 mg; and
- minimum wear value – 500 cycles.

4.5.3 Plastics

Plastics shall meet the applicable requirements of 4.1, 4.3, 4.4, and 4.5.1.

4.5.4 Welding

Welded seams and deposited weld material shall meet the applicable requirements of 4.1, 4.2, 4.3, and 4.4.

4.5.5 Gaskets and sealants

Gaskets and sealants shall be closed cell, durable, resistant to cleaning and disinfecting agents, and resistant to general use. They shall be made of materials that do not release halogens and are non-hardening, nontoxic, stable, odor free, not detrimentally absorbent, and unaffected by exposure to gases, liquids, cleaning compounds, and decontamination agents listed in 4.5.2.

Exposed surfaces of gaskets for all access panels, doors, structural seams, and sashes/windows shall be skinned and smooth. Gaskets supplied with HEPA/ULPA filters shall be exempt from this requirement.

4.5.6 Sound dampening

Sound-dampening materials shall conform to the requirements for the area in which they are used. They shall not be used in areas subject to contamination. Non-hardening and porous types shall not be accepted.

4.5.7 Hard solder

Hard (silver) solder shall be formulated to be corrosion-resistant.

5 Design and construction

5.1 General

Cabinets shall be designed and constructed to function properly and operate in a safe manner, minimize contamination, provide personnel and product protection, and be capable of being cleaned and decontaminated. Exposed burrs and sharp edges (including, but not limited to, sheet metal screws) shall be eliminated from surfaces of the cabinet that are subject to normal operation, field certification, and maintenance (including those maintained with simple tools).

5.2 Cleanability

Interior work, exposed interior, and the other interior surfaces subject to splash or spillage shall be readily accessible and easily cleanable as assembled or when removed. Interior work, exposed interior, and other interior surfaces, including plenums, shall be capable of being vapor or gas decontaminated.

5.3 Decontamination[19]

Cabinets shall be designed to be decontaminated with an inactivating agent (such as formaldehyde gas) without being moved. Closure to contain decontaminating agents should be limited to gas-tight sealing of air intake and exhaust openings with metal plates, or plastic film and tape, or equivalent.

Pressure tight valves, if provided, suitable for decontamination shall be located on the clean side of the HEPA/ULPA filter.

[19] See Annex G.

5.4 Canopy connect exhaust

Type A1 or A2 cabinets may be connected to an exhaust system via a canopy connection and exhausted by a remote fan. The external exhaust shall draw air sufficient to capture using a visible medium to verify all exhaust from the BSC is captured and to maintain a flow of air into the exhaust connection through the openings or gaps. The flow of air through the openings or gaps provides a buffer between the BSC exhaust and variation in the external exhaust system assuring consistent BSC performance and/or containment of volatile chemicals used in the BSC. Properly sized canopy openings or gaps also provide enough relief open area, so that if the facility (external) exhaust system fails, the BSC will continue to function (maintain inflow velocity above the lowest level as verified by NSF biological testing) as if it was not connected to an exhaust system and continue to provide biological and particulate containment only. The canopy connection type of BSC exhaust connection is required for externally vented Class II, type A1 or A2 BSCs.

5.5 Direct connect exhaust

The external exhaust shall draw air sufficient to capture all exhaust from the BSC and maintain a negative pressurization in the exhaust duct. The direct connection type of BSC exhaust connection is required for Class II, Type B1 or B2 BSCs.

5.6 Duct and plenum design

All biologically contaminated ducts and plenums in Types A1, A2, B1, and B2 cabinets shall be maintained under negative pressure or enclosed within a negative pressure zone.

5.7 Internal corners and angles

5.7.1 Interior work surfaces

5.7.1.1 Two-plane intersection

An internal angle of 2 rad (110°) or less formed by the intersection of two planes, which is subject to manual cleaning, shall have a minimum continuous and smooth radius of 0.13 in (3.2 mm) (see figure 2).

5.7.1.2 Three-plane intersection

An internal corner formed by the intersection of three planes at 2 rad (110°) or less, subject to manual cleaning, shall have a minimum continuous and smooth radius of 0.25 in (6.3 mm) for a vertical or horizontal intersection. The alternate intersections shall have a minimum continuous and smooth radius of 0.13 in (3.2 mm) (see figure 2).

5.7.1.3 Fillet material

Parent material or hard solder may be used as fillet material in structurally sound seams.

5.8 External corners and angles

All external corners and angles subject to splash or spillage or both shall be sealed as smooth as the surfaces being joined, and formed to eliminate sharp edges that may interfere with use, cleaning, or maintenance (see figure 3).

5.9 Joints and seams

5.9.1 Interior work and exposed interior surfaces

All joints and seams subject to routine manual cleaning shall be sealed as smooth as the surfaces being joined. Perimeter drain spillage trough joints and seams shall be welded and sealed. All other seams shall be sealed. Equipment parts shall be stamped, extruded, formed, or cast in one piece. Joints shall be fabricated to eliminate dirt-catching horizontal ledges.

5.9.2 Other interior and exterior surfaces

All joints and seams subject to routine splash or spillage or both shall be sealed and smooth. All joints and seams subject to exposure to vapor or toxic volatile substances or both shall be sealed. All other seams shall be closed.

5.10 Fastening methods

5.10.1 Exposed fastenings

Exposed screw threads, projecting screws, and studs shall not be used on interior work surfaces. They shall only be used on exposed interior and other interior surfaces when other fastening methods are impractical. All metal fasteners and studs subject to maintenance shall not be subject to excessive overspray.

5.10.2 Exterior fastenings

Fasteners for exterior removable panels that are gasketed and subject to pressure shall be studs with solid acorn nuts, or equivalent, so that the gasket is sealed. Fasteners for other removable panels may be low profile-type fasteners (truss, round counter sunk, flat counter sunk head [see figure 4]), or studs with solid acorn nuts. All metal fasteners and studs subject to maintenance shall not be subject to excessive overspray.

5.10.3 Interior fastenings

In areas subject to cleaning, interior fastenings and joinings shall be fabricated to minimize projections, ledges, and recesses. All metal fasteners and studs subject to maintenance shall not be subject to excessive overspray.

5.11 Welds

Welds shall meet the smoothness requirements of the applicable surface.

5.12 Solder

Solder shall only be used to seal structurally sound seams or as a fillet material (see 5.7.1.3).

5.13 Removable panels

All maintenance panels to access the blower/motor assemblies and filters shall be front access. Panels shall remain in place when sealing fasteners are removed. All cabinets shall be provided with a blower access panel. Cabinets fabricated without an access panel large enough to allow removal of the blower motor assembly as one piece shall be prohibited. The design and construction of removable panels shall minimize projections and openings. Removable panels for access into contaminated areas shall be designed so that upon reassembly, a seal is provided as required in 6.2.

5.14 Stability

Cabinets shall stand on the floor or bench top in a stable and secure manner and not tip or fall when tested in accordance with Annex A, section A.7.

5.15 Provision for mounting

Provision shall be made for cleaning, and where necessary, cleaning underneath the unit. All cabinets shall be designed and constructed with one of the following provisions for mounting.

5.15.1 Mounting

The cabinet base shall be designed to be sealed to the mounting surface (floor, raised base, bench top).

5.15.2 Clear space beneath

The cabinet shall be mounted on adjustable legs, or other acceptable means, to ensure a minimum of 4 in (10 cm) of unobstructed clearance beneath the unit. A 2 in (5 cm) minimum clearance beneath the ends of the cabinet is acceptable if the front is open for cleaning and the side panel is equal to or less than 2 in (5 cm) thick (see figures 5, 6, and 7).

5.16 Legs and feet

Legs and feet shall be sufficiently rigid to provide support with a minimum of cross bracing. They shall be fastened to the cabinet and shaped at floor or bench top contact to minimize the accumulation of splash and spillage. Legs and feet shall be of simple design, with no exposed threads. The minimum contact diameter of the foot shall be 0.75 in (19 mm). The foot shall be fabricated with a smooth material to prevent floor damage.

5.17 Reinforcing and framing

Reinforcing and framing members, not totally enclosed or within walls, shall be easily cleanable. Reinforcing and framing members shall not provide harborage for vermin. The ends of all hollow sections, not subject to gas decontamination, shall be closed. Reinforcing and framing members subject to splash or spillage or both shall be sealed. Horizontal angle reinforcing and gussets shall not be placed where soil may accumulate. Where angles are used horizontally, they shall have one leg turned down wherever the equipment permits or be formed integrally with the sides. All vertical channel sections shall be completely closed or open.

5.18 Fixed panels

Fixed panels shall be designed, constructed, and fastened to eliminate projections and openings.

5.19 Doors and covers

Doors and covers shall fit properly and close completely. Horizontal sliding doors shall not be used for the work area. When used for storage areas, doors shall slide easily and be readily removable. Piano and butt-type hinges are acceptable. Handles shall be designed, constructed, and installed to eliminate sharp edges or unnecessary projections. Latches and hold-open mechanisms shall provide even and secure support.

5.19.1 Single panel

Single panel doors (see figure 8) and covers shall be fabricated to minimize the collection of foreign matter and be designed without channel sections at the bottom. Channel sections, if used, shall be

inverted or shallow and wide enough to be easily cleanable. Clean-out holes shall be provided in all channels that are not inverted.

5.19.2 Double panel

Double panel doors and covers shall be fabricated to minimize the collection of foreign matter. Openings to hollow sections shall be closed. If subject to splash, spillage, or both, openings shall be sealed.

5.19.3 Viewing panel

Viewing panels shall be fabricated to prevent particles from entering the workspace by induction through joints, tracks, or guides.

5.19.4 Sliding sash alarm

Sliding sash enclosures shall include an audible and visual alarm activated when the sash is raised (1.0 in (25 mm)) above or positioned (1.0 in (25 mm)) below the manufacturer's specified opening height.

5.20 Louvers and openings

All louvers and openings outside the work area and air plenums shall comply with one or more of the following:

- be of drip deflecting design;
- not be subject to routine splash, spillage, or overhead drippage;
- be designed and constructed to be readily accessible and the space behind easily cleanable; or
- louvers through double panel doors and covers shall be sleeved.

5.21 Tracks and guides

All tracks and guides for doors, sash covers, and access panels shall be designed and constructed to be easily cleaned.

5.22 Filters

- HEPA or ULPA filters shall be required for the downflow and exhaust air systems.

- HEPA and ULPA filters for downflow and exhaust systems shall conform to the materials, construction, and aerosol efficiency requirements of IEST-RP-CC-001.4 for type C, type J, type K, or type F filters. Filter media shall be tested in accordance with the methods of IEST-RP-CC021 with performance levels to meet the minimum efficiency requirements as specified above and the pressure drop requirements as required by the specific application. In addition, HEPA and ULPA filters shall be scan tested for a leakage not to exceed 0.01% when tested in accordance with Annex A, section A.2.

The cabinet shall be designed to provide accessibility for filter installation, testing, and sealing.

- HEPA and ULPA filters shall be mounted to prevent air bypass of the filters. When required, one or more plugged penetrations shall be located in the plenum upstream of the HEPA or ULPA filters and accessible from under the work surface. In the case of a Type B2 cabinet where the downflow plenum is not contaminated, the sample port may terminate anywhere that is accessible from the front of the cabinet. If a Type B2 cabinet is equipped with an exhaust sample port, that sample port shall be accessible from under the work surface. Sample ports shall be capped and labeled. The label shall include the purpose of the penetration (upstream aerosol sampling). Sample ports coming from the plenum to the area under the work surface shall have a minimum inside diameter of ¼ inch (7 mm). The tube shall be short enough that it cannot break the plane of the sash. These penetrations

are used to measure the aerosol concentration upstream of the HEPA and ULPA filters during the HEPA or ULPA filter leak test (see 6.3). When the penetration enters a potentially contaminated space, it shall be labeled "Decontaminate Cabinet Before Opening."

– Cabinets exhausting into the room shall be provided with a perforated exhaust filter guard (see figure 9) to prevent damage to the filter and blockage of exhaust air.

NOTE – An additional airflow sensor may be provided to indicate blockage of exhaust air.

– HEPA and ULPA filter patches shall not exceed 3% of the total face area of the side being patched. The maximum width of any one patch shall not exceed 1.5 in (4.0 cm).

5.23 Gaskets and sealants

Exposed surfaces of gaskets shall be easily cleanable and shall not contain internal angles (angles less than 2.4 rad [135°]). All corner joints and hollow sections of gaskets shall be sealed.

– Fixed gaskets shall be securely fastened and sealed in place.

– HEPA/ULPA filter seals shall be leakproof when tested in accordance with Annex A, section A.3. Gaskets on HEPA/ULPA filters shall have interlocking corners or sealed joints.

– Gaskets used in cabinet seams or on the facing of service panels shall have sealed joints. Structural strength of seams and service panel joints shall be independent of the seal produced by the gasket.

– The structural strength of joints or assemblies where sealant bonding has been applied shall be independent of the sealants.

5.24 Stopcocks and service outlets

Stopcocks and service outlets shall be readily accessible. Electrical outlets on exposed interior surfaces shall have drip-proof caps or gasket seal blade openings.

5.25 Alarms

5.25.1 Sliding sash alarm

Sliding sash enclosures shall include an audible and visual alarm, activated when the sash is raised (1.0 in (25 mm)) above or positioned (1.0 in (25 mm)) below the manufacturer's specified opening height.

5.25.2 Internal cabinet supply/exhaust fan interlock alarm

When a cabinet contains both an internal downflow and exhaust fan, they shall be interlocked so that the downflow fan shuts off whenever the exhaust fan fails. An audible and visual alarm shall signal the failure. If the downflow fan fails, the exhaust fan shall continue to operate, and an audible and visual alarm shall signal the failure.

5.25.3 Type B exhaust alarm

Type B cabinets shall be exhausted by a remote fan. Once the cabinet is set or certified in its acceptable airflow range, audible and visual alarms shall be required to indicate a 20% loss of exhaust volume within 15 s. The internal cabinet fan(s) shall be interlocked to shut off at the same time the alarms are activated. Type B cabinets shall not initiate cabinet blower startup until sensors determine appropriate exhaust flow.

5.25.4 Type A1 or A2 exhaust alarm

Type A1 or A2 cabinets may be connected to an exhaust system via a canopy connection and exhausted by a remote fan. Once the cabinet and canopy is set or certified in its acceptable airflow range, audible and visual alarms shall be required to indicate within 15 s a loss of capture of room air using a visible medium to verify at the canopy air intake(s). The cabinet fan(s) must remain in operation when the alarm is activated.

5.26 Electrical components

5.26.1 Motor

- A thermal protector shall be provided. It shall not trip at 115% of the rated voltage under maximum load and ambient temperature conditions. The motor shall be rated for continuous operation.

- Fan motors shall be sized to operate at a static pressure sufficient to meet the requirements of 6.13.

- All fan motors shall be variable speed and shall have controls that can be secured. Controls shall be installed behind a removable or locked panel. Motor controls shall permit the adjustment of fan speeds to achieve proper airflow balance.

- Motors and lights shall be separately protected from the receptacles. Circuit overload protection conforming to the National Electrical Code shall be provided. Flexible power cords for single-phase power shall be 3 wire, with the ground wire connected to the frame, unless otherwise specified and sized in accordance with the National Electrical Code for the specified load(s).

5.26.2 Electrical wiring, switches, etc.

Replaceable electrical components shall not be located in contaminated air plenums, except for fan motors, sealed nonporous or jacketed wiring, and necessary airflow sensors. All wiring penetrations of contaminated spaces shall be sealed in accordance with 6.2. Circuit overload protection shall be provided for all receptacles. Switches shall be mounted outside the work area. A wiring diagram showing connection of all electrical components shall be permanently attached to the unit in an accessible location outside of air plenum systems. A statement providing starting current, running power, and circuit requirements shall be provided with the installation instructions.

5.27 Lighting

5.27.1 Work lighting

The light intensity at the work surface shall conform to 6.5. Lamps, ballasts, and starters shall be accessible and not installed in contaminated areas. Lamps shall be located so reflection does not interfere with visibility through the sash, and the operator's eyes are shielded from direct radiation.

5.27.2 Ultraviolet lighting[20]

UV lighting is not recommended in Class II (laminar flow) biosafety cabinetry. If requested by the purchaser, it shall be installed in such a manner that it does not reduce the required performance as specified in 6. This Standard does not provide any performance verification of UV lighting.

[20] UV irradiation can cause erythema of skin and eye damage.

5.28 Gauges

Pressure gauges indicating the differential pressure across the recirculated air filter, if provided, shall be installed in accordance with the manufacturer's instructions. Hose connections to the gauge and sampling port shall be secured by positive compression clamps. If threaded connections are used to penetrate the plenum, an engagement of three continuous threads shall be required.

5.29 Drain spillage trough

A drain spillage trough shall be provided below the work surface to retain spillage from the work area; the trough shall be easily cleanable. A drainpipe shall be connected to the drain spillage trough and fitted with a 0.37 in (0.94 cm) or larger ball valve. The drainpipe and valve shall conform to the material requirements of the drain pan or trough. The drain spillage trough shall accommodate at least 1 gal (4 L). The drain valve shall be identified with a label and operating instructions placed in close proximity to, or on, the valve.

5.30 Diffuser placement

Removable diffusers shall be designed and constructed to ensure reassembly in the proper operating position.

5.31 Work area components placement

Readily removable interior work area work surfaces, intake air grills, and exhaust air grills shall be designed and constructed to ensure fixed reinstallation in their proper operating positions.

5.32 Height and width

The cabinet, excluding removable light fixtures, exhaust filter housings and guards, and adjustable legs or feet, shall be sized to fit through a 79 by 35 in (201 x 89 cm) doorway using commonly available furniture moving equipment (jacks and dollies) (see figure 10).

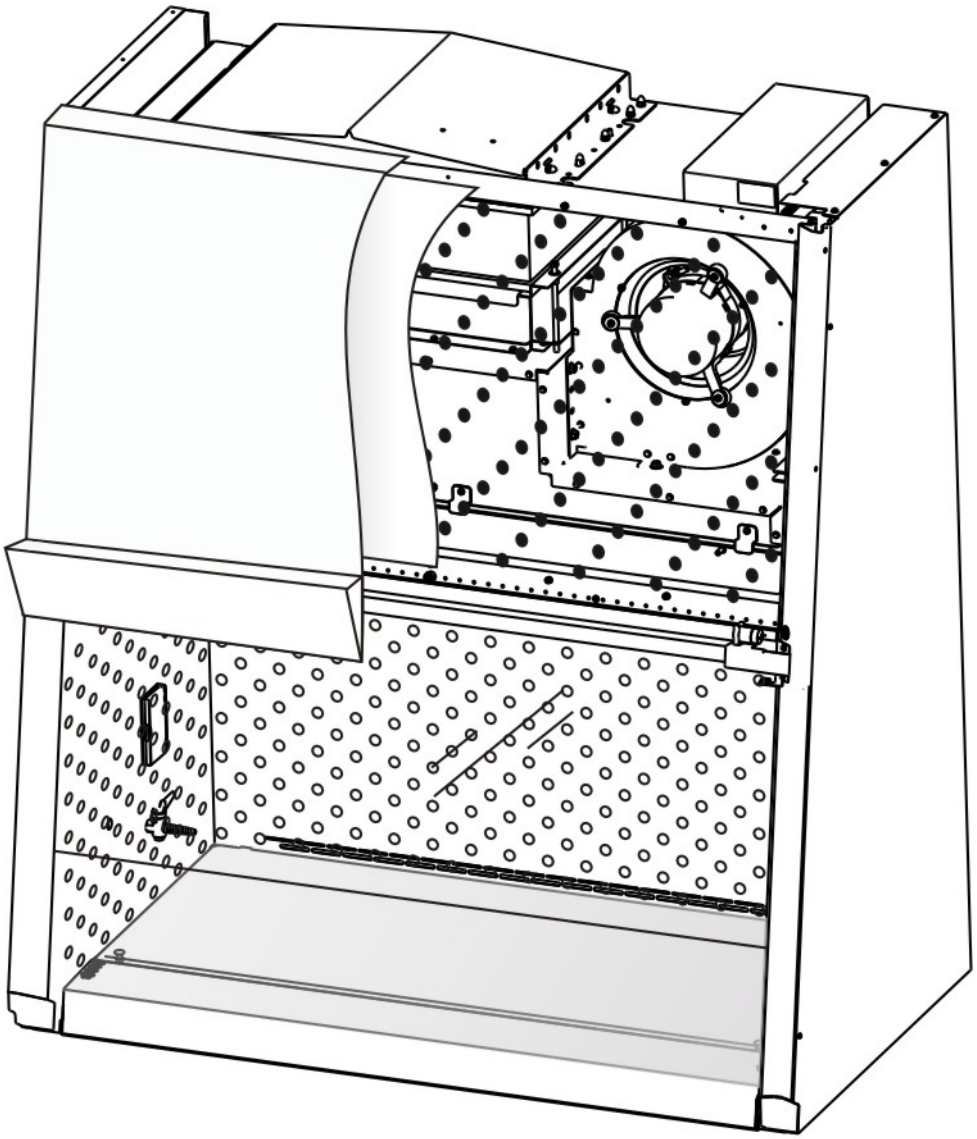
5.33 Data plate(s)

A data plate(s) indicating the following shall be readily visible on the front of the cabinet:

- manufacturer's name and address;
- cabinet model;
- cabinet serial number;
- nominal set point for downflow and inflow velocities (DIM and thermal anemometer);
- type classification;
- downflow velocity test grid dimensions (Annex A, section A.8.3);
- indication that the cabinet has potentially contaminated plenums that are at positive pressure directly to the room (if applicable);
- voltage requirements; and
- inflow velocity test grid and method (Annex A, section A.9.3).

5.34 Routine maintenance adjustment fixtures

Adjustments required during routine recertification shall be possible without entering any contaminated areas of the cabinet, or potentially releasing contaminants. For example, the exhaust damper adjustment fixture may not be located such that it can only be adjusted by exposing a potentially contaminated zone inside the cabinet.



Interior work surfaces

Exposed interior surfaces

Exterior surfaces

Other interior surfaces

Figure 1 - Surfaces

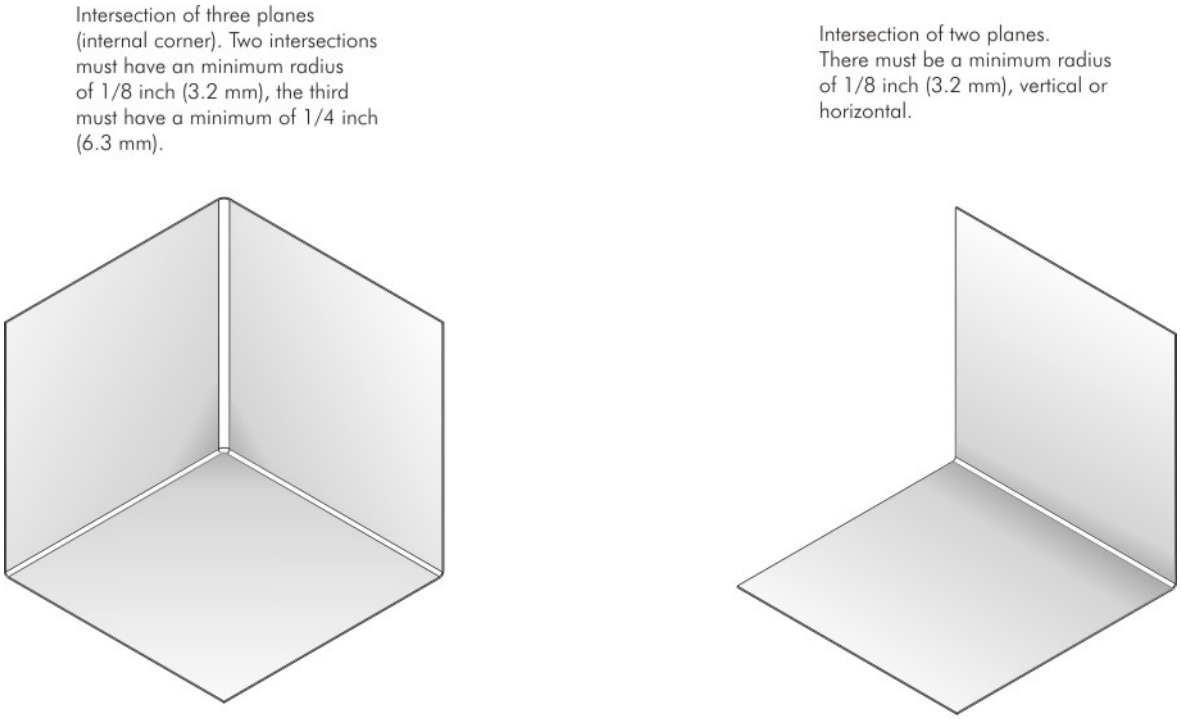


Figure 2 - Internal corners and angles.

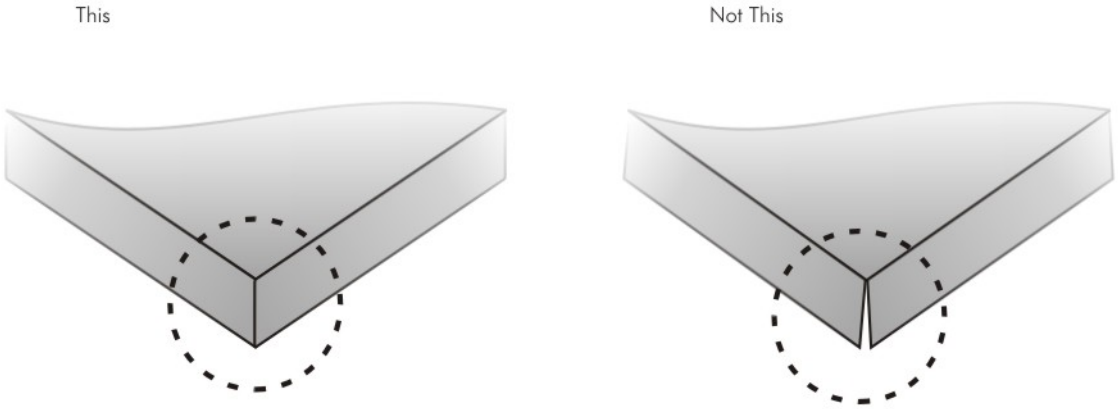


Figure 3 - External corners and angles.

SECTION A-A

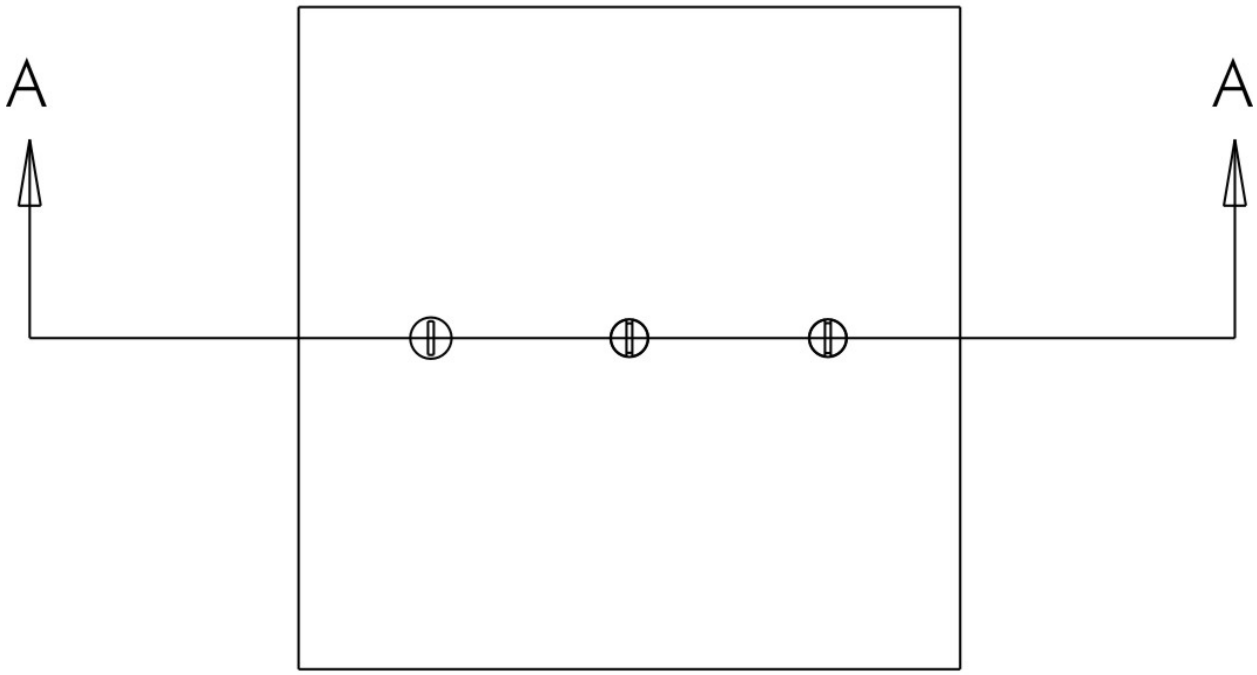


Figure 4 - Low profile - type fasteners

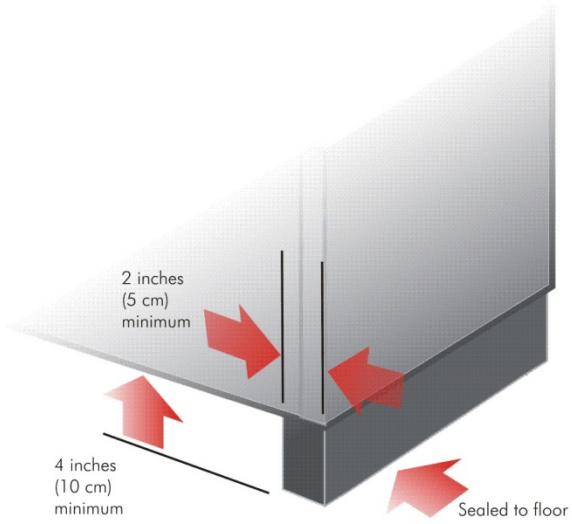


Figure 5 - Clear space beneath

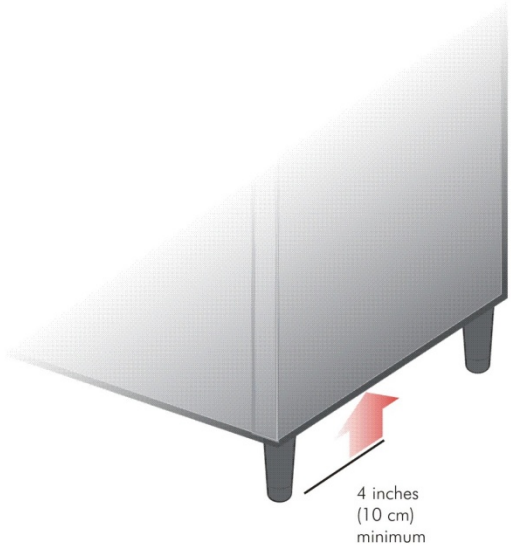


Figure 6 - Clear space beneath

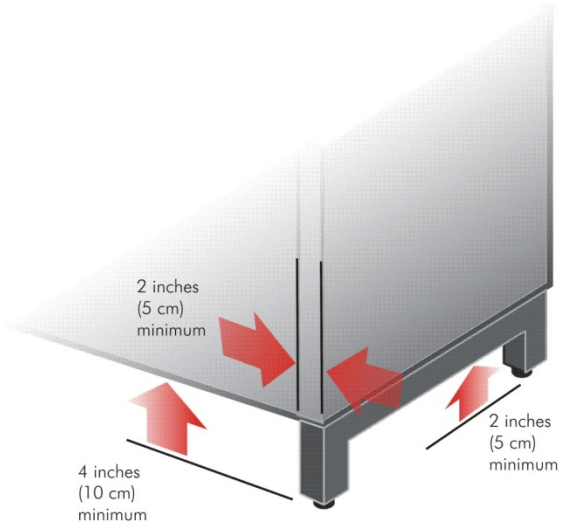


Figure 7 - Clear space beneath

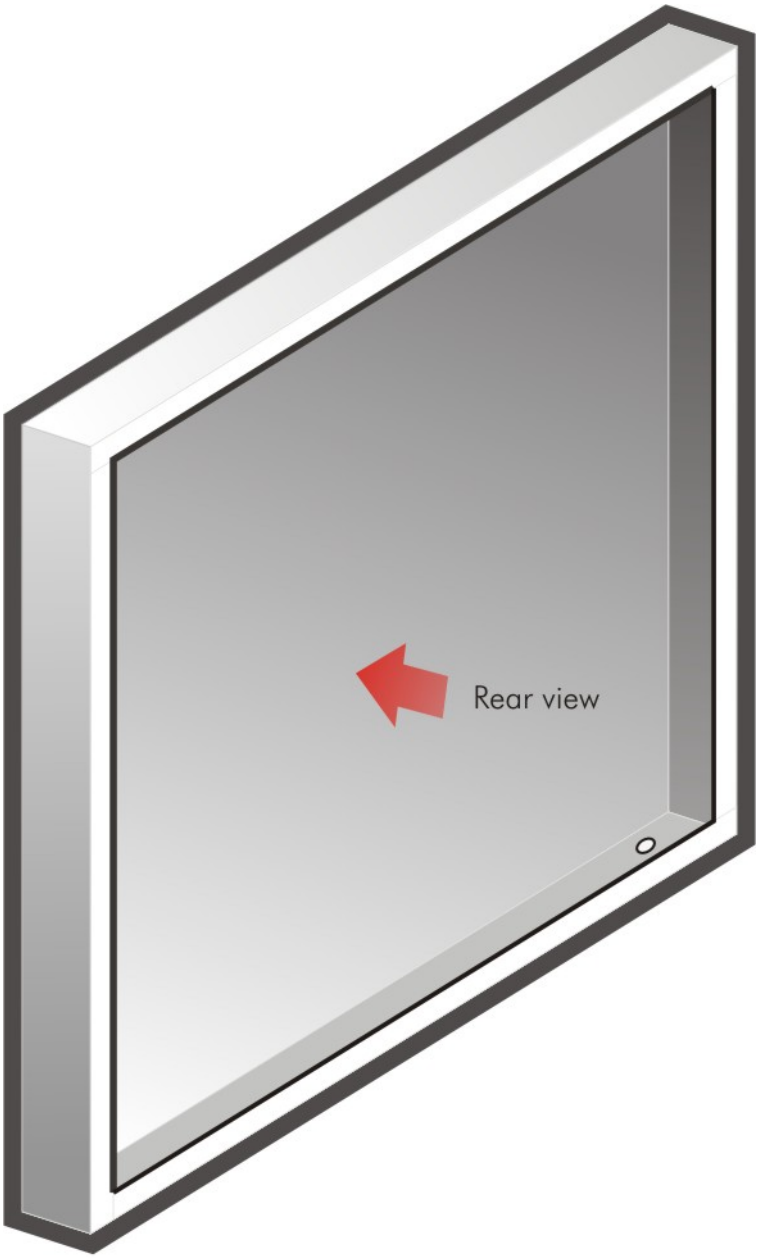


Figure 8 - Single panel door

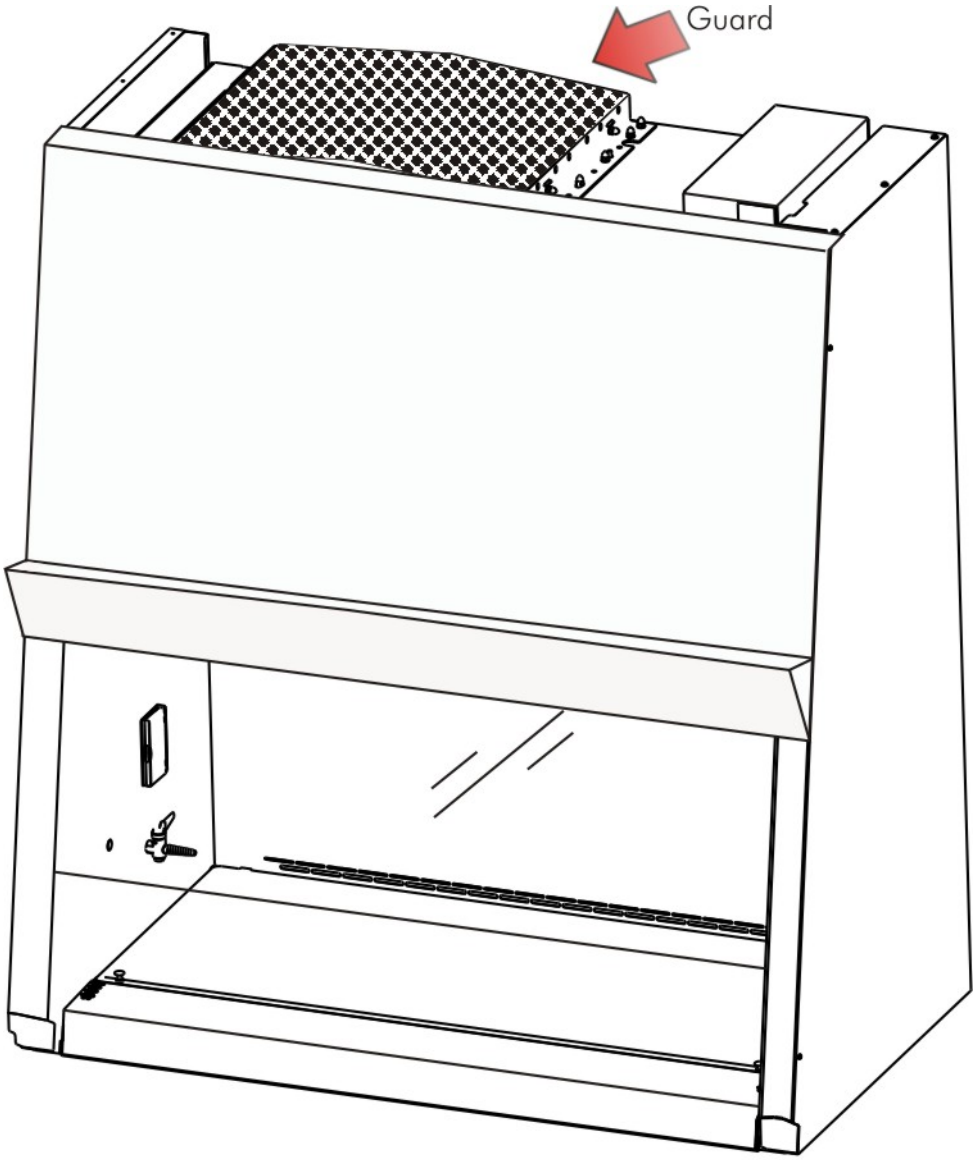


Figure 9 - Exhaust filter guard

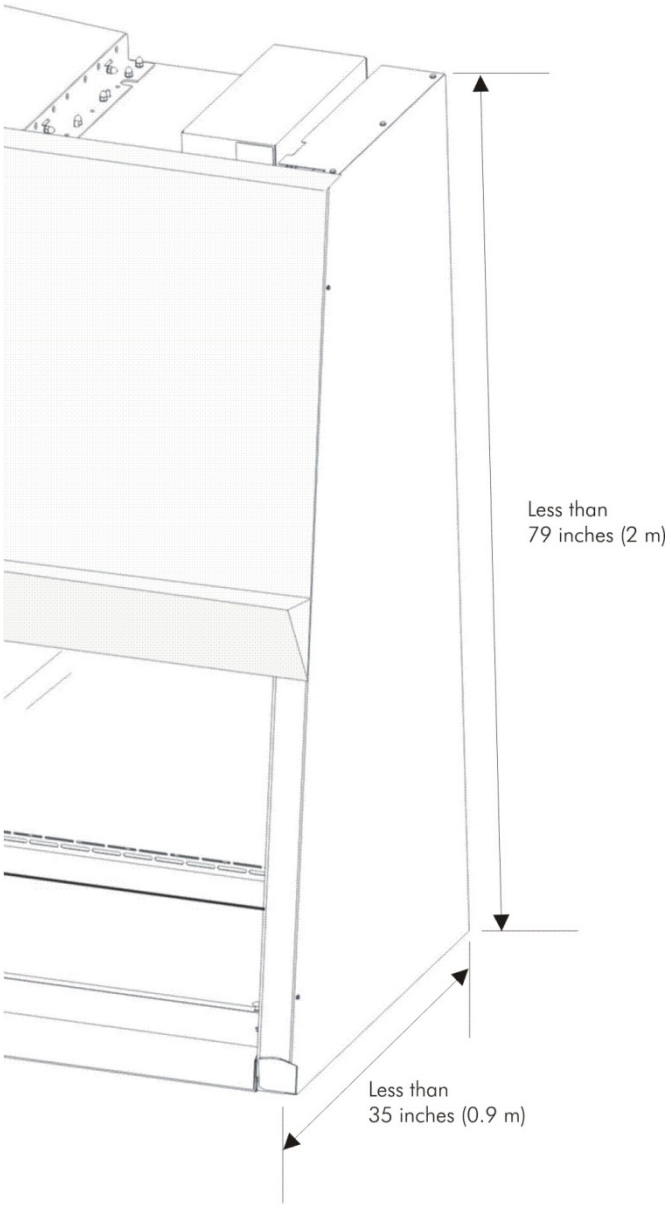


Figure 10 - Height and width

6 Performance

6.1 General

For qualification by the testing organization, biosafety cabinetry shall meet the performance requirements listed in 6.2 through 6.15 when tested in accordance with Annex A. All removable components within the cabinet that are offered as optional equipment by the manufacturer shall be in place during testing except during nominal set point downflow velocity determination.

6.2 Pressure decay / soap bubble / tracer gas leak

The periphery and penetrations of all plenums shall be leak tight when tested by the pressure decay or soap bubble test (see Annex A, section A.1).

6.2.1 The cabinet shall hold 2 in w.g. (500 Pa) within ± 10% for 30 min or all welds, gaskets, penetrations, or seals on exterior surfaces of air plenums shall be free of soap bubbles when at 2 in w.g. (500 Pa) ± 10% pressure above atmospheric.

6.3 HEPA/ULPA filter leak

6.3.1 HEPA/ULPA filters, filter housings, and mounting frames shall be tested with dioctyl phthalate (DOP) or equivalent and determined to be leak tight when cabinet is operating at the nominal set point velocities.

6.3.2 Polydisperse DOP or equivalent sustained penetration shall not exceed 0.01% of the upstream concentration at any point when measured on a linear or logarithmic scale photometer (see 3.19).

6.4 Noise level

6.4.1 The noise level shall be determined with the cabinet operating at the nominal set point velocities.

6.4.2 The overall noise level 12 in (30 cm) in front of the cabinet and 15 in (38 cm) above the plane of the work surface at the vertical centerline of the cabinet shall not exceed 67 dbA with a maximum background level of 57 dbA.

6.5 Lighting intensity

6.5.1 The lighting intensity at the work surface shall be determined with a background lighting intensity in the room of 10 ± 5 fc (110 ± 50 lux) at the work surface elevation.

6.5.2 The average lighting intensity shall be a minimum of 60 fc (650 lux). Individual readings shall be a minimum of 40 fc (430 lux).

6.6 Vibration

The net displacement shall not exceed 2×10^{-4} in (5×10^{-6} m) rms amplitude at frequencies between 10 Hz and 10 kHz in the center of the work surface when the cabinet is operating at the nominal set point velocities.

6.7 Personnel, product, and cross-contamination protection

The cabinet shall meet the requirements of 6.7.1, 6.7.2, and 6.7.3 and Annex A, section A.7, when operating with the airflows specified in that annex.

6.7.1 Personnel protection

The system shall be challenged by 1 x 10^8 to 8 x 10^8 *Bacillus subtilis* var. *niger (B. subtilis)* spores for 5 min. The number of *B. subtilis* colony-forming units (CFU) recovered from the collection suspension of all six glass impinger samplers (AGI-30) shall not exceed 10 CFU per test. Total slit-type air sampler plate counts shall not exceed five *B. subtilis* CFU for a 30 min sampling period. Three replicate tests shall be performed. The control plate shall be positive for *B. subtilis* CFU.

6.7.2 Product protection

The system shall be challenged by 1 x 10^6 to 8 x 10^6 *B. subtilis* spores for 5 min. The number of CFU recovered on agar settling plates shall not exceed 5 CFU for each test. Three replicates shall be performed. The control plate shall be positive for *B. subtilis* CFU.

6.7.3 Cross-contamination protection

The system shall be challenged by 1 x 10^4 to 8 x 10^4 *B. subtilis* spores for 5 min. Some agar plates within 14 in (36 cm) from the challenge sidewall will recover *B. subtilis* CFU and shall be used as positive controls. The number of CFU recovered on agar plates with centers greater than 14 in (36 cm) shall not exceed 2 CFU per test. Three replicates each shall be performed from the left and right sides of the cabinet.

6.8 Stability

The cabinet shall be designed and constructed to resist overturning and distortion under applied forces, resist deflection of the work surfaces under load, and resist tipping under workload.

6.8.1 Resistance to overturning

Cabinets shall conform to the requirements of UL 61010-1 or current edition, section 7.3.

6.8.2 Resistance to distortion

The top front edge and the top of the sides shall not move forward more than 0.063 in (1.6 mm) from the static position when a 250 lb (110 kg) lateral force is applied to the top rear edge and top of the opposite side, respectively.

6.8.3 Resistance to deflection of work surface

The work surface shall not be permanently deflected by a 50 lb (23 kg) test load distributed uniformly over an area 10 x 10 in (25 x 25 cm) in the center of the work surface.

6.8.4 Resistance to tipping

The rear bottom of the cabinet shall not lift off the floor more than 0.062 in (1.6 mm) when a 250 lb (110 kg) test load is applied to the leading edge of the cabinet.

6.9 Downflow and inflow velocities

6.9.1 The average downflow velocity (uniform downflow) or velocities (non-uniform downflow) and the calculated and measured average inflow velocities of the cabinet shall be set at the nominal set points ± 3 ft/min (0.02 m/s) for testing unless otherwise noted. Subsequent production models of the test cabinets of the initial model and size conforming to 6.7 may also qualify when the inflow and average downflow velocity (or velocities, if so specified) operate within ± 5 ft/min (± 0.025 m/s) (see Annex A, section A.9) of the nominal set points of the unit being tested.

6.9.2 Downflow velocity

The downflow velocities are measured in a horizontal plane located 4 in (10 cm) above the bottom edge of the sash in its normal operating position (certified height).

6.9.3 Non-uniform (zoned) downflow velocity

The manufacturer shall designate the test point locations and average downflow velocity in each zone. In each zone, the individual downflow velocities shall not vary more than ± 20% or ± 16 ft/min (± 0.08 m/s), whichever is greater, from the overall average velocity of that particular zone.

6.9.4 Uniform downflow

In a uniform downflow velocity profile, the individual point velocities vary no more than 20% or 16 ft/min (0.08 m/s) (whichever is greater) from the overall average velocity.

6.10 Inflow velocity

The velocity of the inflow air through the work access opening shall be determined. Subsequent production cabinets of the initial model and size conforming to 6.7 may also qualify if the directly measured and calculated inflow velocities are within ± 5 ft/min (± 0.025 m/s) of the nominal set point velocities.

6.10.1 The minimum directly measured and calculated inflow velocities of Type A1 cabinets shall be 75 ft/min (0.38 m/s).

6.10.2 The minimum inflow quantity per 1 ft (0.3 m) of work area width of Type A1 cabinets shall be 45 ft^3/min (0.02 m^3/s) (see 6.7 and 6.9).

6.10.3 The minimum directly measured and calculated inflow velocities of Type A2 cabinets shall be 100 ft/min (0.51 m/s).

6.10.4 The minimum inflow quantity per 1 ft (0.3 m) of work area width of Type A2 cabinets volume rate shall be 65 ft^3/min (0.03 m^3/s) (see 6.7 and 6.9).

6.10.5 The minimum directly measured and calculated inflow velocities of Type B1 and B2 cabinets shall be 100 ft/min (0.51 m/s).

6.10.6 The minimum inflow quantity per 1 ft (0.3 m) of work area width of Type B1 and B2 cabinets volume rate shall be 65 ft^3/min (0.03 m^3/s).

6.11 Airflow smoke patterns

Smoke patterns shall be determined with the cabinet operating at the nominal set point velocities.

6.11.1 Airflow within the work area of the cabinet shall be downward, with no dead spots, reflux, or escape from the cabinet.

6.11.2 Airflow along the entire perimeter of the work access opening shall be inward, with no reflux out of the cabinet or smoke penetration over or onto the work surface.

6.11.3 Airflow within the work area of cabinets shall be downward (no reflux), with no escape to the outside of the cabinet at the sides and top of the sash.

6.12 Drain spillage trough leakage

Drain spillage troughs shall hold a minimum of 1 gal (4 L) of water with no visible leakage after a 1 h holding period.

6.13 Motor/blower performance

When the cabinet is operated at the nominal set point velocities and without readjusting the fan speed control, a 50% increase in pressure drop across the new filter shall not decrease total air delivery more than 10%.

6.14 Electrical safety

The cabinet shall be tested by a National Recognized Testing Laboratory (NRTL) for compliance to the requirements of the current edition of any national standard that is based on IEC 61010-1. Compliance is demonstrated by cabinet listing, i.e. UL, CSA or IECEE CB Scheme certificate.

6.15 Performance data

The manufacturer shall provide a performance data sheet with each cabinet. The following quality control tests shall be conducted in accordance with Annex A and reported for each unit:

- pressure decay / soap bubble / tracer gas leak;
- HEPA/ULPA filter leak;
- downflow velocity;
- inflow velocity; and
- airflow smoke patterns.

The following additional quality control tests shall be conducted in accordance with Annex A and reported on every tenth unit produced:

- noise;
- lighting; and
- vibration.

6.16 Record maintenance

Quality control test results shall be maintained on file at the plant location for a minimum of three years. Current calibration records (obtained within one year) for all quality control test instruments shall be maintained on file at all times.

6.17 Air velocity stability

Air velocity stability shall be determined with the cabinet operating at the nominal set point velocities +/- 3 fpm (0.015 m/s).

6.17.1 When the cabinet is subjected to a 1.0 cm free fall drop on each side, the cabinet inflow velocity and downflow velocity (where applicable) shall not change by more than 5 fpm (0.025 m/s). There shall be no visible damage to the cabiet followig the shock.

6.17.2 When the supply voltage to the cabinet is reduced or increased by 10 percent, the cabinet inflow velocity and downflow velocity (where applicable) shall not change by more than 5 fpm (0.025 m/s).

6.17.3 When the cabinet has been disconnected from power for a minimum of 1 hour, the cabinet inflow velocity (where applicable) and/or downflow velocity (where applicable) shall not change by more than 3 fpm (0.015 m/s) when power is restored. The cabinet shall come on in the same state it was in when

power was lost (lights on, blower on, alarm parameters set, etc.) when power is restored. The cabinet shall provide the user with a visual indication that there was a power loss.

Annex A
(normative)

Performance tests

NOTE – Before any performance tests are run, the cabinet shall be properly installed and leveled and airflows adjusted to the nominal set point (± 3.0 ft/min [± 0.015 m/s]). These tests are intended for the qualification of a new cabinet model by the testing organization. The testing organization also requires and performs appropriate tests during periodic requalification. Cabinet models undergoing major redesign shall be requalified as stated in 1.3 of this Standard. Field tests are provided in Annex F.

Until certified under NSF/ANSI 49 - 2002, all new cabinets shall be factory tested using the procedures described in NSF/ANSI 49, Annex A - 2002, with the exception of the downflow velocity test. When the downflow velocity test is performed, the procedure in NSF 49, 1992 should be used; however, the acceptance criteria outlined in the 2002 standard shall be applied. These are factory testing requirements and may be more stringent than field testing in Annex F relating to variability in the field (ideal conditions).

A.1 Pressure decay / soap bubble

A.1.1 Pressure decay or soap bubble test

A.1.1.1 Purpose

This test on exterior surfaces of all plenums determines whether welds, gaskets, plenum penetrations, and seals are free of leaks.

A.1.1.2 Apparatus

- manometer, pressure gauge, or pressure transducer system with a minimum range of 0 – 2 in w.g. (0 – 500 Pa) and accurate to ± 0.02 in w.g. (5 Pa);
- liquid leak detector;
- plastic sheet (0.02 in [0.5 mm] extruded high-impact styrene); and
- duct tape.

A.1.1.3 Method (pressure decay)

a) Prepare the cabinet as a sealed system, i.e., seal the front sash and exhaust port.

b) Remove decorative panels and other access obstructions, where necessary, to expose plenums to be tested.

c) Attach a manometer, pressure gauge, or pressure transducer system to the test area to indicate the interior pressure.

d) Pressurize the cabinet with air to a reading of 2 in w.g. (500 Pa), turn off the pressurizing air, and measure the pressure after 30 min. A leakage of 10% of the original pressure is allowable. If a cabinet does not hold 2 in w.g. (500 Pa), use the soap bubble method to locate leaks.

A.1.1.4 Method (soap bubble)

a) Prepare the cabinet as a sealed system, i.e., seal the front sash and exhaust port.

b) Remove decorative panels and other access obstructions, where necessary, to expose plenums to be tested.

c) Attach a manometer, pressure gauge, or pressure transducer system to the test area to indicate the interior pressure.

d) Pressurize the cabinet with air to ensure a continuous reading of 2 in w.g. (500 Pa) ± 10%.

e) Spray or brush the liquid leak detector along all welds, gaskets, penetrations, and seals on exterior surfaces of cabinet plenums. Small leaks will be indicated by bubbles. Large leaks will occur that blow the detection fluid from the hole without forming bubbles and may be detected by slight feel of airflow or sound.

A.1.1.5 Acceptance

The cabinet shall hold 2 in w.g. (500 Pa) ± 10% for 30 min or all welds, gaskets, penetrations, and seals on exterior surfaces of air plenums shall be free of soap bubbles when at 2 in w.g. (500 Pa) ± 10% pressure above atmospheric.

A.2 HEPA/ULPA filter leak test

A.2.1 Purpose

This test determines the integrity of downflow and exhaust HEPA/ULPA filters, filter housings, and filter mounting frames. The cabinet shall be operated within ± 3.0 ft/min (0.015 m/s) of the nominal set point, with the exception of the downflow HEPA/ULPA filters on B1 cabinets.

A.2.2 Apparatus

A.2.2.1 An aerosol photometer with linear or expanded logarithmic scale shall be used. The instrument shall be capable of indicating 100% upstream concentration with an aerosol of 10 µg/L of polydisperse dioctylphthalate (DOP) particles, or an equivalent fluid, which provides the same particle size distribution (e.g., polyalpha olefin [PAO], di[2-ethylhexyl], sebecate, polyethylene glycol, and medicinal-grade light mineral oil)[21] produced by the generator described in Annex A, section A.2.2.2. It shall also be capable of detecting an aerosol of 1×10^{-3}% of the same particles. The sampling rate of air shall be at least 1 ft^3/min (5×10^{-4} m^3/s) ± 10%. The probe area shall have a maximum open area of 1.7 in^2 (11 cm^2) and a minimum dimension of 0.5 in (1.3 cm). The photometer shall be calibrated in accordance with the photometer manufacturer's instructions or with IEST-RP-CC-013 if instructions are not provided.

A.2.2.2 An aerosol generator of the Laskin Nozzle type conforming to Annex A, figure A1 or equivalent shall be used to create an aerosol by flowing air through liquid DOP or an equivalent substitute. When a Laskin nozzle generator is used, the compressed air supplied to the generator should be adjusted to a minimum of 20 psi (140 kPa), if using DOP or 23 psi (160 kPa) if using PAO, measured at the generator manufacturer's recommended location. The nozzles shall be covered with liquid to a depth not to exceed 1.25 in (31 mm).

A.2.2.3 A pressure gauge for the generator having a maximum range of 0 – 80 psi (0 – 550 kPa) with a resolution and accuracy of 1 psi (7 kPa) calibrated by the manufacturer or in accordance with the manufacturer's instructions shall be used.

[21] Hinds, W., Macher, J., First M. W. *Size Distributions of Aerosols Produced from Substitute Materials by the Laskin Cold DOP Aerosol Generator*. 16th Dept. of Energy Nuclear Air Cleaning Conference; and Yan, X., First, M. W., Rudnicks, S. N. *Characteristics of Laskin Nozzle Generated Aerosols*. Proc. 21st Nuclear Air Cleaning Conference. M. W. First, Ed., N. T. I. S., Springfield, VA, Feb. 1991. p. 116

A.2.3 Method

A.2.3.1 Filters that can be scanned

a) Turn on the cabinet blower and lights (types A1/A2 and B2 – downflow filter test). Remove filter diffusers and protective covers if they are present. Place the generator so the aerosol is introduced into the cabinet, as specified by the manufacturer, to provide uniform distribution upstream of the HEPA/ULPA filter. When the manufacturer has not identified the aerosol introduction point(s), introduce the aerosol in such a manner as to ensure thorough mixing in the cabinet airflow. For example, a T-connection can be fitted to the aerosol generator output to enable distribution of challenge into both entrances of a single blower, or entrances of multiple blowers. The manufacturer shall determine the aerosol introduction point that provides the most uniform distribution (reference IEST-RP-CC-034[22]). The location of the aerosol introduction point shall be clearly described or indicated in a manner readily available to the certifier. The location should be described either on the cabinet data plate or with the electrical schematic if the schematic is affixed to the cabinet.

b) Turn on the photometer and adjust in accordance with the manufacturer's instructions.

c) Sample the aerosol concentration upstream of the HEPA/ULPA filter and verify that the concentration gives a light scattering intensity at least equal to that produced by 10 µg/L of DOP.

- For linear readout photometers (graduated 0 – 100), adjust the instrument to read 100 on the 100% scale.

- For logarithmic readout photometers, adjust the upstream concentration to 1 x 104 above the concentration needed to produce one scale division (use the instrument calibration curve).

d) With the nozzle of the probe held not more than 1.0 in (2.5 cm) from the area being tested, scan the entire downstream side of the HEPA/ULPA filters, and the perimeter of each filter pack, by passing the photometer probe in slightly overlapping strokes at a traverse rate of not more than 2 in/s (5 cm/s). Separate passes shall be made around the entire periphery of the filter, along the bond between the filter pack and frame, and around the seal between the filter and the device.

A.2.3.2 Filters that cannot be scanned

When a cabinet is ducted so that the exhaust filter cannot be scanned, it may be leak tested by drilling a hole approximately 0.3 in (1 cm) in diameter in the duct at a downstream location that will produce a well-mixed aerosol, and inserting the photometer sampling probe with rigid extension tubing through the hole.

A.2.4 Acceptance

A.2.4.1 Filters that can be scanned

Sustained aerosol penetration shall not exceed 0.01% of the upstream concentration at any point.

A.2.4.2 Filters that cannot be scanned

Sustained aerosol penetration shall not exceed 0.005% of the upstream concentration.

[22] *HEPA/ULPA and ULPA Filter Leak Tests*, Institute of Environmental Sciences and Technology, 940 East Northwest Highway, Mount Prospect, IL 60056 <www.iest.org>

A.3 Noise level test

A.3.1 Purpose

This test provides a uniform method for measuring the noise level produced by the cabinet. The methods can be performed in most acoustically ordinary rooms, such as a factory, where walls are neither sound absorbing nor completely sound reflecting. The cabinet shall be operated at the nominal set point velocities within ± 3.0 ft/min (± 0.015 m/s).

A.3.2 Apparatus

The measuring instrument shall be a type/class 1 sound level meter with a minimum range of 50 to 100 db and an "A" weighting scale set up in accordance with the manufacturer's instructions.

A.3.3 Method

a) Turn on the cabinet blower and lights.

b) Set the instrument to the "A" weighting mode.

c) Measure the noise level 12 in (30 cm) in front of the cabinet leading front edge of the access opening and 15 in (38 cm) above the plane of the work surface, in line with the vertical centerline of the cabinet (see Annex A, figure A2).

d) To measure the ambient noise level, turn the cabinet blower and lights off, and if applicable, leave the remote exhaust blower on and measure as in c) above.

A.3.4 Acceptance

Overall noise level in front of the cabinet shall not exceed 67 dbA when measured where the maximum ambient sound level is 57 dbA. When the ambient sound level is greater than 57 dbA, the reading obtained in Annex A, section A.4.3 c) shall be corrected in accordance with curves or tables provided in the instrument operator's manual. If this information is not available, use standard correction curves or tables (see below).

Correction chart for sound level readings

Difference between total and background sound readings in dbA	Number to subtract from total to yield corrected noise level
0-2	reduce background levels
3	3
4-5	2
6-10	1
>10	0

A.4 Lighting intensity test

A.4.1 Purpose

This test determines the light intensity on the work surface of the cabinet in fc (lux).

A.4.2 Apparatus

A portable photoelectric illuminance meter, as described in The Lighting Handbook[23], section 9.8.1. The meter shall be accurate within ± 10%, cosine and color corrected. The illuminance meter shall be calibrated in accordance with the manufacturer's instructions.

A.4.3 Method

a) With the cabinet lights off, measure the background lighting intensity along the side-to-side centerline of the work tray on a uniform linear pattern in increments close to but no greater than 12 in (30 cm), starting 6.0 in (15 cm) from the side walls (see Annex A, figure A3).

b) Turn on the lights and blower.

c) Measure the cabinet light intensity along the side-to-side centerline of the work tray on a uniform linear pattern in increments close to but not greater than 12 in (30 cm), starting 6.0 in (15 cm) from the side walls (see Annex A, figure A3).

A.4.4 Acceptance

Lighting intensities shall average a minimum of 60 fc (650 lux) on the work surface, and individual readings shall not be below 40 fc (430 lux) when measured where the background light levels average 10 ± 5 fc (110 ± 50 lux) at the work surface.

A.5 Vibration test

A.5.1 Purpose

This test determines the amount of vibration in the operating cabinet. The cabinet shall be operated within ± 3.0 ft/min (± 0.015 m/s) of the nominal set point velocities.

A.5.2 Apparatus

A vibration analyzer with an accuracy of 5% of full scale and a minimum reliable reading of 1.0×10^{-4} in (2.5 µm) rms amplitude or the ability to detect differences of this magnitude, set up in accordance with manufacturer's instructions.

A.5.3 Method

a) To determine the vibration displacement on the vertical axis, affix the sensing element of the vibration pickup unit firmly onto the geometric center of the work surface(s) by:

- clamping;
- bolting; or
- using an integral magnet with petroleum jelly film, or a double-faced adhesive tape.

The test position is shown in Annex A, figure A4.

b) Determine the gross vibration amplitude with the cabinet operating.

c) Determine the background vibration amplitude with the cabinet blower(s) off and, if applicable, the exhaust blower on.

[23] Illuminating Engineering Society, 120 Wall Street, Floor 17, New York, NY 10005-4001 <www.iesna.org>

d) Subtract the background from the gross vibration amplitude to determine the net vibration amplitude attributable to the cabinet.

A.5.4 Acceptance

Net displacement shall not exceed 2 x 10^{-4} in (5 µm) rms amplitude at 10 Hz to 10 kHz in the center of the work surface(s).

A.6 Personnel, product, and cross-contamination protection (biological) tests

A.6.1 Purpose

These tests determine whether aerosols will be contained within the cabinet, outside contaminants will not enter the cabinet work area, and aerosol contamination of other equipment in the cabinet will be minimized. The cabinet shall be operated at the airflow velocities indicated in the specific test methods with removable equipment installed. The cabinet shall be turned on at least 30 min before the start of any test and operated continuously throughout all test methods. Cabinets meeting these test requirements shall then meet airflow characteristics as measured in Annex A, sections A.8 and A.9.

A.6.2 Materials

- spores of *Bacillus subtilis* var. *niger* (*B. subtilis*), ATCC 9372[24], or NCTC No. 10073[25]; and
- sterile diluent prepared as follows:

a.1) Step 1: concentrated diluent phosphate buffer solution (PBS):

- dissolve 34 g KH_2PO_4 in 500 ml distilled water;
- adjust pH to 7.2 ± 0.5 with 1 N NaOH at 77°F (25°C); and
- dilute to 1 L with distilled water.

a.2) Step 2: final diluent PBS:

- distilled H_2O – 1 L;
- stock PBS step 1 – 1.25 ml;
- final pH – 7.2 ± 0.5;
- autoclave at 250°F (120°C) for 15 min; and
- optional – magnesium sulfate (50 g $MgSO_4 \cdot 7H_2O$ per L distilled water) – 5.0 ml.

Or

b)

- distilled water – 1 L;
- adjust pH to 7.0 ± 0.1 at 77°F (25°C);
- autoclave at 250°F (120°C) for 15 min;

[24] American Type Culture Collection, Rockville, MD <www.atcc.org>

[25] National Collection Type Culture, London, England <www.ukncc.co.uk/>

NOTE – Formula b) is suitable for diluent when spore suspension is prepared for immediate use. When storage of diluent suspension at 39.2°F (4°C) is required, formula a) should be used;

– petri plates (100 x 15 mm and 150 x 22 mm) containing nutrient agar, trypticase (tryptic) soy agar (TSA)[26,27], or other suitable growth medium with no inhibitors or other additives;

– six AGI-30 samplers (flow rate calibrated at 12.3 to 12.6 L/min) containing 20 ml of sterile diluent. The AGI-30 samplers shall be Ace Glass, Inc., Vineland, NJ, Catalog Number 7540-10, air sampling impingers, or equivalent;

– two slit-type air samplers operating at a rated flow of 1.0 ± 0.05 ft^3/min (28 ± 1.4 L/min);

– refluxing 6-jet modified MRE-type short-form colision nebulizer (available as Model CN-38 Nebulizer [Model NSF CN-31/I] from BGI, Inc., Waltham, MA) or any other nebulizer demonstrated to produce a bacterial aerosol of equivalent characteristics;

– one 2.5 in (63 mm) outside diameter stainless steel, steel, or aluminum cylinder with closed ends shall be used to disrupt the airflow. The length is to be determined by the size of the cabinet interior. One end butts against the back wall of the work area and the other end protrudes at least 6.0 in (15 cm) into the room through the work access opening of the cabinet;

– a pressure gauge having a minimum range of 0 – 30 psi (0 – 210 kPa) maximum range of 0 – 50 psi (0 – 340 kPa) with a resolution and accuracy of 1 psi (7 kPa) calibrated by the manufacturer or in accordance with the manufacturer's instructions shall be used for operation of the Nebulizer;

– suspension of *B. subtilis* var. niger spores prepared as follows:

  – Method A (using previously harvested *B. subtilis* spores)

    a) Aseptically inoculate (by streak plating technique) several TSA petri plates (100 x 15 mm).

    b) Incubate for 48 ± 2 h at 99 ± 1°F (37 ± 0.5°C).

    c) Remove characteristic (pigmented dark orange) colonies and transfer them to ten 220-ml sterile screw-capped bottles each containing approximately 50 ml of TSA.

    d) Incubate for 48 ± 2 h at 99 ± 1°F (37 ± 0.5°C).

    e) Add 10 ml of PBS to each slant and gently wash the bacteria from the agar surface.

    f) Combine the bacterial suspensions to yield approximately 100 ml in a sterile 150-ml screw-cap bottle. Heat the stock culture at 149 ± 1°F (65.0 ± 0.5°C) for 15 min. If cell debris interferes with nebulizer dissemination, the suspension may be clarified by

[26] BBL Microbiological Systems, Cockeysville, MD 21030 <www.bd.com>

[27] Difco Laboratories, P.O. Box 331058, Detroit, MI 48232-7058 <www.vgdllc.com>

washing three times in PBS by centrifugation at 2500 rpm for 15 min. Re-suspend in PBS to the original volume.

g) Determine spore concentration by standard dilution-plate methods[28] using PBS and TSA. Spores prepared as above should yield an average count of 2 x 10^9 to 4 x 10^9/ml.

h) Incubate plates for 48 ± 2 h at 99 ± 1°F (37 ± 0.5°C).

i) Dilute the spore suspension with PBS to obtain a final spore concentration of 5 x 10^8 to 8 x 10^8/ml if the spores are to be used immediately.

j) Store the stock spore suspension (2 x 10^9 to 4 x 10^9/ml) at 39°F (4°C) or divide it into aliquots to store in screw-capped vials at -94°F (-70°C). Make frequent checks of spore viability by surface plating and of spore predominance by an acceptable spore staining technique.[29]

– Method B

a) Inoculate 250-ml portions of sterile tryptose broth with aliquots of previously harvested *B. subtilis* spores, or rehydrated freeze-dried cultures per ATCC or NCTC instructions.

b) Incubate on a reciprocating shaker for 48 ± 2 h at 99 ± 1°F (37 ± 0.5°C).

c) Heat the stock cultures at 149 ± 1°F (65 ± 0.5°C) for 15 min.

d) Transfer the suspensions to screw-cap test tubes and wash at least three times in sterile distilled water by centrifugation at 2500 rpm for 15 min. Use PBS in the last washing if storage is required.

e) Determine spore concentration by standard dilution-plate methods using PBS and TSA. Spores prepared as described above should average 1.5 x 10^9/ml.

f) Incubate the plates for 48 ± 2 h at 99 ± 1°F (37 ± 0.5°C).

g) If the spore suspension is to be used promptly, dilute the spore suspension with PBS to obtain a final suspension concentration of 5 x 10^8 to 8 x 10^8/ml.

h) To store the stock spore culture, divide it into aliquots and store it at 39°F (4°C) in sterile screw-cap vials or store it in a freezer at -94°F (-70°C). Before use, check the viability of the spore suspension as described in Annex A, section A.7.3.1.

[28] Standard Methods for the Examination of Water and Wastewater, Twenthieth Edition, American Public Health Association, 1015 Eighteenth Street NW, Washington, DC 20036 www.apha.org

[29] APHA Intersociety/Agency Committee on Microbiological Methods for Foods, "Compendium of Methods for Microbiological Examinations of Foods," 1976, pp. 92-93 <www.apha.org>

A.6.3 Personnel protection test (system challenged with 1 x 10^8 to 8 x 10^8 *B. subtilis* spores in 5 min)

A.6.3.1 Method

a) Set the cabinet at the nominal set point airflow velocities.

b) A nebulizer containing up to 55 ml of spore suspension (5 x 10^8 to 8 x 10^8/ml) shall be centered between sidewalls of the cabinet. The horizontal spray axis shall be placed 14 in (35 cm) above the work surface; the opening of the nebulizer shall be 4 in (10 cm) behind the sash. The spray axis shall be parallel to the work surface and directed toward the sash (see Annex A, figure A5).

c) The cylinder shall be placed at the cabinet center. The axis of the cylinder shall be 2.75 in (7.0 cm) above the work surface. Around the cylinder, 4 AGI-30s shall be positioned with the sampling inlets 2.5 in (6.3 cm) outside the cabinet front. Two AGI-30s shall be placed so that their inlet axes are 6.0 in (15 cm) apart and in a horizontal plane tangent to the top of the cylinder. Two AGI-30s shall be positioned so that their inlet axes are 2.0 in (5.0 cm) apart and lie in a horizontal plane 1.0 in (2.5 cm) below the cylinder. As a positive control, an agar plate shall be placed under the center of the cylinder, and supported a minimum of 0.50 in (1.3 cm) above or below the front intake grill, to minimize the obstruction of airflow into the grill (see Annex A, figures A6 and A7).

d) Two slit-type air samplers shall be placed so that the horizontal plane of the air inlets is at the work surface elevation, and the vertical axes of the inlets are 6.0 in (15 cm) in front of the cabinet and 8.0 in (20 cm) from each interior sidewall. When the nominal width of the test cabinet is less than 3 ft, the two slit-type air samplers shall be placed so that the horizontal plane of the air inlets is at the work surface elevation, and the vertical axes of the inlets are 6.0 in (15 cm) in front of the cabinet and 2.0 in (5.1 cm) from each interior sidewall. Two AGI-30 samplers shall be placed so that the horizontal plane of the air inlets is 14 in (36 cm) above the work surface, the vertical axes are 2.0 in (5.0 cm) outside the front edge of the cabinet, and there are 6.0 in (15 cm) on each side of the cabinet centerline (see Annex A, figure A7).

e) Duration of the test shall be 30 min. The test sequence shall be as follows:

Time remaining (min)	Activity
30	start slit samplers
25	start nebulizer
24	start impingers
19	stop impingers
18.5	stop nebulizer
0	stop slit samplers

Three replicate tests shall be performed.

f) Filter the sampling fluid from all of the AGI-30 samplers[30] through a 1.85 in (47.0 mm) diameter 0.22 µm membrane filter, remove the filter aseptically, and place it on appropriate media. Incubate plates containing the filters and plates from the slit-type air samplers at 98.6°F (37.0°C). Examine them at 24 – 28 h, and if negative, re-incubate and read at 44 – 48 h.

g) For new and major modification redesign cabinet models, repeat the above steps after setting the cabinet airflow velocities at -10 ± 3.0 ft/min (-0.051 ± 0.015 m/s) inflow using a direct airflow reading instrument and +10 ± 3.0 ft/min (+0.051 ± 0.015 m/s) downflow above and below the nominal set points.

[30] For research and field applications, the sampling fluid may be filtered separately from each AGI sampler to provide information on specific areas within the cabinet.

– Airflow velocity readjustments shall be made per the manufacturer's procedure.

– The overall average downflow velocity shall be used in making downflow adjustments.

– Removable equipment not essential to cabinet operation shall be removed to set the downflow velocity.

h) For new and major modification redesign cabinet models, repeat the above steps setting the airflow velocities at -10 ± 3.0 ft/min (0.051 ± 0.015 m/s) from the nominal set point for both downflow and inflow.

A.6.3.2 Acceptance

The number of *B. subtilis* CFU recovered from the 6 AGI-30 samplers shall not exceed 10 CFU per test. Total slit-type air sampler plate counts shall not exceed five *B. subtilis* CFU for a 30 min sampling period. Three replicate tests shall be performed. The control plate shall be positive. A plate is "positive" when it contains greater than 300 CFU of *B. subtilis*.

A.6.4 Product protection test (system challenged by 1 x 10^6 to 8 x 10^6 *B. subtilis* spores in 5 min)

A.6.4.1 Method

a) Set the cabinet at nominal set point airflow velocities.

b) Cover the work surface with open agar plates 100 x 15 mm with the cylinder at the midpoint (see Annex A, figure A8).

c) Position the horizontal spray axis of the nebulizer containing 55 ml of 5 x 10^6 to 8 x 10^6 spores/ml at the level of the top edge of the work opening, and center it between the two sides of the cabinet, with the opening of the nebulizer 4 in (10 cm) outside the sash. The spray axis shall be parallel to the work surface and directed toward the open front of the cabinet.

d) A 2.5 in (6.3 cm) outside diameter cylinder, with closed ends, shall be placed in the center of the cabinet. The cylinder shall be positioned in the cabinet so that one end butts against the back wall of the work area, the other end extends at least 6.0 in (15 cm) into the room through the front opening of the cabinet, and the axis of the cylinder is 2.75 in (7.0 cm) above the work surface.

e) As a positive control, an agar plate shall be placed under the center of the cylinder and supported 0.5 in (1 cm) above or below the front intake grill to minimize the obstruction of airflow into the grill (see Annex A, figure A9).

f) The nebulizer shall be operated for 5 min. 5 min after nebulization is terminated, lids shall be placed on the agar plates.

g) The plates shall be incubated at 98.6°F (37.0°C) and examined at 24 – 28 h. If negative, they shall be re-incubated and read at 44 – 48 h.

h) For new and major modification redesign cabinet models, the above steps shall be repeated after the cabinet airflow velocities are set at ± 10 ±3.0 ft/min (+0.051 ± 0.015 m/s) inflow using a direct airflow reading instrument and -10 ± 3.0 ft/min (-0.051 ± 0.015 m/s) downflow from nominal set points.

– Airflow velocity readjustments shall be made per the manufacturer's procedure.

– The overall average downflow velocity shall be used in making downflow adjustments.

– Removable equipment not essential to cabinet operation shall be removed to set the downflow velocity.

A.6.4.2 Acceptance

The number of *B. subtilis* CFU on agar settling plates shall not exceed 5 CFU for each test. Three replicates shall be performed. The control plates shall be positive. A plate is "positive" when it contains more than 300 CFU of *B. subtilis*.

A.6.5 Cross-contamination test (system challenged by 1 x 10^4 to 8 x 10^4 *B. subtilis* spores for 5 min)

A.6.5.1 Method

a) Set the cabinet at the nominal set point airflow velocities.

b) Position the horizontal spray axis of the nebulizer containing 55 ml of 5 x 10^4 to 8 x 10^4 spores/ml 3.0 – 5.0 in (76 – 130 mm) above the work surface, with the back of the nebulizer located against the midpoint of the left interior side wall. The spray axis shall be parallel to the work surface and directed toward the opposite sidewall.

c) Place open agar settling plates (100 x 15 mm) on the work surface in the following manner (see Annex A, figure A10):

– two rows of control plates with the centerline under the outlet of the nebulizer;

– one row of plates with their centers on a line drawn front to back 14 in (36 cm) from the side wall being tested; and

– at least one more row of plates nested beyond the 14 in (36 cm) row; two rows when there is room.

d) Start the nebulizer. After 5 min, stop the nebulizer.

e) After 15 min, place the covers on the open agar plates. Incubate the plates at 98.6°F (37.0°C) and examine them at 24 – 28 h. If negative, re-incubate and read at 44 – 48 h.

f) Perform the same procedure [a) to e)], but place the nebulizer against the midpoint of the right interior wall.

A.6.5.2 Acceptance

Some agar plates, from the challenge sidewall to 14 in (36 cm) from the sidewall, will recover *B. subtilis* CFU and shall be used as positive controls. The total number of CFU recovered on agar plates with centers greater than 14 in (36 cm) shall not exceed 2 CFU per test. Three replicates each shall be performed from the left and right sides of the cabinet.

A.7 Stability tests

A.7.1 Purpose

These tests demonstrate the structural integrity and stability of a biosafety cabinet for the following:

– resistance to overturning under applied forces (refer to UL 61010-1 or current edition) cited in 6.8.1 of this Standard);

- resistance to distortion under applied forces;
- resistance to deflection of work surfaces under load; and
- stability with respect to tipping under load.

Tests are performed by applying static force loads, as described below, and measuring the distortion or deflection within the cabinet.

A.7.2 Apparatus

- compression force gauge or extension spring balance, calibrated in pounds, with an accuracy of ± 1% full scale; or

  NOTE – Where an extension type spring balance is used; force shall be applied as "pull" at opposite side of device from that specified in methods below.

- test loads;
  - 250 lb (114 kg) uniformly distributed over an area of 10 x 10 in (250 x 250 mm);
  - 50 lb (23 kg) uniformly distributed over an area 10 x 10 in (250 x 250 mm); and
  - dial indicator with a minimum accuracy of 0.001 in (0.02 mm).

A.7.3 Resistance to overturning

A.7.3.1 Method

a) Block the cabinet (adjusted to manufacturer's tallest rated service position on stand if applicable) at front or rear bottom edge to prevent lateral movement.

b) Tilt the cabinet 10° from horizontal in the direction most likely to cause overturn.

A.7.3.2 Acceptance

The cabinet shall not initiate overturn when tilted 10° from horizontal in the direction most likely to cause overturn.

A.7.4 Resistance to distortion under applied forces

A.7.4.1 Method

a) Bolt the device securely to a firm base or floor to prevent overturning and lateral movement.

b) Apply a force of 250 lb (1120 N) at top rear and one top side edge. Measure the forward deflection of the top front edge and opposite top side edge with a dial indicator (see Annex A, figures A11 and A12, respectively).

Report the deflection.

A.7.4.2 Acceptance

The top front edge and the top of the sides shall not move forward more than 0.062 in (1.6 mm) from a static position when a 250 lb (1120 N) lateral force is applied to the top rear edge and top of the opposite side, respectively.

A.7.5 Resistance to deflection of work surface under load

A.7.5.1 Method

a) Secure the dial indicator to a rigid stand and position it at the front edge of the work tray, as shown in figure A13. The stand shall be positioned on the floor in front of the cabinet.

b) Zero the dial indicator and place the 50 lb (23 kg) test load at the center of the work tray, distributed over an area 10 x 10 in (250 x 250 mm). Remove the test load and record the distortion measured by the dial indicator (see Annex A, figure A13).

A.7.5.2 Acceptance

Permanent deflection of the work tray shall not exceed 0.001 in (0.02 mm) after the 50 lb (23 kg) test load is applied and removed.

A.7.6 Resistance to tipping under load (applicable only to freestanding devices with work surfaces)

A.7.6.1 Method

Place the 250 lb (114 kg) test load centered from right to left of the work area on the leading edge of the cabinet (see Annex A, figure A14).

A.7.6.2 Acceptance

The rear bottom of the cabinet shall not lift off the floor more than 0.062 in (1.6 mm) when a 250 lb (114 kg) test load is applied.

A.8 Downflow velocity

A.8.1 Purpose

This test measures the velocity of air moving through the cabinet work space 4 in (10 cm) above the bottom edge of the sash, and is performed on all cabinets accepted under Annex A, section A.6. Individual point readings shall be taken and reported on a specified grid with removable components removed (nominal set point set up), and the average for each designated zone shall be calculated (uniform downflow represents a single zone).

A.8.2 Apparatus

A thermal anemometer with an accuracy of ± 3.0 ft/min (± 0.015 m/s) or 3% of the indicated velocity, whichever is larger, shall be used. The device shall be calibrated in accordance with the thermal anemometer manufacturer's instructions, or with IEST-RP-CC-013 if instructions are not provided. When barometric pressure and air stream temperature (where velocity readings are taken) deviate from standard conditions listed for the thermal anemometer being used, correction factors from the manufacturer's manual for the thermal anemometer shall be consulted for the appropriate correction calculation.

A.8.3 Method: Setting nominal set point

The removable equipment non-essential to cabinet operation (acceptable option components) shall be removed prior to setting the nominal set points. The air measurement probe shall be held rigidly in a freestanding fixture that permits accurate positioning and does not distort the airflow pattern (ring-stand and clamp).

A.8.3.1 Downflow velocity

Measure the air velocity at multiple points across the workspace or zones defined by the manufacturer in the horizontal plane defined 4 in (100 mm) above the bottom edge of the sash frame (certified height). Manufacturer's instructions shall include locations of downflow grid or zone boundaries and their respective number of measurement points.

Downflow grid or zone requirements are the following:

- a uniform rectangular equidistant grid with spacings not less than 4 in (100 mm), nor more than 8 in (200 mm) and containing a minimum of three rows or as defined by the zone(s);

- for cabinets with a nominal width of 3 ft (0.9 m) or greater, there shall be a minimum seven readings per row or zone;

- for cabinets with a nominal width less than 3 ft (0.9 m), there shall be a minimum four readings per row or zone;

- the area defined by the perimeter of the test points must equal at least 30% of the total area of the plane in which the readings are taken, except as noted below;

- perimeter or zone air velocity readings shall be taken at least 6.0 in (150 mm) away from the walls and sash enclosing the work area (see Annex A, figure A15). When the requirement above for covering at least 30% of the area in the grid plane cannot be met due to the size of the cabinet, grid spacing shall start 6 in (150 mm) away from the walls and sash. However, if the grid spacing of 6 in (150 mm) from the walls and sash results in not being able to meet the above grid requirements of not less than 4 in (100 mm) with a minimum of 4 readings per row or zone, then equidistant spacing shall be used with the minimum of 4 readings per row or zone; and

- when a cabinet model has a sloped sash and is certified with more than one access opening height, there may be different downflow grids or zone(s) for each opening or those grids or zone(s) may be unified meeting the above requirements.

Reported values shall be each of the readings included in the applicable grid or zone(s) and the average of these readings within the downflow grid or zone(s). The nominal set point shall be based on the above data in accordance with the manufacturer's instructions.

A.8.4 Acceptance

The average downward airflow velocity through the cross section of the unobstructed work area (with removable acceptable option components removed) at the level 4 in (10 cm) above the bottom of the sash of cabinets meeting the requirements of Annex A, section A.6 shall be the values specified by the manufacturer. Subsequent production cabinets of the initial model and size conforming to Annex A, section A.6 may also qualify if the measured downflow velocity set points are within ± 5 ft/min (± 0.025 m/s) of the nominal downflow velocity set point and any additional velocity readings agreed to by the testing organization are provided. Individual point readings in cabinets with uniform downflow shall not vary more than ± 20% or ± 16 ft/min (± 0.081 m/s) from the average downflow velocity, whichever is greater, as determined in Annex A, section A.8.3. Individual point readings shall not vary more than ± 20% or ± 16 ft/min (± 0.081 m/s) from the average of each zone, whichever is greater, as determined in Annex A, section A.8.3, when the downflow is specified as non-uniform downflow (zoned) by the manufacturer.

A.9 Inflow velocity (face velocity) test

A.9.1 Purpose

This test determines the measured and calculated inflow velocity through the work access opening and the calculated exhaust flow volume rates. A minimum of five individual volumetric readings shall be taken and averaged using a direct reading instrument and the calculated average intake velocity.

NOTE – Include instructions for the validated secondary method for measuring intake velocity.

A.9.2 Apparatus

The following devices may be used to carry out inflow velocity testing:

– a direct inflow measurement (DIM) instrument with an accuracy of ± 3% of reading ± 7 ft^3/min (± 0.003 m^3/s) or another acceptable source or in accordance with Annex B;

– a thermal anemometer with an accuracy of ± 3.0 ft/min (±0.015 m/s) or 3% of the indicated velocity (whichever is larger); and

– a pitot tube constructed according to the dimensions given in the Industrial Ventilation Manual.[31]

The direct inflow measurement instrument shall be used to obtain direct measurement of inflow volume (primary method). Thermal anemometers or pitot tubes or both shall be used to determine calculated inflow velocity (secondary method).

A.9.3 Methods

A.9.3.1 General

The nominal set point average inflow velocity shall be determined by a direct inflow reading instrument measurement. After the nominal set point is determined by a direct inflow reading instrument measurement, readings shall be taken by the appropriate alternate calculated or measured method recommended by the manufacturer. Both of these set point values shall meet the requirements of Annex A, section A.9.4.

A.9.3.2 Direct inflow measurement method (primary method)

a) Seal by taping the device to the center of the front opening of a biosafety cabinet. Seal the open areas on either side of the capture hood portion of the DIM as necessary.

b) All cabinet and exhaust blowers must be operating. Take at least five non-back pressure compensated readings, and average them to determine inflow volume rate. Care should be taken not to restrict the airflow through the instrument intake area. Care should be taken not to restrict the airflow through the instrument intake area.

c) Calculate the average inflow velocity in ft/min (m/s) by dividing the average inflow volume rate in ft^3/min (m^3/s) by the work access opening area in ft^2 (m^2).

d) Determine the inflow quantity per linear foot of work area width by dividing the inflow volume rate by the width of the work area in ft (m).

[31] American Conference of Industrial Hygienists, "Industrial Ventilation, A Manual of Recommended Practice," 6500 Glenway Ave., Building D7, Cincinnati, OH 45211 <www.acgih.org>

e) Include the following in the reported data: individual inflow volume rate readings, average inflow volume rate, work access opening dimensions and area, directly measured average inflow velocity, width of the work area, inflow quantity per 1 ft (0.3 m) of work area width, and the methods used to determine them.

A.9.3.3 Method to determine concurrent air balancing exhaust values for Type B cabinets only

a) This test shall be conducted before any previous test conditions are changed.

b) Measure and calculate exhaust volume by conducting a duct traverse in accordance with American Society of Heating, Refrigerating, and Air-Conditioning Engineers (ASHRAE)[32] standards for air velocity measurements in round or rectangular ducts or with the Industrial Ventilation Manual.

c) Measure exhaust static pressure at a point approximately two duct diameters from the cabinet exhaust connection in accordance with ASHRAE standards for air velocity measurements in round or rectangular ducts or with the Industrial Ventilation Manual.

d) Include exhaust volume rate in ft^3/min (m^3/s) and exhaust static pressure in inches water gauge (Pascal) in the reported data.

A.9.3.4 Alternate inflow measurement methods

These methods, approved by the testing organization, shall be validated and provided by the manufacturer and shall be subject to review by the testing organization. Manufacturer validation procedures shall contain no fewer than ten replicate tests. The testing organization's approval shall be based on review of data and successful reproduction of test results. The following methods have been found to be acceptable on some cabinets:

A.9.3.4.1 Method for Type A1 and A2 cabinets that use a thermal anemometer to measure exhaust velocity to determine inflow velocity

a) Take air velocity measurements at multiple points across the exhaust filter face as described by the manufacturer on a grid no larger than 4 x 4 in (10 x 10 cm), with the grid starting points and height above the filter validated by the testing organization (see Annex A, figure A16). A clear 12 in (30 cm) of space is required above the exhaust HEPA filter face for valid thermal anemometer measurements.

b) The effective open area of the exhaust HEPA/ULPA filter or exhaust port shall be determined and supplied by the manufacturer and validated by the testing organization. Cabinets in which the exhaust filter is not accessible or exhaust port flow is non-uniform, such as caused by a damper or exhaust filter housing design, shall be tested as approved by the testing organization.

c) To obtain the exhaust flow volume rate in ft^3/min (m^3/s), multiply the average exhaust air velocity in ft/min (m/s) by the exhaust area in ft^2 (m^2).

d) Calculate the average inflow velocity in ft/min (m/s) by dividing the average exhaust volume rate in ft^3/min (m^3/s) by the work access opening area in ft^2 (m^2).

e) Include the following in the reported data: individual exhaust velocity readings, average exhaust velocity, exhaust volume rate, exhaust opening dimensions and area, work access opening dimensions and area, calculated average inflow velocity, and the method used to determine them.

[32] American Society of Heating, Refrigerating, and Air-Conditioning Engineers, Inc., 1791 Tullie Circle, N.E., Atlanta, GA 30329 <www.ashrae.org>

A.9.3.4.2 Method for Type A1, A2 and B2 cabinets using a thermal anemometer to measure velocity through a constricted access opening to determine average inflow velocity

a) Restrict the access opening as specified by the testing organization.

b) Air velocity measurements shall be taken at multiple points across the restricted opening as specified on the data plate. No fewer than two readings per 1 ft (0.3 m) of access opening width shall be taken.

c) Average the air velocity measurements. Multiply the average by the listed correction factor to obtain average inflow velocity.

d) Include the following in the reported data: height of restriction, individual velocity readings, average velocity, the listed correction factor, calculated inflow velocity, and methods used to determine them.

A.9.3.4.3 Method for Type B1 cabinets using a thermal anemometer to measure velocity through the access opening to determine average inflow velocity

a) Turn off blower(s) that recirculate air in the cabinet, if specified in the manufacturer's instructions.

b) Set the sash to manufacturer's recommended operating height.

c) Take two rows of air velocity measurements with an anemometer at multiple points in the plane of the access opening. Take one row at a distance below the top of the access opening equal to 25% of the opening height. Take the second row at a distance below the top of the access opening equal to 75% of the opening height (see Annex A, figure A17).

d) Take the indicated velocity measurements every 4 in (10 cm) across the width of the front work access opening but no closer than 4 in (10 cm) from sides of the work opening. The average of all measurements represents the inflow velocity.

e) Include individual inflow velocity readings, average inflow velocity, and the methods used to determine them in the reported data.

A.9.3.4.4 Calculated method for Type B2 cabinets using an anemometer and pitot tube, if applicable

a) Turn on the cabinet downflow blower and exhaust system blower.

b) Set the sash at manufacturer's recommended operating height.

c) Measure and calculate exhaust volume in accordance with the testing organization's verified methodology or with ASHRAE standards for air velocity measurements, in round or rectangular ducts or with the Industrial Ventilation Manual.

d) Measure the supply air velocity on an approximate 4 x 4 in (10 x 10 cm) grid in a horizontal plane 6.0 in (15 cm) below the face of the downflow diffuser, starting 2 in (5 cm) from each perimeter wall. The air measurement probe shall be held rigidly in a freestanding fixture (ring-stand and clamp) that permits accurate positioning and does not distort airflow pattern (see Annex A, figure A18). Average the velocity readings and multiply the average by the area in ft^2 (m^2) of the plane in which the velocities were measured to determine the total filtered air supply in ft^3/min (m^3/s).

e) Subtract the supply air volume rate in ft^3/min (m^3/s) from the total exhaust volume rate in ft^3/min (m^3/s); the difference represents the calculated inflow volume rate in ft^3/min (m^3/s).

f) Divide the calculated inflow volume rate by the area of the access opening in ft^2 (m^2) to determine the average inflow velocity in ft/min (m/s).

g) Reported the individual exhaust velocity readings, calculated average exhaust velocity, exhaust duct area, calculated exhaust volume, individual supply velocity readings, average supply velocity, effective supply area, calculated supply air volume, area of the work access opening, calculated inflow air volume, calculated access opening average inflow velocity, and the methods used to determine them.

A.9.4 Acceptance

Acceptance criteria shall be based on inflow determined by the direct measurement. Subsequent production cabinets of the initial model and size may also qualify as meeting Annex A, section A.6 when the directly measured inflow velocities are provided within ± 5 ft/min (± 0.025 m/s) of the nominal set point velocities.

The minimum inflow velocity of Type A1 cabinets shall be 75 ft/min (0.38 m/s). The minimum inflow volume shall be 45 ft^3/min (76 m^3/h) per foot (meter) of work area width (see Annex A, sections A.6 and A.8).

The minimum inflow velocity of Type A2, B1, and B2 cabinets shall be 100 ft/min (0.51 m/s). The minimum inflow volume shall be 65 ft^3/min (110 m^3/h) per 1 ft (0.3 m) of work area width (see Annex A, sections A.6 and A.8).

A.10 Airflow smoke patterns test

A.10.1 Purpose

This test determines that the airflow along the entire perimeter of the work access opening is inward, that airflow within the work area is downward with no dead spots or refluxing, that ambient air does not pass on or over the work surface, and that there is no escape to the outside of the cabinet at the sides and top of the sash.

A.10.2 Apparatus

A visible aerosol or mist that is close to neutrally buoyant in air. The generation process should not create a velocity sufficient to interfere with the air patterns being observed.

NOTE – Titanium tetrachloride is corrosive and should be handled with care.

A.10.3 Method

A.10.3.1 Downflow test

Smoke shall be passed from one end of the cabinet to the other, along the centerline of the work surface, at a height of 4 in (10 cm) above the top of the access opening.

A.10.3.2 View screen retention test

Smoke shall be passed from one end of the cabinet to the other, 1 in (2.5 cm) behind the view screen, at a height 6.0 in (15 cm) above the top of the access opening.

A.10.3.3 Work opening edge retention test

Smoke shall be passed along the entire perimeter of the work opening edges, approximately 1.5 in (3.8 cm) outside the cabinet. Particular attention should be paid to corners and vertical edges.

A.10.3.4 Sash seal test

Smoke shall be passed up the inside of the sash 2 in (5 cm) from the sides and along the top of the work area.

A.10.4 Acceptance

A.10.4.1 Downflow test

The smoke shall show smooth downward flow with no dead spots or reflux (upward flow).

A.10.4.2 View screen retention test

The smoke shall show smooth downward flow with no dead spots or reflux. No smoke shall escape from the cabinet.

A.10.4.3 Work opening edge retention test

No smoke shall be refluxed out of the cabinet once drawn in, nor shall smoke billow over the work surface or penetrate onto it.

A.10.4.4 Sash seal test

There shall be no escape of smoke from the cabinet.

A.11 Drain spillage trough leakage test

A.11.1 Purpose

This test demonstrates the containment capability of the spillage trough under the work surface.

A.11.2 Method

Fill the drain spillage trough with a minimum of 1 gal (4 L) of water and hold it for 1 h. Check for visible signs of water leakage after 1 h.

A.11.3 Acceptance

The drain spillage trough shall hold a minimum of 1 gal (4 L) of water and have no visible leakage after a 1 h holding period.

A.12 Motor/blower performance

A.12.1 Purpose

This test demonstrates that the motor/blower will operate at a static pressure sufficient to meet the requirements of 6.13.

A.12.2 Apparatus

Instrumentation required in Annex A, sections A.9 and A.10 shall be used. A manometer with an accuracy of at least ± 2% of reading shall be used.

A.12.3 Method

a) Set the cabinet at the nominal set point, ± 3.0 ft/min (± 0.015 m/s).

b) Measure the total airflow volume rate, ft^3/min (m^3/s), and determine that the cabinet blower is delivering at the nominal set point (see Annex A, sections A.8 and A.9). The cabinet supply air volume shall be determined as in Annex A, section A.9.3.4.4 (b).

c) Locate the testing organization approved[33] positive and negative pressure taps. The manufacturer shall locate the positive pressure tap (see Annex A, figure A19) directly above the downflow HEPA/ULPA filter to allow conversion of velocity pressure to static pressure. The positive pressure tap shall not be located in the face of the blower outlet (see Annex A, figure A19). If more than one pressure tap is used, as in a piezometer ring, pressure taps may be connected together for an average reading. The manufacturer shall locate the negative pressure tap not less than one-half equivalent diameter from the blower inlet. In the case of double inlet blowers, static measurements shall be made in both blower inlets and connected together for an average static pressure (see Annex A, figure A20). If it is not possible to mount both static pressure taps due to cabinet design, one tap will be sufficient. For negative pressure tap, use a series pressure tap (see Annex A, figure A21). Attach manometers to each pressure tap and record result. The positive pressure reading is the initial static pressure reference point. The sum of the positive and negative readings without reference sign is the total cabinet static pressure.

d) Increase the initial negative pressure reading by 50% or more of the initial positive pressure reading by restricting the cabinet's negative airflow. To accomplish this, monitor the cabinet's initial negative pressure, and load or restrict the cabinet's negative airflow area (i.e., type A1, A2, B1-front grill or type B2-supply air inlet) until the initial negative pressure has increased by 50% of the initial positive pressure reading. In the case where the first loaded HEPA/ULPA filter is under negative pressure (type B1), the 50% positive pressure value shall be considered 50% of the pressure drop of the first HEPA/ULPA filter.

e) Measure the total volume of airflow (ft^3/min [m^3/s]) the restricted cabinet blower is delivering (see Annex A, sections A.9 and A.9.3.4.4 (d)).

f) Record the initial negative and positive pressures, the final negative pressures, and the initial and final airflow volume rates.

A.12.4 Acceptance

The total airflow volume rate, ft^3/min (m^3/s), shall not decrease more than 10% meeting the requirements of 6.13.

A.13 Cabinet airflow stability

A.13.1 Purpose

This test demonstrates the ability of the cabinet to maintain proper airflow following cabinet physical shock, during line voltage fluctuations, and following loss of power to the cabinet.

[33] Manufacturer to supply positive and negative pressure taps (see Annex A, figure A21) on units submitted for laboratory certification.

The test methods used for these requirements are set up to minimize work by measuring airflow in the simplest way possible; where downflow velocity measurements are required, only 6 points on the downflow velocity grid are considered representative of the downflow air movement.

For Type A1 and A2 cabinets that employ the traditional single blower design, airflow is quantified using only DIM inflow measurements.

For Type A1 and A2 cabinets that employ separate blowers to provide the downflow and exhaust airflow, the inflow and downflow velocities shall be measured as part of the airflow stability measurement.

For Type B1 cabinets, only the downflow velocity shall be considered. A change in the cabinet motor speed will not affect the inflow velocity for a type B1 cabinet.

For Type B2 cabinets, only the downflow velocity shall be considered. The inflow velocity will be affected by a change in the downflow velocity for a type B2 cabinet but measurement of the downflow velocity captures this effect without adding in the potential error caused by the facility exhaust system.

A.13.2 Apparatus

- Instrumentation required in Annex A, sections A.9 and A.10 shall be used.

- A power source capable of being adjusted between 90 and 253 AC volts 50 and 60 Hz. The power source shall be stable within 0.1 volt once set. Voltage shall be measured with a calibrated volt meter accurate to 0.1 volt at the cabinet plug rather than relying on the power source display, even if the power source is fully calibrated.

- A voltage meter with a minimum range of 0 – 300 AC volts and accurate to 0.1 volt.

A.13.3 Method

A.13.3.1 Shock stability

a) Measure the inflow velocity for Type A1 and A2 cabinets. Measure a minimum of 6 points on the downflow velocity grid for Type B1 and B2 cabinets and for Type A1 and A2 cabinets with separate downflow and exhaust blowers. Location of downflow velocity points shall be at least one column in from the sides and include at least 2 points in each row. One point in each row shall be to the left of the cabinet center line and one point shall be to the right of the cabinet center line. The average of those points shall be considered representative of the downflow velocity and used to determine compliance with the requirements of this test. Measure the ambient temperature in the test laboratory.

b) Lift one side of the cabinet off the floor 1 cm and then drop it. Repeat this on the opposite side of the cabinet. The cabinet shall be installed on the stand (if provided) during this test.

c) Repeat the inflow velocity measurement for Type A1 and A2 cabinets. Repeat the downflow velocity measurement for Type B1 and B2 cabinets and for Type A1 and A2 cabinets with separate downflow and exhaust blowers at the same points used for the initial measurement. The same instruments used to make the initial velocity and airflow measurements shall be used to make the repeat measurements. The repeat air measurements shall be completed on the same work day as the initial measurements. Measure the ambient temperature in the test laboratory. Ambient temperature shall be maintained within 4 degrees Fahrenheit (2 degrees Celsius) during the test.

A.13.3.2 Input voltage stability

a) Measure the inflow velocity for Type A1 and A2 cabinets. Measure a minimum of 6 points on the downflow velocity grid for Type B1 and B2 cabinets and for type A1 and A2 cabinets with separate

downflow and exhaust blowers. Location of downflow velocity points shall be at least one column in from the sides and include at least 2 points in each row. One point in each row shall be to the left of the cabinet center line and one point shall be to the right of the cabinet center line. The average of those points shall be considered representative of the downflow velocity and used to determine compliance with the requirements of this test. Measure the ambient temperature in the test laboratory.

b) Increase the supply voltage by 10 percent +/- 0.2 volts from the line voltage measured during the as-set airflow measurement.

c) Repeat the inflow velocity measurement for Type A1 and A2 cabinets. Repeat the downflow velocity measurement for Type B1 and B2 cabinets and for Type A1 and A2 cabinets with separate downflow and exhaust blowers at the same points used for the initial measurement.

d) Decrease the supply voltage by 10 percent +/- 0.2 volts from the line voltage measured during the as-set airflow measurement.

e) Repeat the inflow velocity measurement for Type A1 and A2 cabinets. Repeat the downflow velocity measurement for Type B1 and B2 cabinets and for Type A1 and A2 cabinets with separate downflow and exhaust blowers at the same points used for the initial measurement. The same instruments used to make the initial velocity and airflow measurements shall be used to make the repeat measurements. The repeat air measurements shall be completed on the same work day as the initial measurements. Measure the ambient temperature in the test laboratory. Ambient temperature shall be maintained within 4 degrees Fahrenheit (2 degrees Celsius) during the test.

A.13.3.3 Power failure stability

a) This test shall be completed only after the motor speed has been adjusted and set at least once. The cabinet blower shall be running and the lights shall be on when power is disconnected. Alarm parameters (if so equipped) shall be set and recorded at the time the power is disconnected.

b) Measure the inflow velocity for Type A1 and A2 cabinets. Measure a minimum of 6 points on the downflow velocity grid for Type B1 and B2 cabinets and for Type A1 and A2 cabinets with separate downflow and exhaust blowers. Location of downflow velocity points shall be at least one column in from the sides and include at least 2 points in each row. One point in each row shall be to the left of the cabinet center line and one point shall be to the right of the cabinet center line. The average of those points shall be considered representative of the downflow velocity and used to determine compliance with the requirements of this test. Measure the ambient temperature in the test laboratory.

c) Disconnect power to the cabinet for a minimum of 1 hour.

d) Reconnect power to the cabinet. Repeat the inflow velocity measurement for Type A1 and A2 cabinets. Repeat the downflow velocity measurement for Type B1 and B2 cabinets and for Type A1 and A2 cabinets with separate downflow and exhaust blowers at the same points used for the initial measurement. The same instruments used to make the initial velocity and airflow measurements shall be used to make the repeat measurements. The repeat air measurements shall be completed on the same work day as the initial measurements. Measure the ambient temperature in the test laboratory. Ambient temperature shall be maintained within 4 degrees Fahrenheit (2 degrees Celsius) during the test.

A.13.4 Acceptance

A.13.4.1 Shock stability

The difference between the initial inflow velocity and the final inflow velocity shall not exceed 5 fpm (0.025 m/s). The difference between the initial downflow velocity and the final downflow velocity shall not exceed 5 fpm (0.025 m/s). There shall be no visible damage observed to the cabinet following the test.

A.13.4.2 Input voltage stability

The difference between the initial inflow velocity and the inflow velocity measured at both the increased and decreased supply voltage shall not exceed 5 fpm (0.025 m/s). The difference between the initial downflow velocity and the downflow velocity measured at both the increased and decreased supply voltage shall not exceed 5 fpm (0.025 m/s).

A.13.4.3 Power failure stability

The difference between the initial inflow velocity and the final inflow velocity shall not exceed 3 fpm (0.015 m/s). The difference between the initial downflow velocity and the final downflow velocity shall not exceed 3 fpm (0.015 m/s). The cabinet blower and lights shall come back on automatically when power is restored. Alarm parameters (if so equipped) shall remain unchanged from the set points prior to power loss. The cabinet shall provide the user with a visual indication that there was a loss of power.

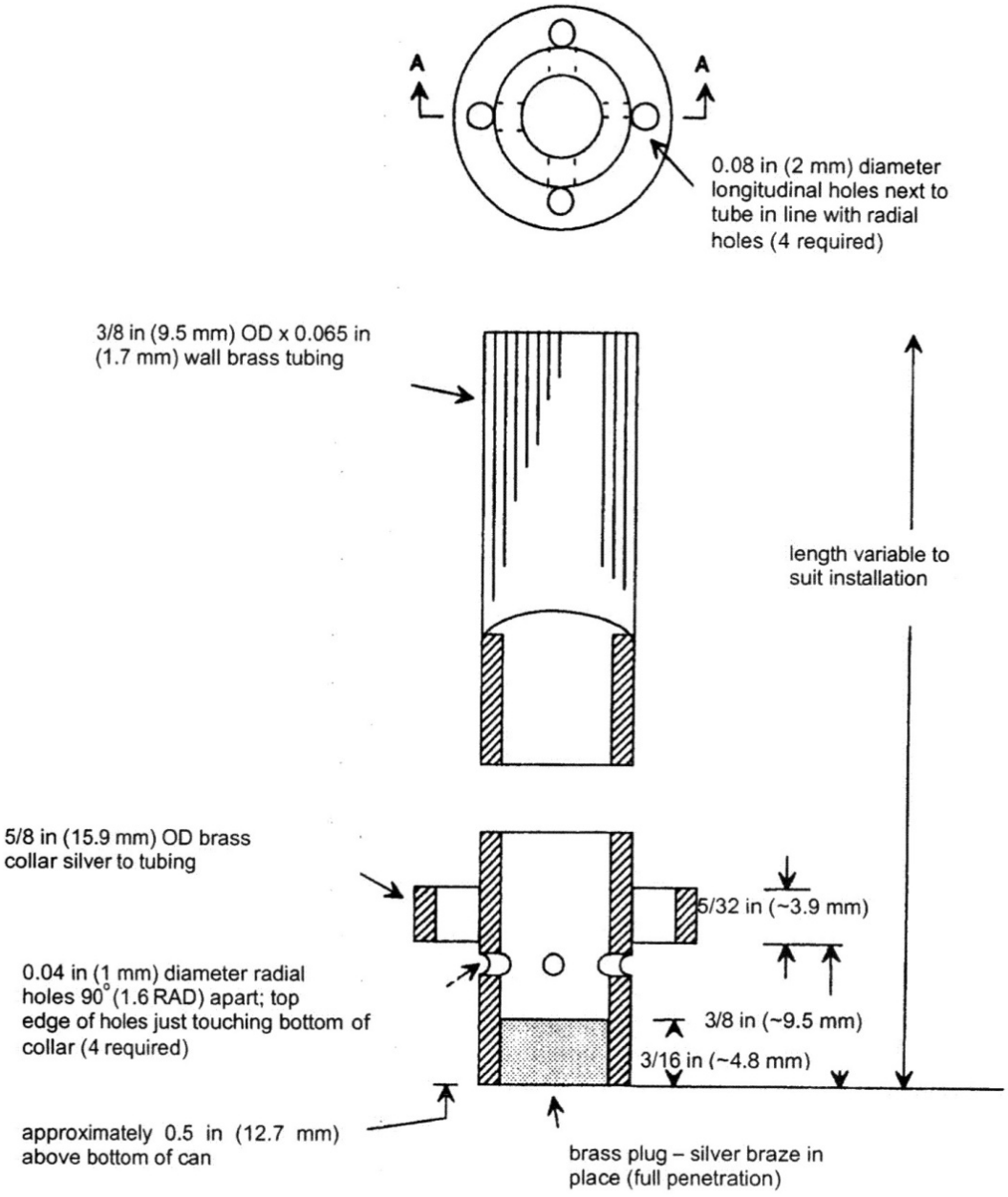


Figure A1 - Detail of Laskin nozzle

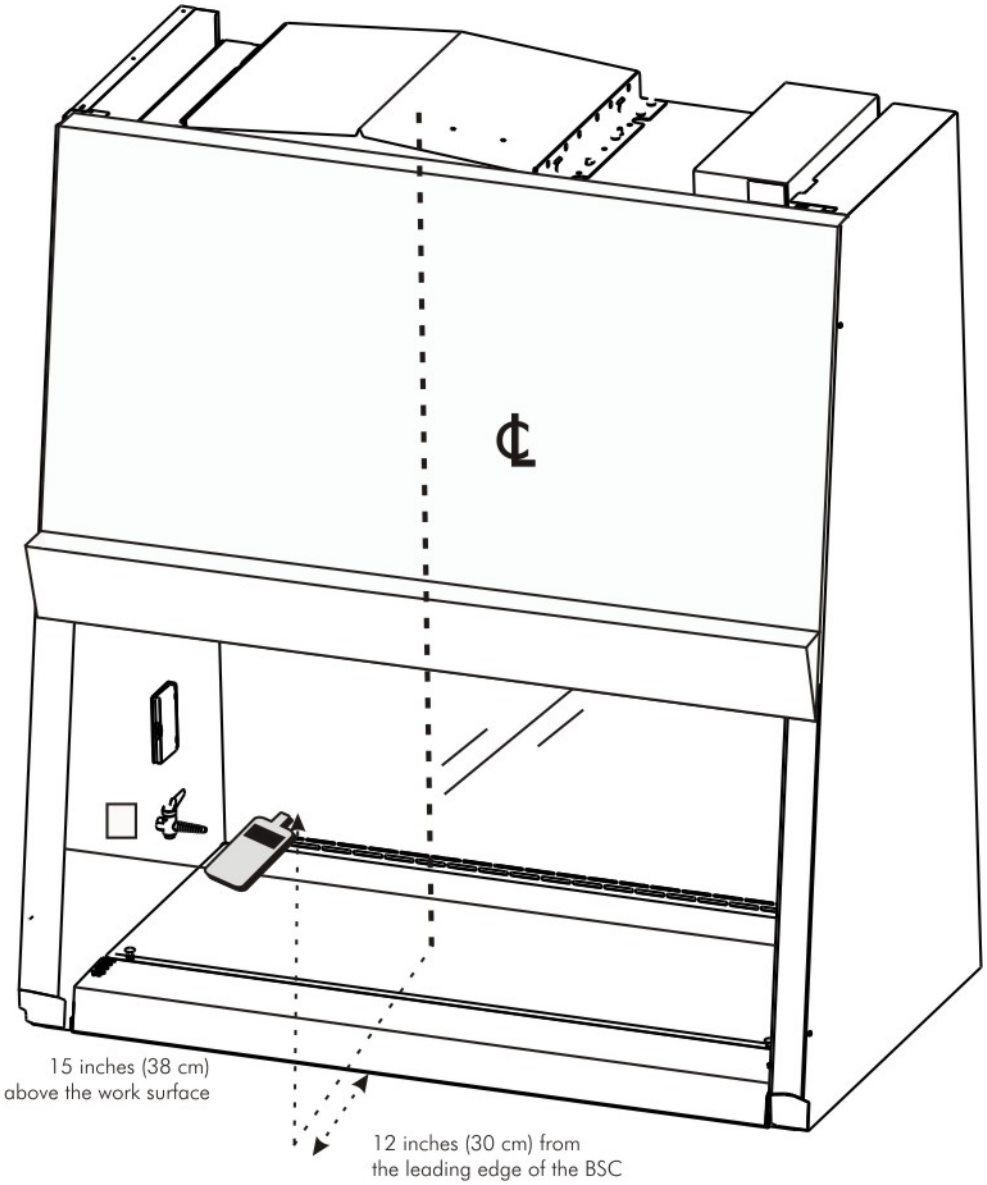


Figure A2 - Noise Level Test

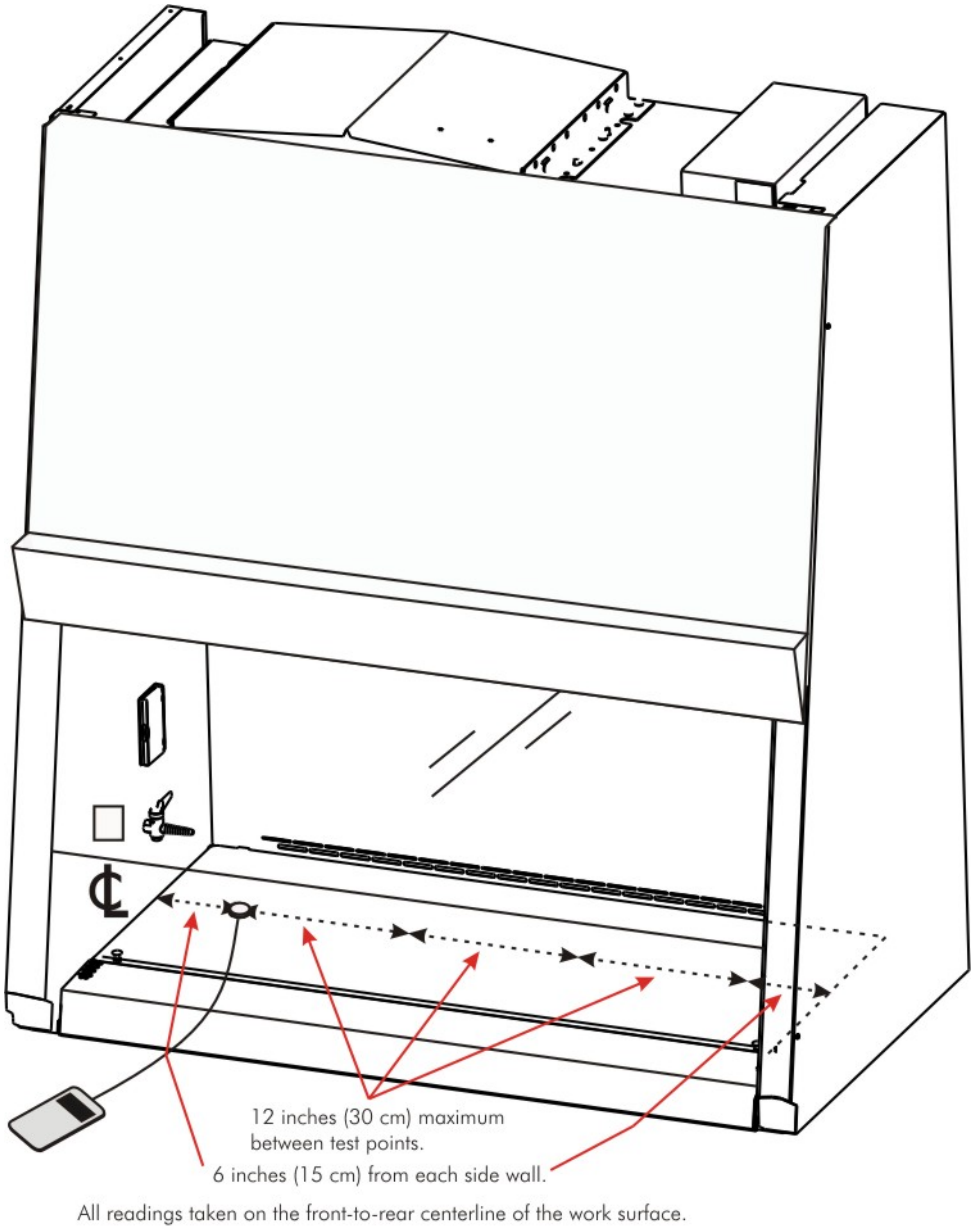


Figure A3 - Light Level Test

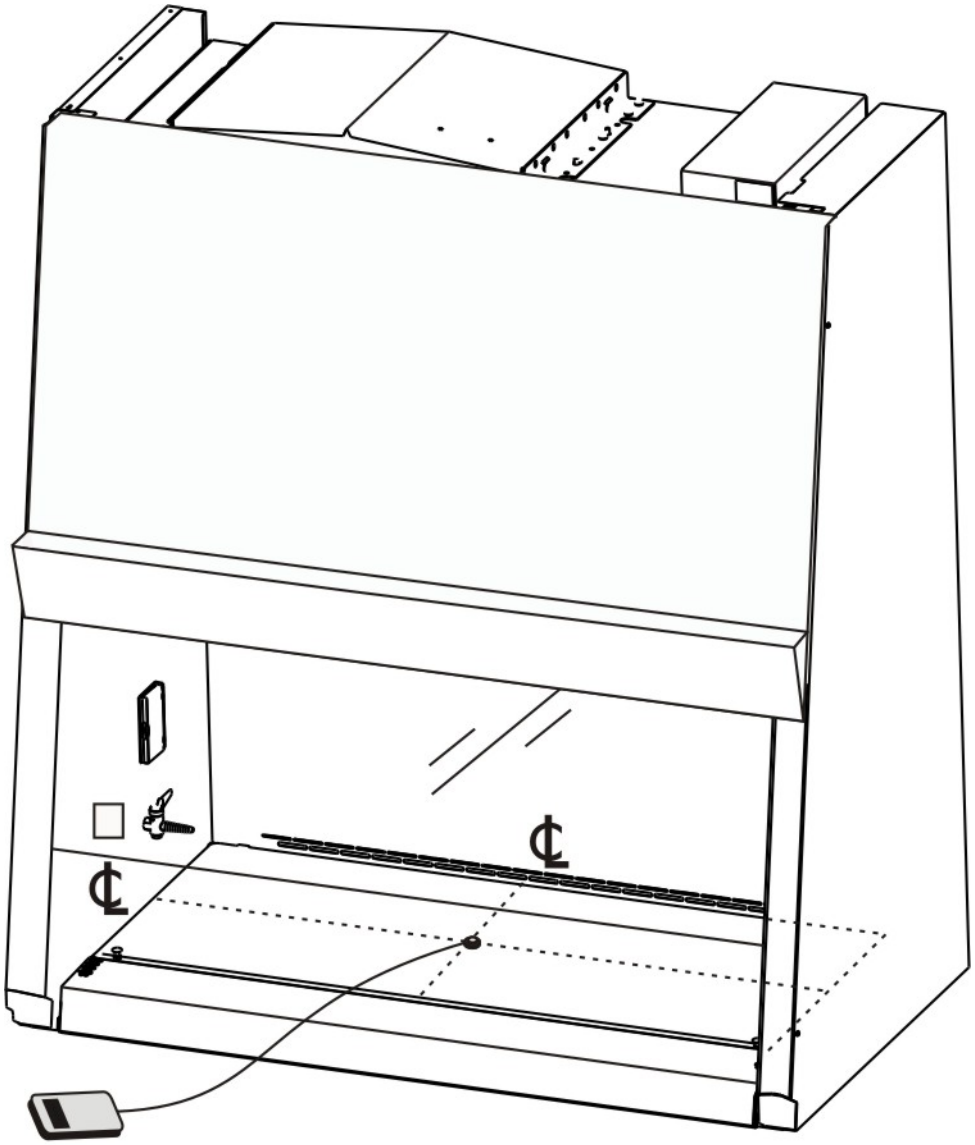


Figure A4 - Vibration Test

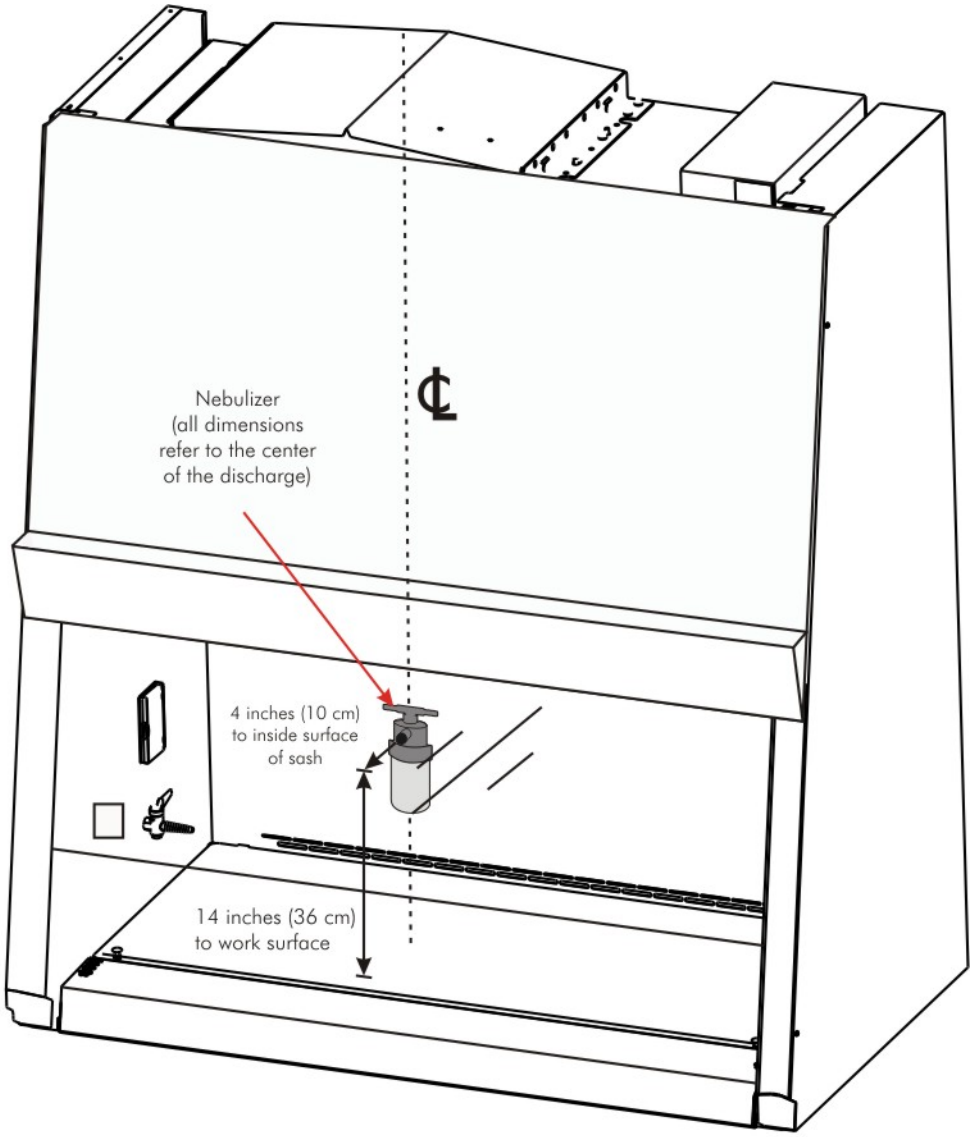


Figure A5 - Personnel Protection Test

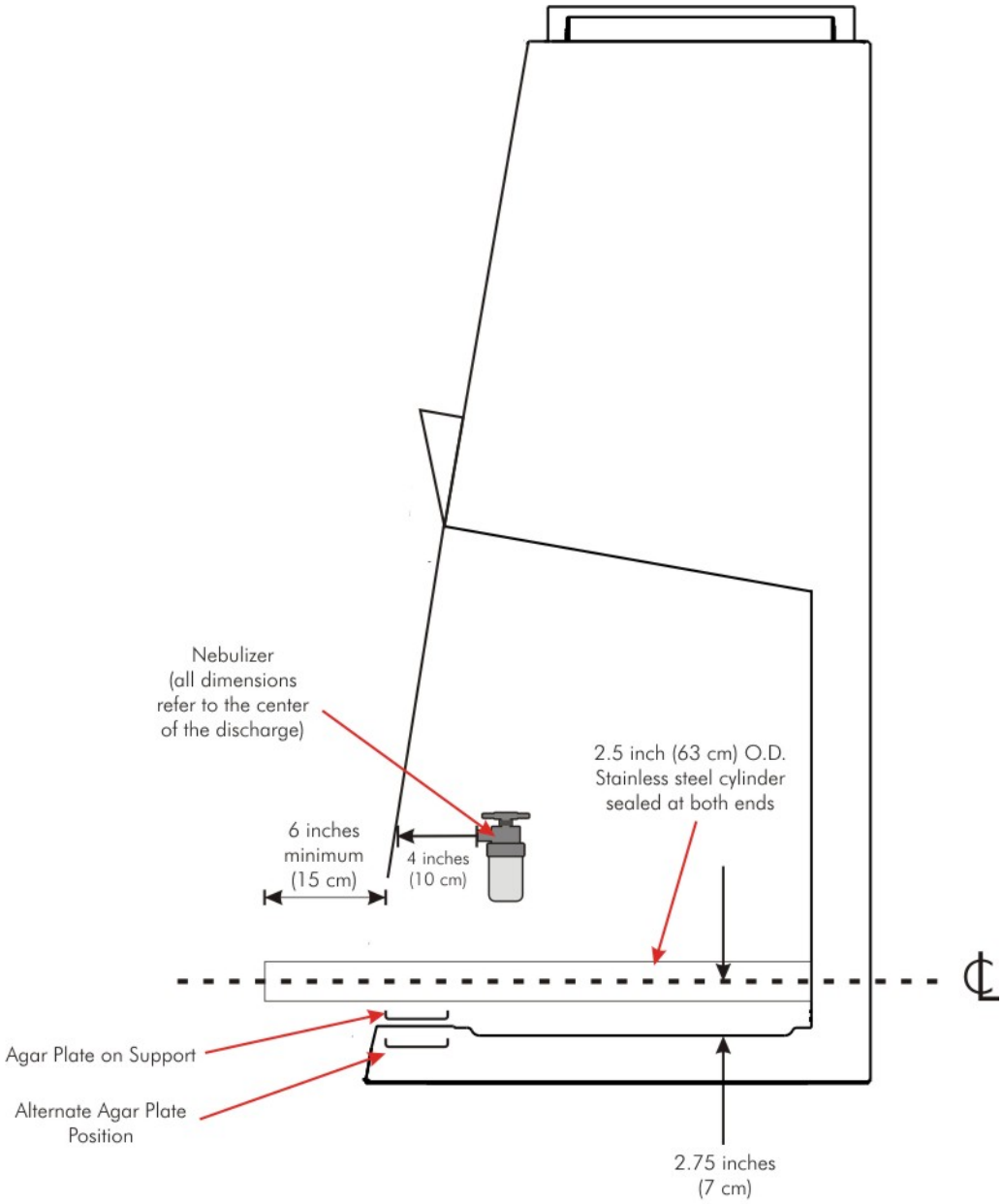


Figure A6 - Personnel Protection Test

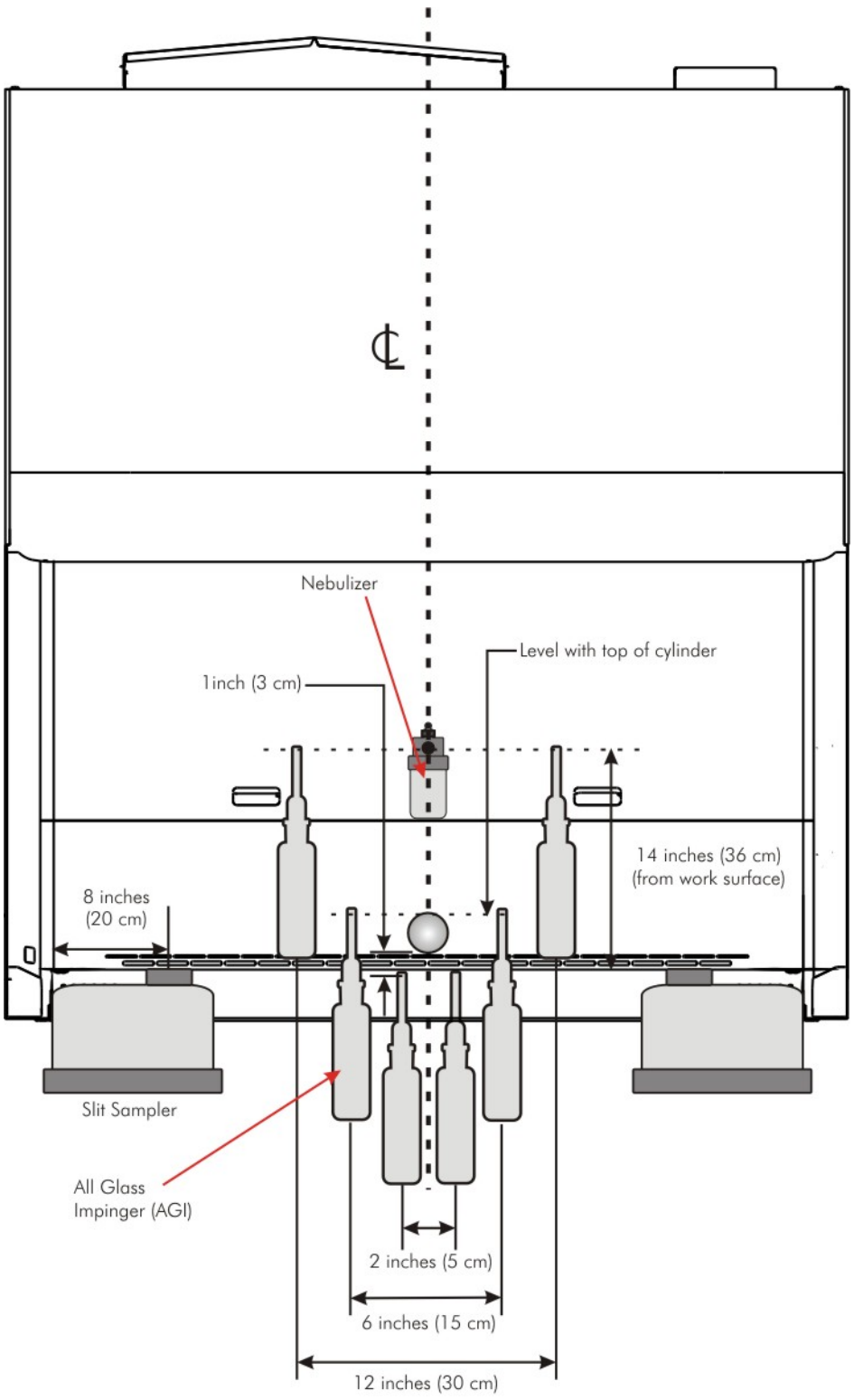


Figure A7 - Personnel Protection Test

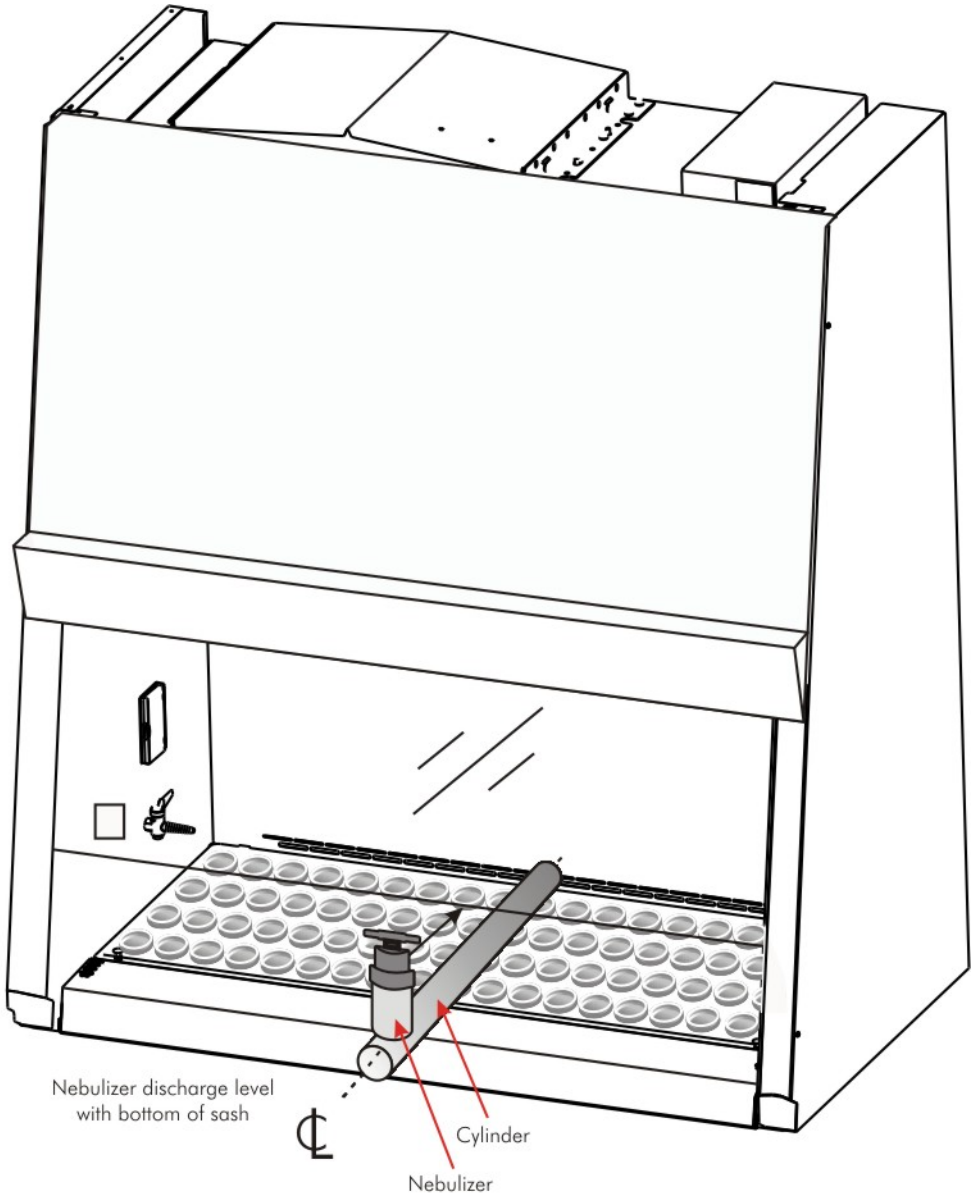


Figure A8 - Product Protection Test

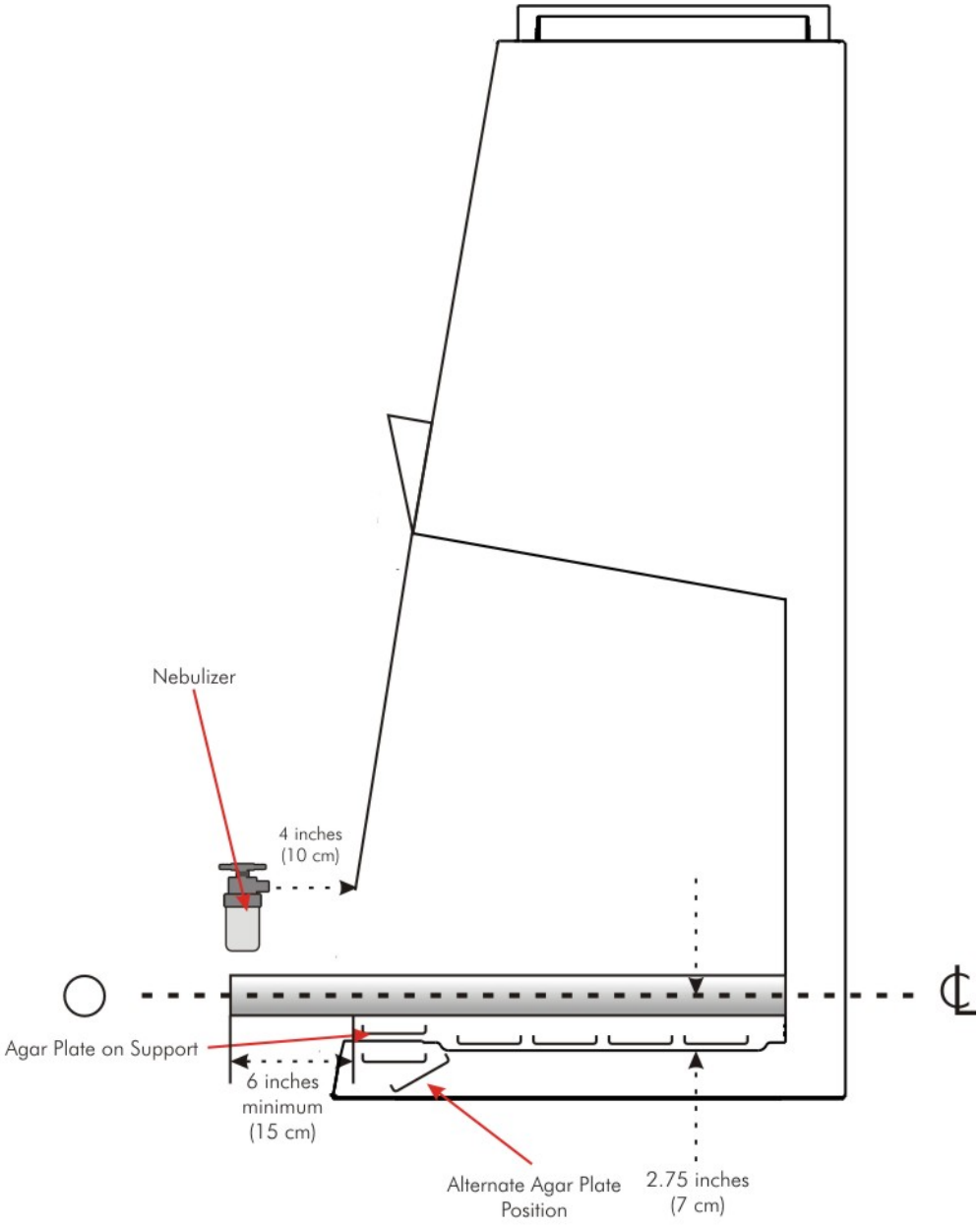


Figure A9 - Product Protection Test

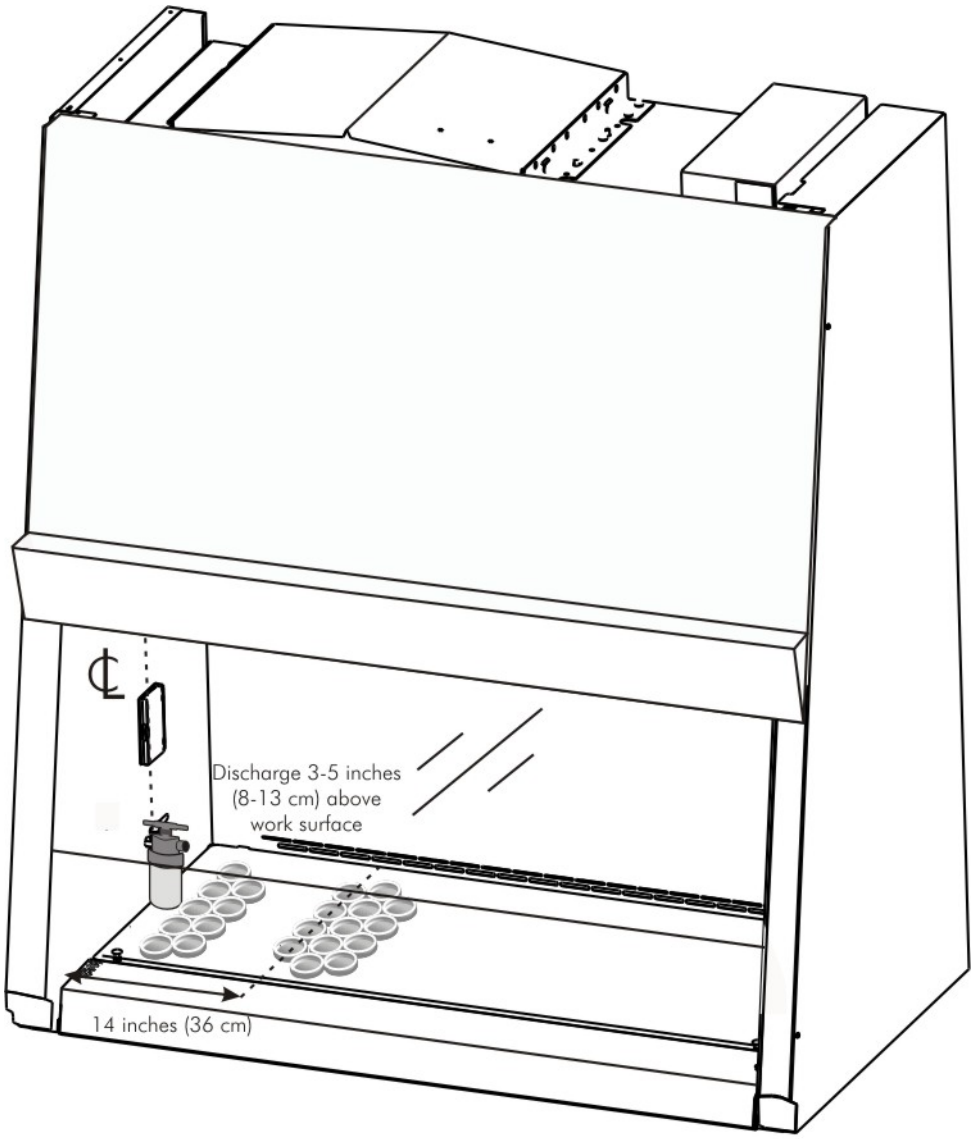


Figure A10 - Cross Contamination Protection Test

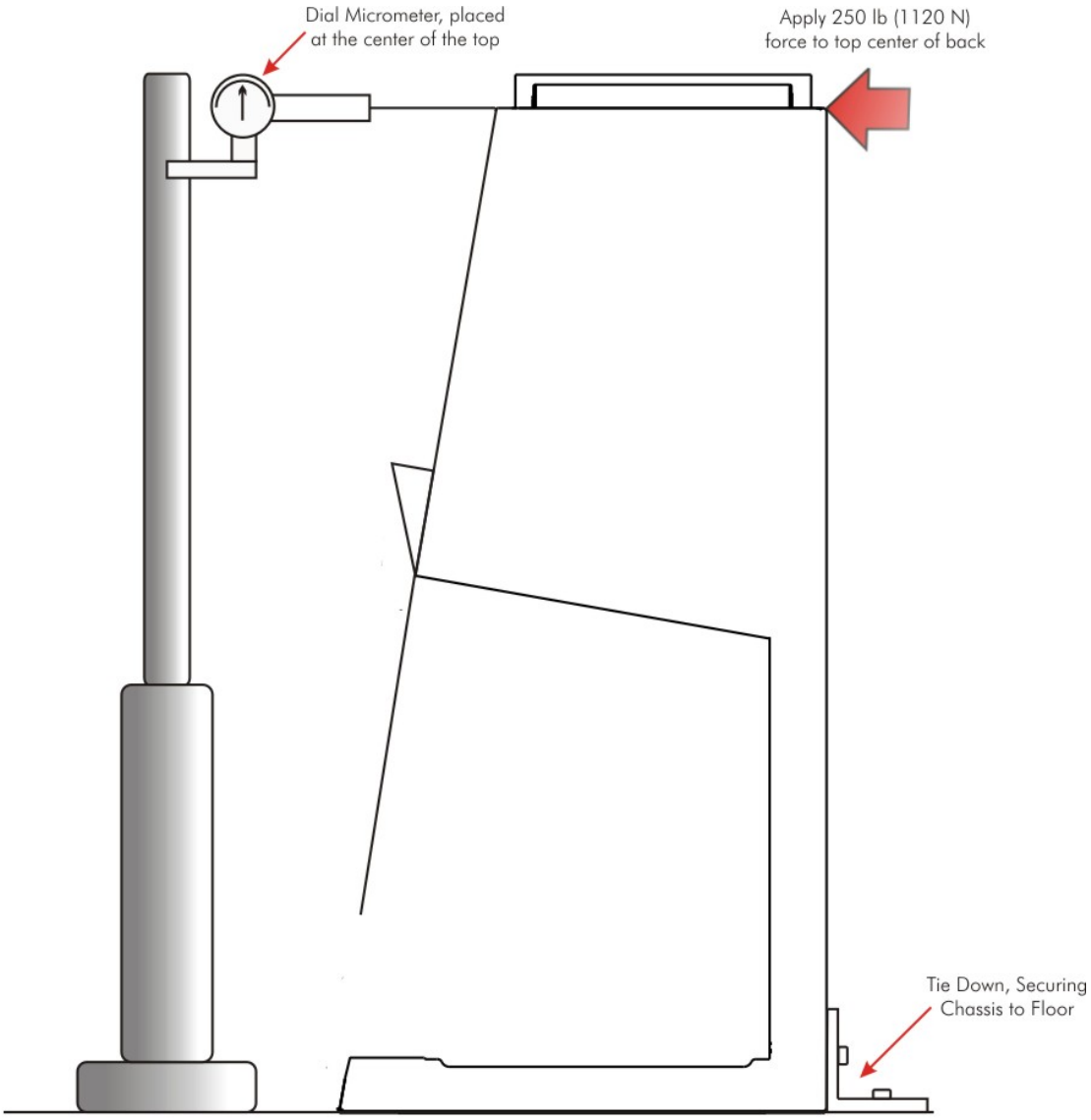


Figure A11 - Resistance to Distortion Test

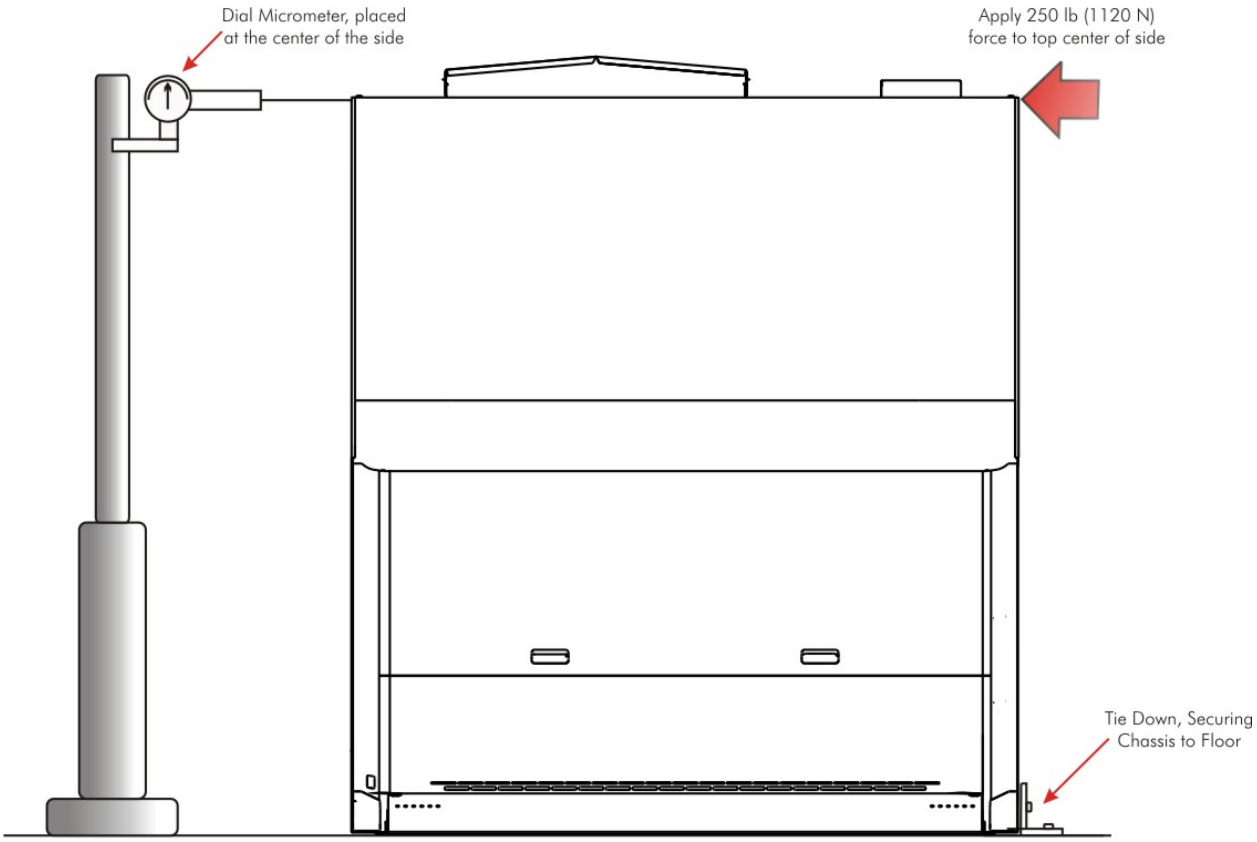


Figure A12 - Resistance to Distortion Test

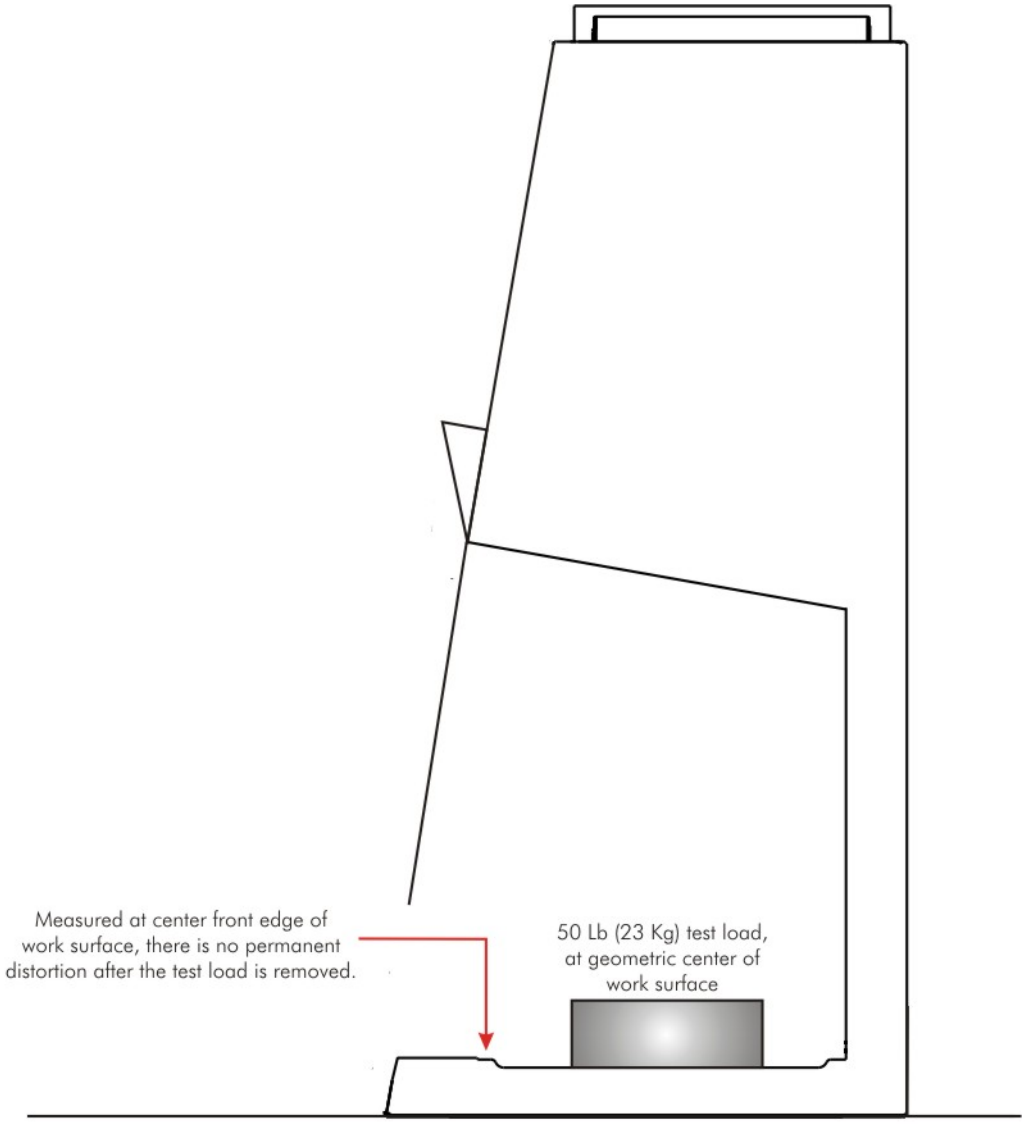


Figure A13 - Resistance to Deflection Test

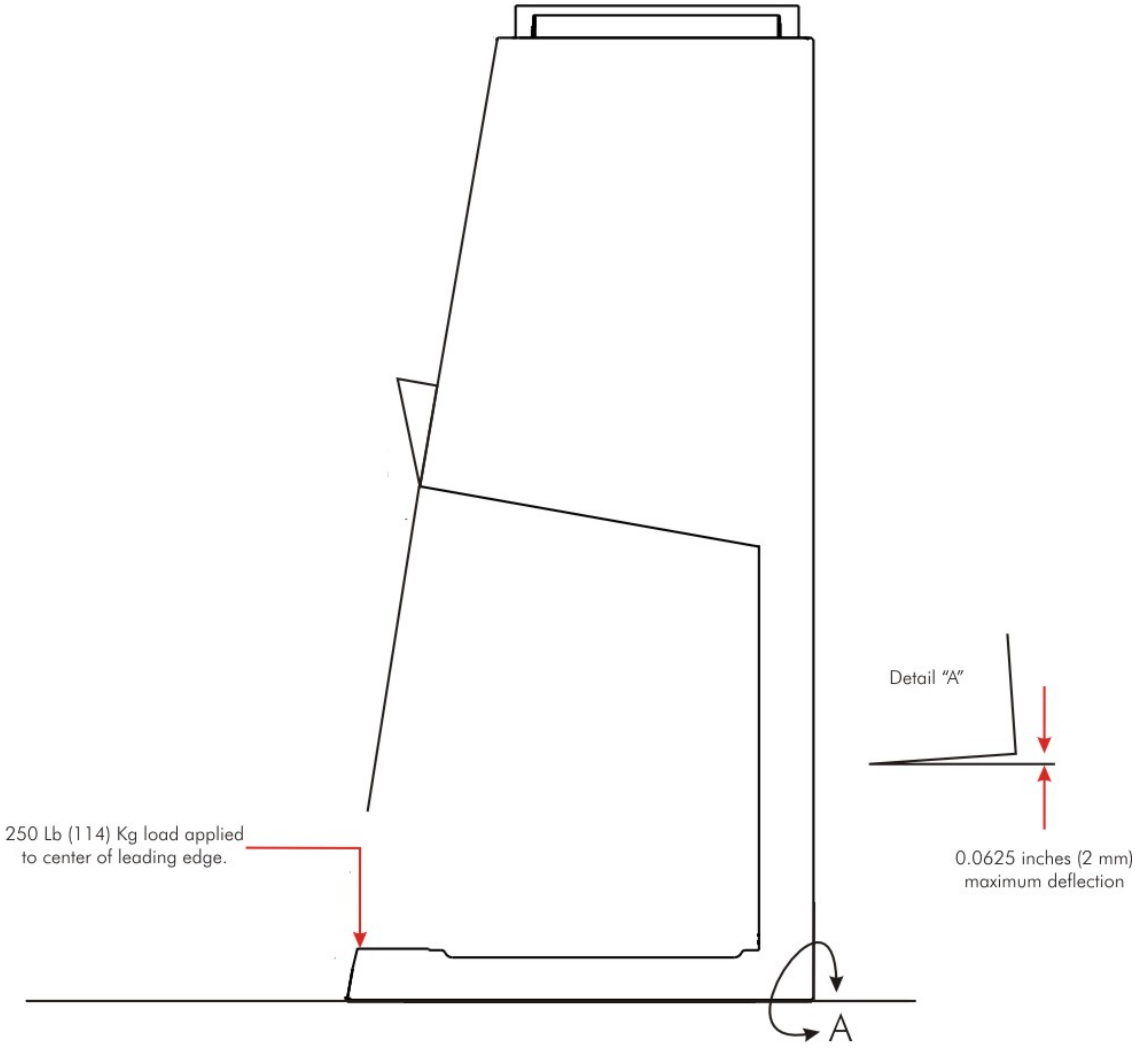


Figure A14 - Resistance to Tipping Test

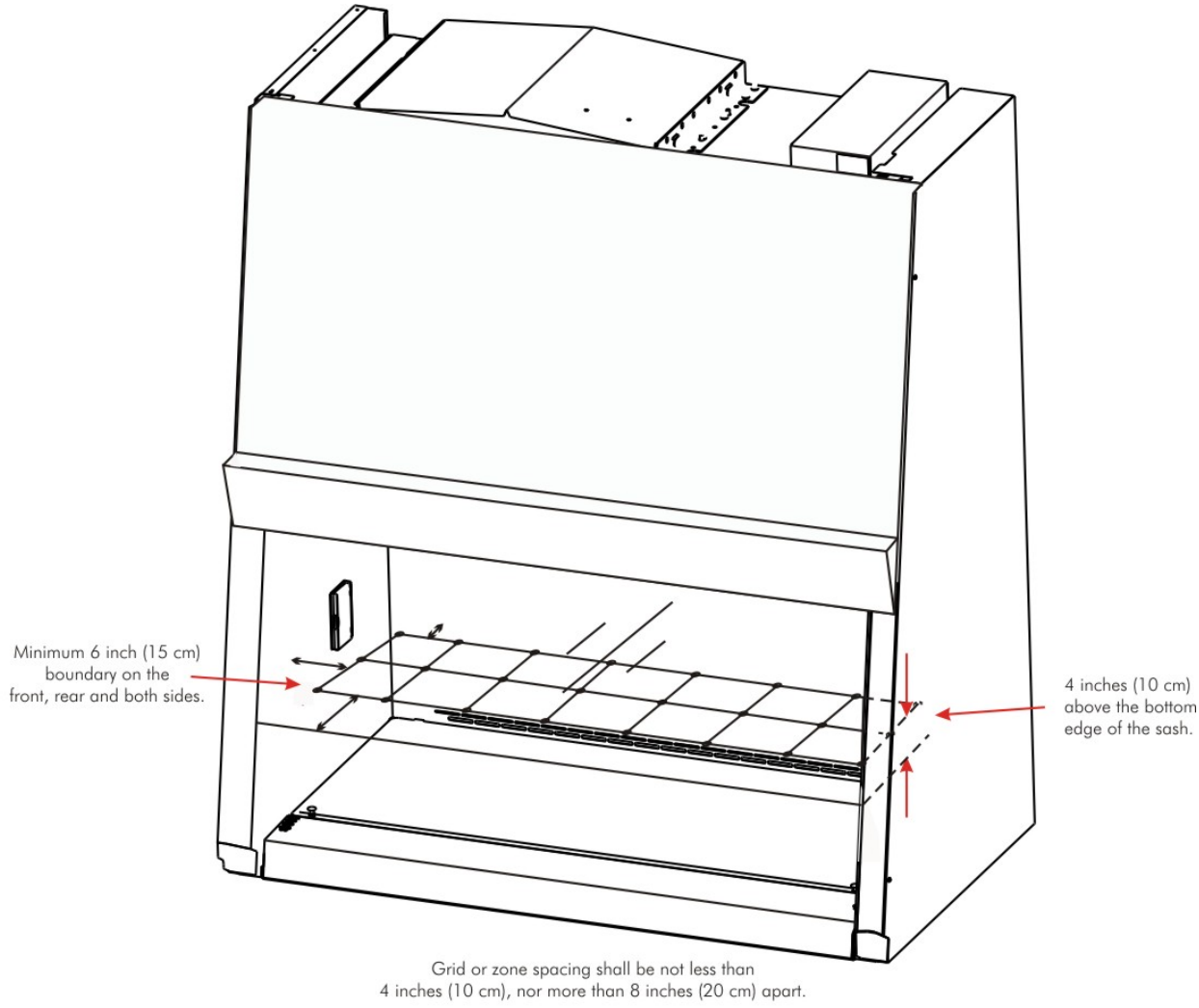


Figure A15 - Velocity Profile Test

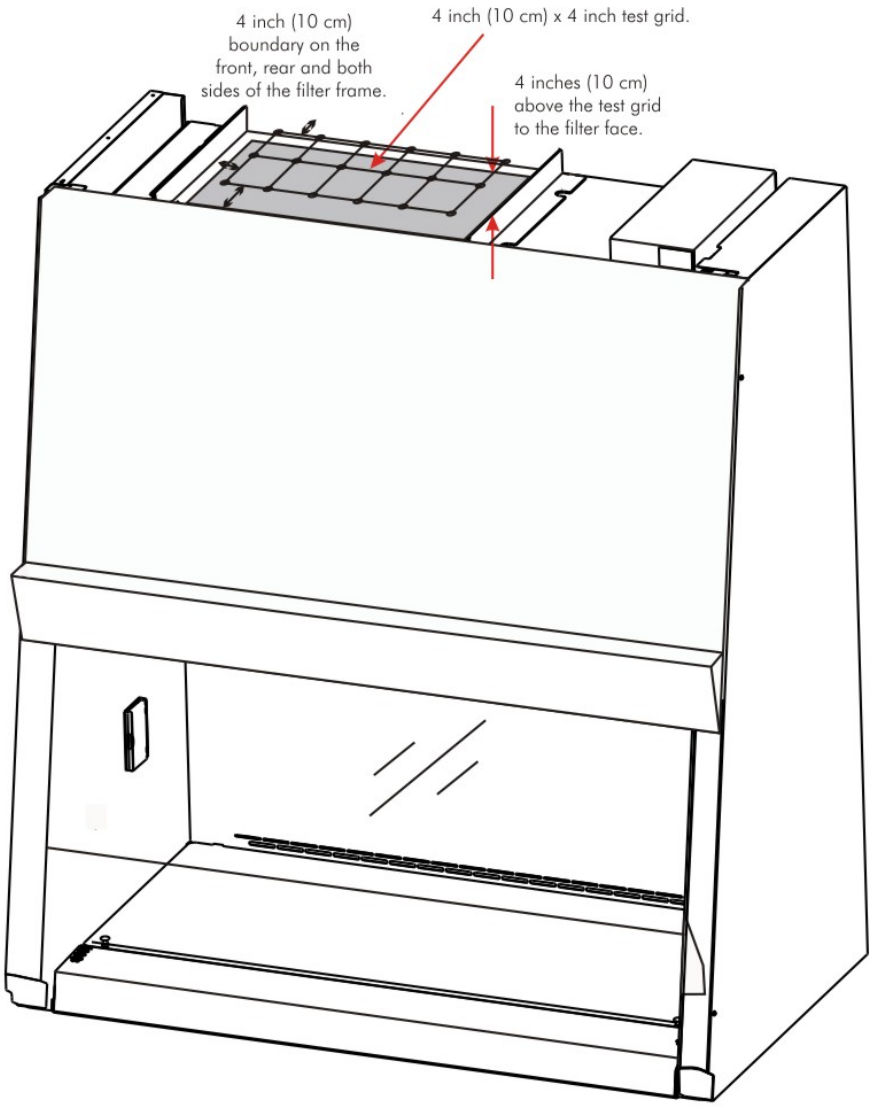


Figure A16 - Calculated Inflow Velocity Profile Test for Type A Cabinets

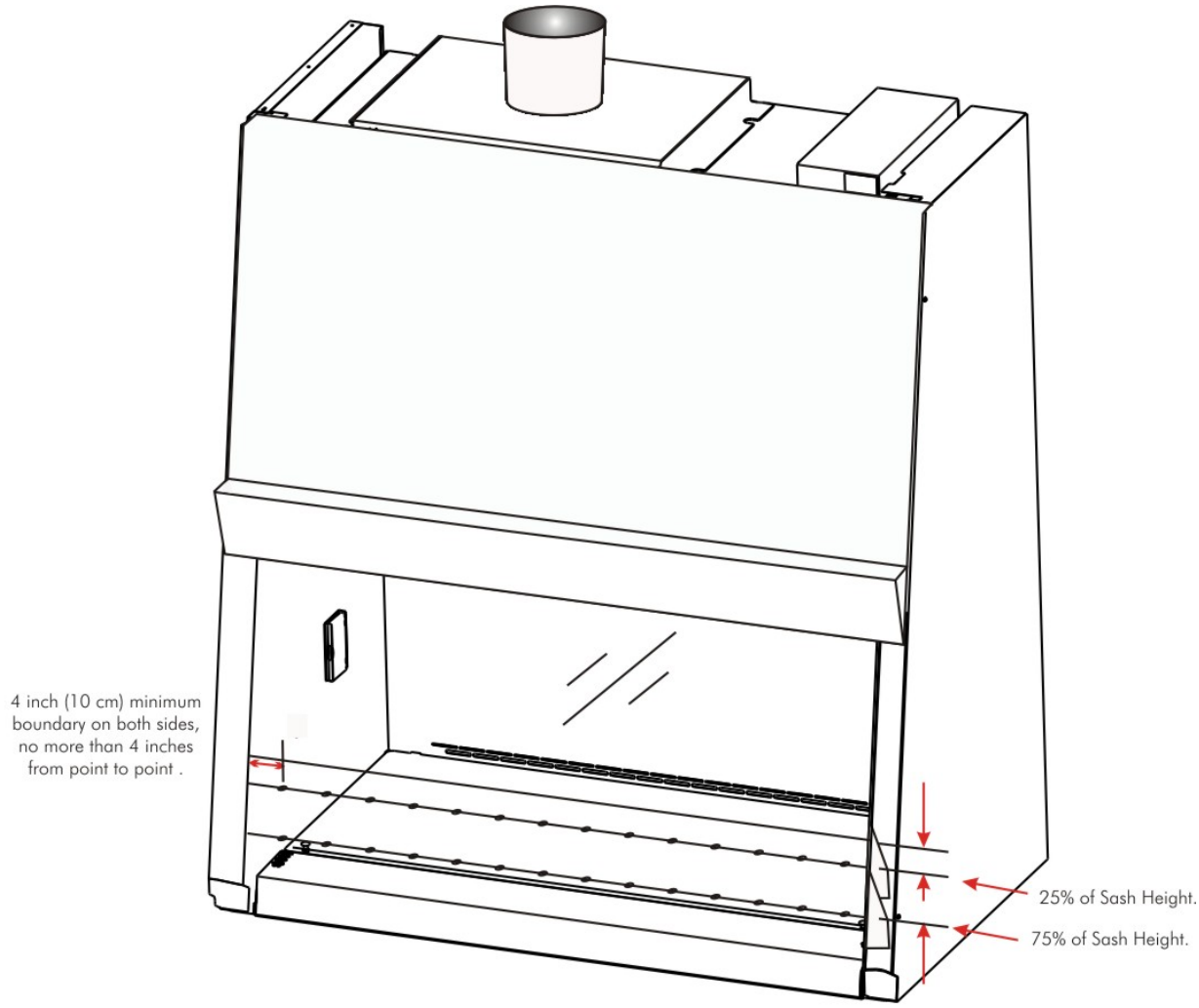


Figure A17 - Another Example of Inflow Velocity Test, Type B1

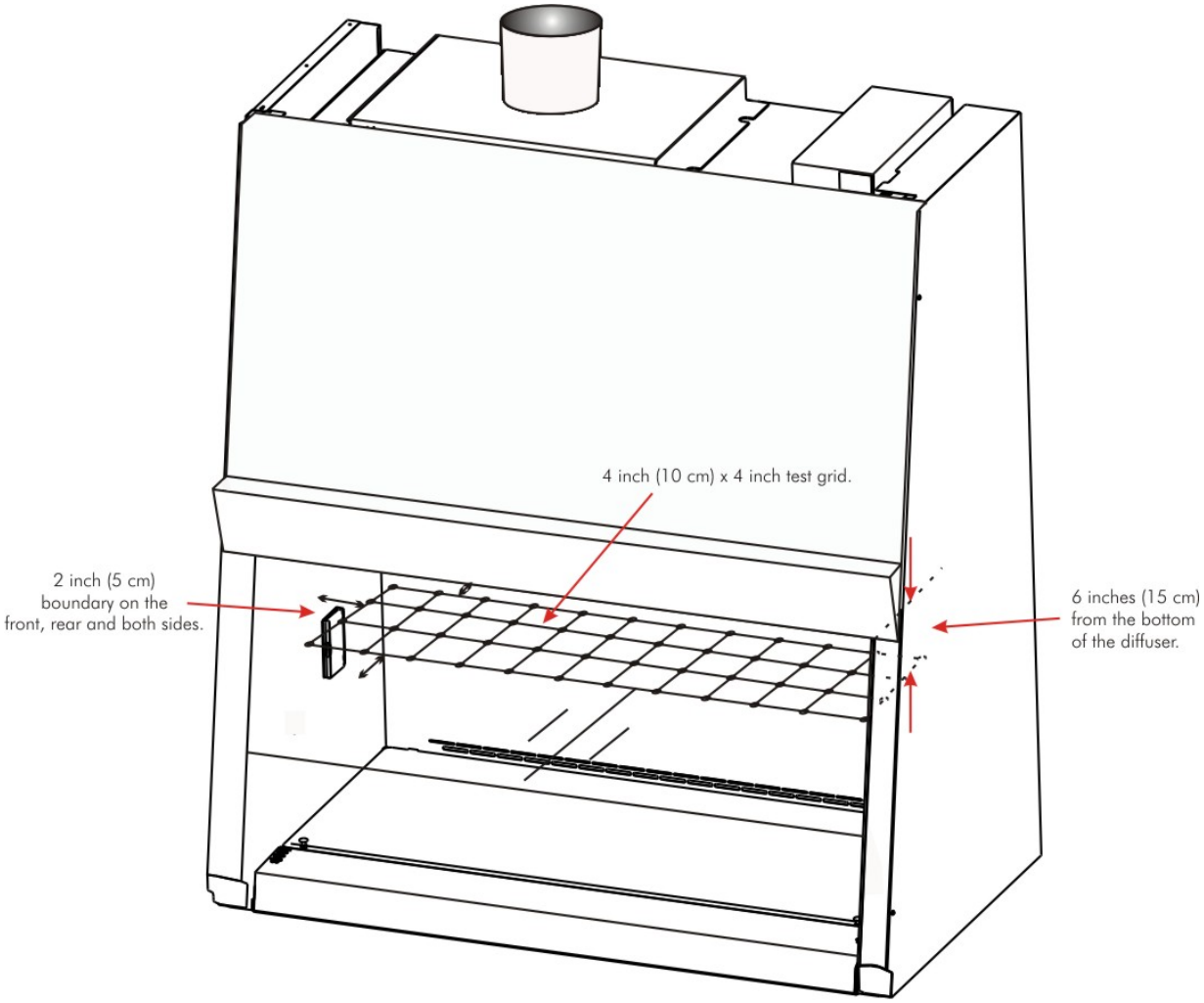


Figure A18 - Supply Air Volume Test

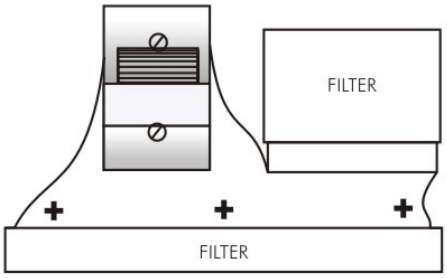


Figure A19.1 - Flexible Style Plenum

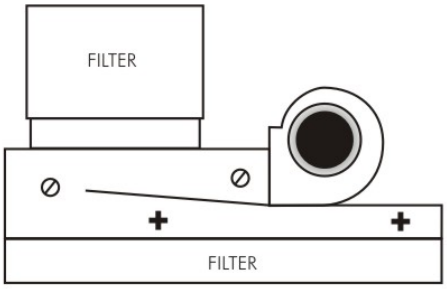


Figure A19.2 - Rigid Style Plenum

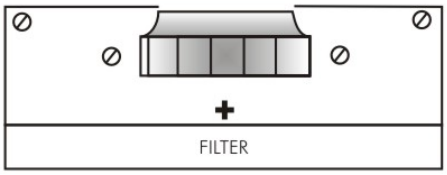


Figure A19.3 - Backward Curved Fan Style Plenum

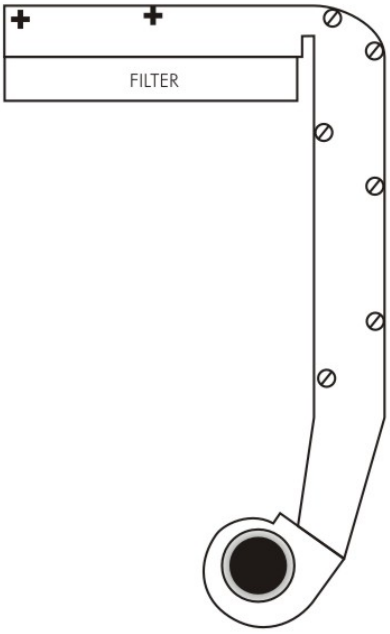


Figure A19.4 - Console Style Plenum

✚ = Appropriate pressure tap location

⊘ = Inappropriate pressure tap location

Figure A19 - Positive Pressure Tap Placement - Motor/Blower Performance Test

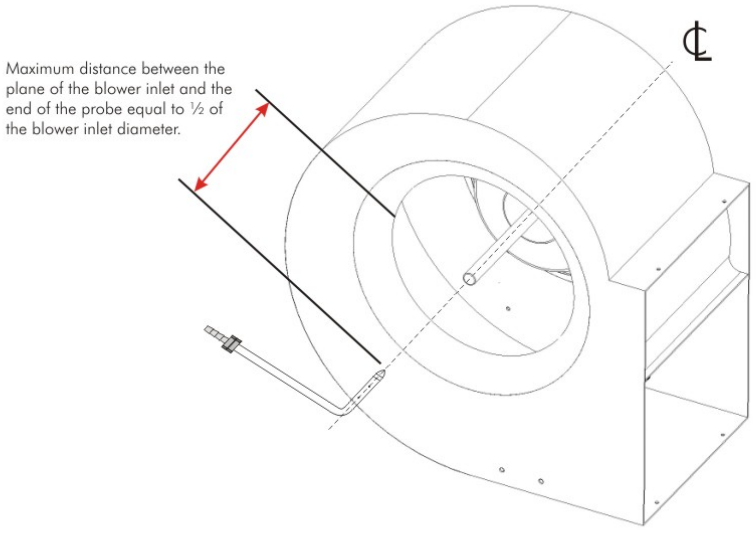


Figure A20.1 - Forward Curved Blower

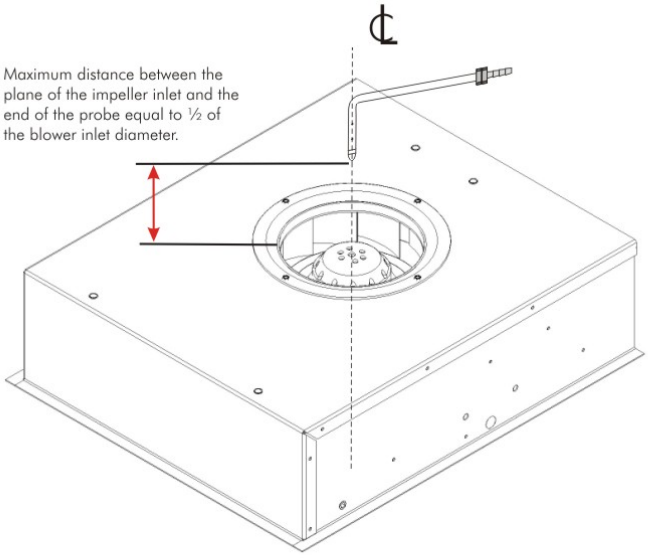


Figure A20.2 - Backward Curved Impeller

Figure A20 - Negative Pressure Tap Placement - Motor/Blower Performance Test

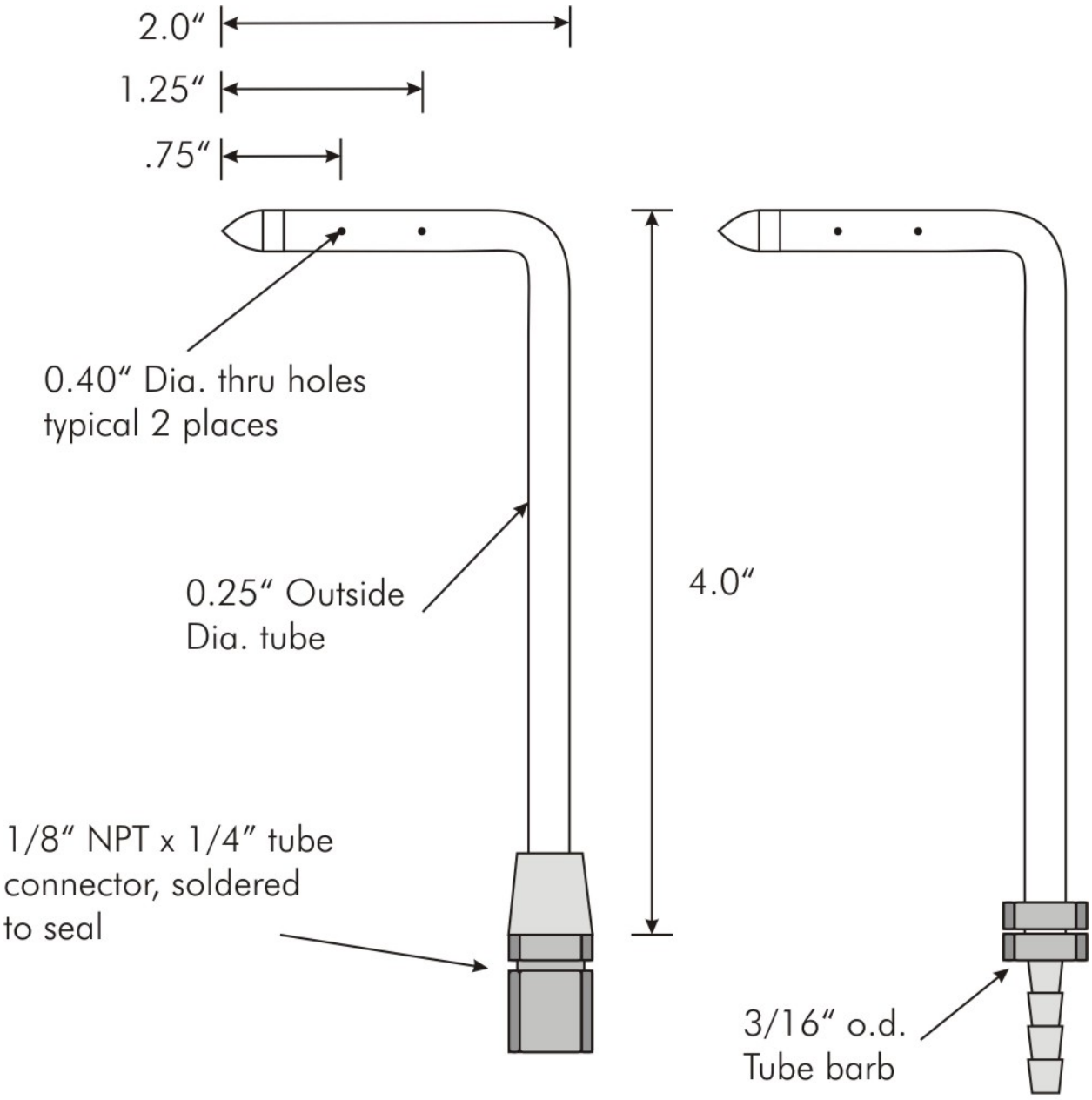


Figure A21 - Pressure Tap

Annex B
(normative)

B.1 Method to verify fitness for use of potential direct inflow measurement devices

B.1.1 Calibrate the basic measuring portion of the device in a wind tunnel with National Institute of Standards and Technology (NIST) traceable calibration (i.e., for devices with removable hoods, calibrate the device without a hood installed; for devices using thermal anemometer, calibrate the thermal anemometer). A pitot tube constructed according to the dimensions given in the Industrial Ventilation Manual is a primary standard and needs no other verification.

B.1.2 Install the device using one of the two following methods:

- Method 1 (figure B1)

  a) Seal the device to the front opening of a Class II Type B2 biosafety cabinet hard connected.

  b) Connect the exhaust of the cabinet to a duct containing an orifice meter or other flow meter calibrated traceable to NIST.

  c) Turn off the downflow blower and seal the downflow air opening.

  d) If the cabinet has a moveable sash, seal the sash.

- Method 2

  a) Seal the device to the front opening of a Class II Type A1 or A2 biosafety cabinet intended to be canopy connected.

  b) Seal a calibrated, NIST traceable flow hood, such as Shortridge[34] model CFM-88, to the cabinet exhaust.

  c) If the cabinet has a moveable sash, seal the sash.

  d) The cabinet exhaust filter open area shall be larger than the section of the flow hood where readings are measured (14 x 14 in [36 x 36 cm] for the Shortridge unit).

B.1.3 Using the condition of Annex B, section B.1.2, Method 1, measure the exhaust flow, non-back pressure compensated, both with the device installed and removed. Record at least five readings in each instance. The difference should not exceed 2%. Then run the cabinet at no fewer than three airflow velocities in a range spanning the highest and lowest airflows the device is to be required to measure. Record at least five readings of the device and of the flow meter, or orifice meter, and calculate the difference. The average difference should not exceed 2%.

B.1.4 Using the configuration of Annex B, section B.1.2, Method 2, measure the exhaust flow, non-back pressure compensated, both with the device installed and with it removed. Record at least five readings in each instance. The difference should not exceed 2%. Then, run the cabinet at no fewer than three airflow velocities in a range spanning the highest and lowest airflows the device is to be required to measure. Record at least five readings of the device and of the flow hood on the cabinet exhaust and calculate the difference. The average difference should not exceed 2%.

[34] Shortridge Instruments, 7855 E. Redfield Rd., Scottsdale, AZ 85260 <www.shortridge.com>

B.1.5 The calibration is valid for cabinets of the size used and smaller. It is recommended that 6 ft (2 m) cabinets be used in this procedure.

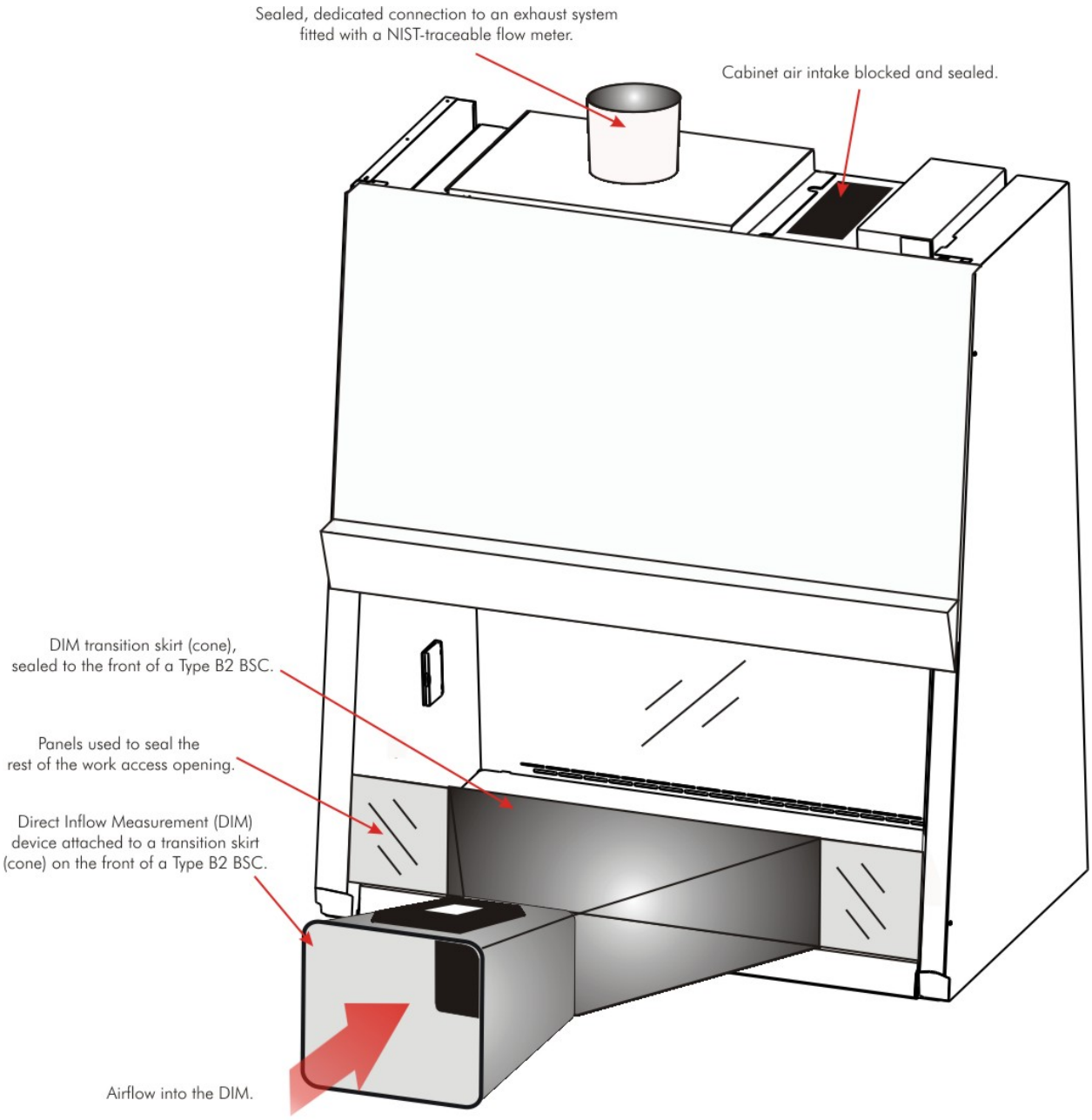


Figure B1 - Verifying fitness for use of potential direct inflow measurement devices

Annex C
(normative)

Nebulizer selection and calibration

C.1 Selection

C.1.1 Criteria

Nebulizers are acceptable when they:

- deliver 1 x 108 to 8 x 108 airborne spores of Bacillus subtilis var. niger in 5 min;
- deliver 94% ± 6% single cell spores; and
- have a spore aerosol discharge velocity of 100 ± 10 ft (30 ± 3 m) per minute.

NOTE 1 – Tests performed by First[35] et al. demonstrated that a stainless steel six-jet collision refluxing nebulizer will deliver the bacterial spore aerosol required in 6.7.1 when the following conditions are met:

- the nebulizer is equipped with a glass flask 2.0 in (5.0 cm) in diameter, 3.5 in (9.0 cm) high and a 0.90 in (2.3 cm), ID horizontal discharge spout on top;
- the nebulizer is operated at 20 psi (140 kPa);
- 55 ml of a 5 x 10^8 to 8 x 10^8/ml spore suspension is placed in the flask;
- the bottom of the six-jet spray head is 0.71 in (1.8 cm) above the bottom of the flask; and
- six rosette patterns created by the air jets form on the inside of the glass flask. (These should be observed frequently for size and contour to verify that the jets are not clogged or obstructed.)

NOTE 2 – The six-jet collision refluxing nebulizer need not be retested for performance before use.

C.2 Calibration

C.2.1 Purpose

The purpose of this section is to demonstrate that a nebulizer conforms to all the criteria cited in Annex C.

C.2.2 Site

The nebulizer shall be calibrated in the laboratory where it is being used.

C.2.3 Frequency

The nebulizer shall be calibrated prior to its first use and periodically thereafter.

C.2.4 Materials

- suspension of 5 x 10^8 to 8 x 10^8 *Bacillus subtilis* var. niger spores per ml;
- nebulizer to be calibrated;

[35] First, M. W., Stuart, D., Webb, T., "Report of NSF Standard Number 49 Ad Hoc Task Group to Recommend Revisions to Appendix C," November 5, 1986.

– one all-glass ACI-30[36] (Ace Glass No 7540-10 impinger) sampler, air sampling impingers (or equivalent);

– switching timer;

– membrane filter funnel (47 mm filter size) with silicone rubber diaphragms sealed to each end with RTV. Diaphragms are perforated to insert the outlet of the nebulizer at the wider end and one impinger sampler at the other end. Insertions shall be tight on the impinger end. Insertion shall be loose on the nebulizer end so that the impinger is operating in atmospheric pressure, not in a closed system;

– flow meters;

– pressure gauge; and

– 37-mm aerosol type membrane filter in sampling cassette with an open face.

C.2.5 Method

a) Measure the nebulizer outlet dimensions and calculate the area in ft^2.

b) Calculate the airflow in ft^3/min (m^3/s) through the nebulizer required to result in 100 ft/min (0.5 m/s) discharge velocity.

c) Add the manufacturer's recommended volume of spore suspension to the nebulizer.

d) Place the outlet of the nebulizer in the rubber diaphragm of the wide end of the filter funnel. Insert the collecting tube of the impinger sampler through the rubber diaphragm on the opposite end of the filter funnel. Ensure a tight fit at impinger end, as shown in figure C1.

NOTE – The all-glass impinger (chemical corps type) comes in two versions: (1) an impinger with the tip submerged in liquid 4 mm from the flask bottom and passing 6.0 Lpm at a pressure drop of 8.0 psig or greater (ACE Glass[36] No. 7541 impinger) and (2) an impinger with the tip above the liquid surface and passing 12.5 Lpm at a pressure drop of 8.0 psig or greater, known as AGI-30, (ACE Glass[36] No. 7540 impinger). Either impinger may be used. When the air delivery rate of the nebulizer is not precisely 6.0 or 12.5 Lpm, select the impinger that samples at a higher rate and bleed in through an opening around the nebulizer insertion an amount of air equal to the difference in the two airflows. If the nebulizer and the impinger are to be operated at the same flow rate, a snug fit in the diaphragm at both ends is recommended.

e) Attach the hose to a pressure gauge attached to flow meter, then to the nebulizer.

f) Simultaneously turn on the nebulizer (maintain airflow through the nebulizer to result in a calculated 100 ft [30 m] per minute output velocity based on airflow [ft^3/min] and diameter of the discharge spout - 12.5 L/m for the six-jet collision described in this annex) and the impinger sampler (operating according to manufacturer's instructions). Operate nebulizer for 5 min (using the switching timer) and the impinger sample for 5.25 min.

g) Aseptically transfer the impinger sampler collecting fluid to a sterile 500-ml graduated cylinder. Rinse the funnel, impinger stem, and bottle with sterile water to insure collection of all spores, and collect all rinse water in the graduated cylinder.

h) Measure and record the volume of fluid in the graduated cylinder. Transfer all the fluid aseptically to a sterile flask containing a magnetic stirrer and mix thoroughly.

[36] Ace Glass, Inc., PO Box 688, 1430 North West Blvd., Vineland, NJ 08362-0688 <www.aceglass.com>

i) Prepare serial dilutions and quantify spore concentration by five replicate platings.

j) Actively sample the bacterial aerosol with membrane filter located in its design mode. After sampling is completed, stain the membrane with an appropriate dye. Count the number of deposits containing single and more than one bacterium in representative fields under a microscope.

C.2.6 Calculations

a) number of spores delivered in 5 min = (dilution factor) x (average number of CFUs on the five plates);

b) velocity of air leaving nebulizer = the air volume measured in C.2.5 b) in ft^3/min (m^3/s) divided by nebulizer outlet area in ft^2; and

c) calculate the percent of single bacteria in the total aerosol sample.

C.2.7 Acceptance

– The average of five replicate calibration tests shall fall between 1×10^8 and 8×10^8 spores per 5 min nebulizer operation.

– The velocity of air leaving the nebulizer shall be 100 ± 10 ft (30 ± 3 m) per minute.

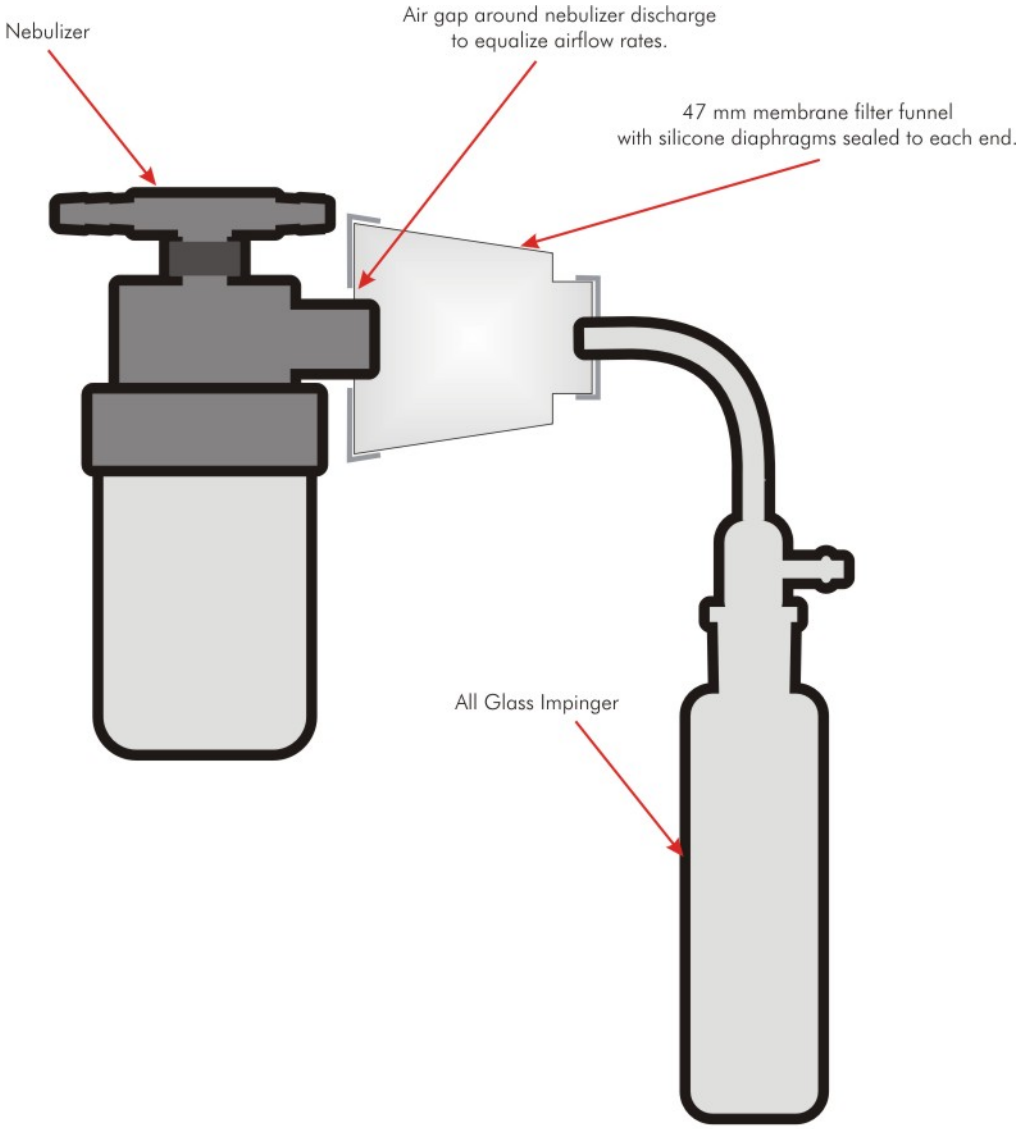


Figure C1 - Nebulizer calibration

Annex D
(normative)

Evaluation of chemical resistance and abrasion resistance of surfaces

D.1 Chemical resistance

D.1.1 Chemicals

The following chemicals shall be used for resistance testing:

- 1N hydrochloric acid;
- 1N sodium hydroxide;
- 1% quaternary ammonium compound;
- 5% formaldehyde;
- 5,000 ppm hypochlorite;
- 2% iodophor;
- 5% phenol; and
- 70% ethyl alcohol.

D.1.2 Method

Chemical spot tests shall be made by applying 10 drops (approximately 0.5 ml) of each reagent to the surface to be tested. Each reagent shall be covered by a watch glass, convex side down, in the center of the puddle, to hold the reagent in place. Reagents shall be allowed to remain on the surface for 4 h, and tests shall be performed so the testing surface is wet throughout the entire test period. After 4 h, the surface shall withstand scrubbing with a stiff brush and hot water at 160°F (72°C). Samples shall be dried before examination. Surface stains of dyes shall be removed with an alcohol wash before examination.

D.1.3 Acceptance

When exposed to the chemicals listed above or special chemicals, the surface shall show no visible effect on the finish, other than a slight change of gloss, slight discoloration, or temporary slight softening of the finish, with no loss of adhesion and film protection.

D.2 Abrasion resistance

D.2.1 Method

A protective coating shall be applied in the recommended manner and properly cured on a panel of the prescribed substrate. It shall be evaluated on a Taber Abrader following the procedures of ASTM[37] D1044-76 using a CS-IOS wheel, and a 1,000-g load for 500 cycles.

D.2.2 Acceptance

The maximum weight loss for 500 cycles shall not exceed 100 mg. The substrate shall not be exposed during the test.

[37] ASTM, 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959 <www.astm.org>

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Annex E[38]
(informative)

Biosafety Cabinet Selection, Installation, Lifespan and Decommissioning

E.1 Biosafety consultation prior to BSC purchase

A biosafety officer or qualified safety professional should be consulted prior to a BSC purchase. Some institutions have biosafety cabinet purchases approved by the biosafety officer or qualified safety professional after consultation with the user, architect and engineer. Biosafety officers or qualified safety professionals that perform this function should have training and field experience that includes methods used to control biohazards and knowledge of the design, application, and testing of biosafety cabinets.

Issues that may be considered include:

- risk assessment;
- selecting which kind of BSC is required and if it should be exhausted; and
- assessment of the laboratory environment and the proper location of BSCs within it.

E.1.1 If there is a window in the laboratory, it should remain closed at all times. Cabinets should not be located where room ventilation air inlets blow across the front opening or onto the exhaust filter.

E.2 Risk assessment procedure

E.2.1 Risk assessments encompass four main elements:

- hazard identification;
- exposure assessment;
- dose-response assessment; and
- risk characterization, and risk management (job analysis)[39].

E.2.2 Risk assessment team members may include:

- investigator/scientist;
- laboratory staff;
- animal care staff when appropriate;
- animal veterinarian when appropriate; and
- occupational health & biosafety professionals.

E.2.3 Risk assessment hazards considered:

- animal hazards;
- agent/pathogen/recombinant hazards;
- chemical hazards; and
- radiological hazards.

[38] The information contained in this annex is not part of this American National Standard (ANS) and has not been processed in accordance with ANSI's requirements for an ANS. Therefore, this annex may contain material that has not been subjected to public review or a consensus process. In addition, it does not contain requirements necessary for conformance to the Standard.

[39] Songer, J.R. 1995. Laboratory safety management and the assessment of risk, p. 257-277. *In* D.O. Fleming, J.H. Richardson, J.J. Tulis, and D. Vesley (ed.), *Laboratory Safety: Principles and Practices*, 2nd ed. ASM Press, Washington, DC.

E.2.4 Agent/pathogen/recombinant's factors associated with risk of disease or injury:

- virulence;
- infectious dose;
- route of infection (portal of entry);
- toxigenicity;
- agent's host range;
- if the agent is endemic or exotic to the environment it is in;
- availability of effective preventive measures; and
- availability of effective treatment.

E.2.5 Factors associated with worker's risk of exposure:

- worker's work activity; diagnostic, research or production scale;
- worker's proficiency, attitude and safety awareness; and
- worker's age, sex, pregnancy, race, immune status and medications.

E.2.6 Risk management plan includes:

- biosafety containment level assignment to the facility and microbiological practices;
- safety equipment;
- engineering controls;
- personal protective equipment;
- work practices – Standard Operating Procedures (SOPs);
- emergency procedures;
- work schedule – calendar; and
- investigation protocols that include all risk management plans.

E.2.7 Investigation protocol review includes:

- committee (IBC/IRB/IACUC) review, as appropriate;
- meetings with workers to discuss approved protocols;
- training;
- dry runs without agent/pathogen/recombinant; and
- regular audits.

E.2.8 Risk management analysis table

Risk Factors	Assessment Level	
	Decrease<	>Increase
Pathogen Disease Potential		
Known, classified		
Suspected, classified		
Known, unclassified		>>>
Unknown		>>>>
Pathogen Aerosol Potential		
Tissue procedure	<<<	
Culture procedure		>>>
Concentration procedure		>>>>>>
Animal/non-shedder	<<<	
Animal/shedder		>>>>>>
Pathogen Infectious route		
Respiratory		>>>>>>
Mucous membrane		>>>
Parenteral	<<<	
Other	<<<	
Disease Severity		
Moderate		>>
Severe		>>>
Life threatening/lethal		>>>>>>>>
Disease Prophylaxis		
None		>>>>>>>>
Vaccine	<<	
Immune globulin	<<<	
Antibiotics	<<<	
Antivirals	<<<	
Other Factors		
Livestock pathogen		>>>
Poultry pathogen		>>>

E.3 Biosafety cabinet selection

E.3.1 Selecting the proper BSC should be done in two stages; first, select the proper class and type of cabinet required, then decide on the size of the cabinet and options that are needed. The various configurations of Class II BSCs are shown in figures E2, E4 and E5. Deciding which class and type is appropriate can be accomplished by answering the following five questions.

E.3.1.1 What needs to be protected?

- Only the material being worked on (product protection)?
- Only the technician and the laboratory (personnel and environmental protection)?
- Or to protect all three (personnel, product, and environmental protection)?

If all that is needed is product protection, then a Clean Bench, which is not a BSC, may be the unit of choice. Clean Benches use High Efficiency Particulate Air (HEPA or ULPA) filter(s) to remove particulates from room air. This filtered, particulate-free air then flows through an enclosed work area, in a horizontal or vertical direction. These devices bathe the materials inside in filtered air, and then the air is typically discharged into the laboratory. While these devices protect the product from airborne contaminants, any aerosol generated in the work area will be discharged into the laboratory. As such, they cannot be used with toxic or biohazardous materials.

For personnel and environmental protection only, the Class I enclosure offers a simple and economical solution. Room air sweeps around the operator and through the work area. This contaminated air is then HEPA or ULPA- filtered and discharged into the laboratory or exhausted outside of the building via an exhaust system. The Class I will protect the operator and the lab, however, because room air constantly washes over the work area, the product is exposed to airborne contaminants.

Personnel, environmental, and product protection can be had most efficiently by a Class II Biosafety Cabinet. The inflow of air around the operator provides personnel protection. HEPA-or ULPA-filtered air flowing downward through the work area provides product protection, and protects the laboratory from biohazardous particulates.

E.3.1.2 What are all of the different types of work to be done in the cabinet?

One of the most difficult tasks in selecting a BSC is trying to foresee all the different types of work that will be taking place in it. It is critical to decide what things need protection, both now and in the future. All too often users purchase a Clean Bench or Class I device for current applications, only to find these devices are unsuitable as their work requirements change.

E.3.1.3 What types and quantities of chemical vapors will be generated in the BSC?

As important as the preceding question, the user must also foresee the types and quantities of chemical vapors that will be generated in the cabinet. Because chemical vapors can freely pass through HEPA or ULPA filters, both Class I and Class II BSCs must be exhausted out of the laboratory when used with these types of chemicals. For the Class II BSCs, Types B1 and B2 must be hard ducted to an external exhaust system in order to operate properly; Types A1 and A2 can be converted to operate in either a ducted or recirculating mode, depending on the users' requirements. The airflow patterns of Types A1, A2, B1 and B2 Biosafety Cabinets are shown in figures E2, E4 and E5, respectively.

E.3.1.4 If the unit requires an exhaust system, is there an appropriate location for the cabinet and its ductwork?

If a BSC is going to recirculate its HEPA- or ULPA-filtered air back into the laboratory, then the user has some freedom as to where the unit can be installed, provided it is out of major traffic areas, and there are no other air handling devices in the area, as shown in figure E1.

When connected to a hard ducted exhaust system, however, the location of the cabinet becomes dependent on the location of the exhaust system. The exhaust duct must be placed so it can penetrate ceilings and floors without disturbing other ventilation or plumbing systems. The exhaust system must also be designed to minimize excessive lengths and elbows. The exhaust system configurations of Type A and Type B BSCs are shown in figures E3 and E6, respectively. Hard ducting Type A cabinets is not acceptable and shall only be exhausted through a properly designed and fitted exhaust canopy.

E.3.1.5 If the volume of air being removed by the BSC's exhaust system is reduced, or eliminated, due to malfunction, what is its effect on BSC performance, and what is preferred by the user?

For a Type A BSC fitted with a properly designed canopy connection, reduction or elimination of the exhaust air should not significantly affect the airflow patterns within the BSC. Personnel and product protection of the BSC will remain unchanged; however, chemical vapors generated in the BSC will be exhausted into the laboratory via the openings or slots in the canopy.

For a Type B BSC, any reduction or elimination of the exhaust air will directly impact the inflow velocity, and thus the personnel protection offered by the BSC. Reduction of exhaust airflow will reduce the inflow, jeopardizing personnel protection. Loss of the exhaust airflow will eliminate the inflow of air into the front of the BSC, negating personnel protection, and allowing materials in the work area of the BSC to escape into the laboratory.

Table E.3.1.6 Characteristics of Type A1 and A2 BSCs

	Type A1 (figure E2)	Type A2 (figure E2)
Intended Purpose	Routine microbiological work. Not appropriate for work generating chemical vapors. If working with malodorous products, the unit may be canopy-connected to external exhaust for odor control.	Routine microbiological work. Work generating minute quantities of chemical vapors required as an adjunct to microbiological research, if the BSC is canopy-connected to external exhaust. Any vapor generated must not interfere with the work when recirculated in the downflow air.
Airflow Pattern	Room air is drawn in through the sash opening, protecting the operator. HEPA- or ULPA- filtered air flows down through the work area, protecting the product. Both bodies of air flow through a common plenum to the cabinet blower(s). A portion flows out of the cabinet via an Exhaust HEPA or ULPA filter, and the remainder recirculates through a Supply HEPA or ULPA filter before flowing down through the work area.	Room air is drawn in through the sash opening, protecting the operator. HEPA –or ULPA-filtered air flows down through the work area, protecting the product. Both bodies of air flow through a common plenum to the cabinet blower(s). A portion flows out of the cabinet via an Exhaust HEPA or ULPA filter, and the remainder recirculates through a Supply HEPA or ULPA filter before flowing down through the work area.
Air Recirculation	Varies by model; typically 70%	Varies by model; typically 70%
Inflow	Minimum 75 FPM Average	Minimum 100 FPM Average
Downflow	Varies by model, typically 50-80 FPM average	Varies by model, typically 50-80 FPM average
Biological Containment	All NSF-Listed BSCs must pass the same Biological Containment Tests.	All NSF-Listed BSCs must pass the same Biological Containment Tests.
Exhaust System	Canopy connection as needed.	Canopy connection as needed.
Exhaust System Type	Due to the superior ability to handle external exhaust variation, canopy connected Type A BSCs may be ganged into a multiple-cabinet exhaust system, if all BSCs are balanced properly.	Due to the superior ability to handle external exhaust variation, canopy connected Type A BSCs may be ganged into a multiple-cabinet exhaust system, if all BSCs are balanced properly.
Exhaust System Function	To convey the BSC exhaust air, plus an additional volume required by the canopy through the ductwork.	To convey the BSC exhaust air, plus an additional volume required by the canopy through the ductwork.
Exhaust System Volume	Greater than Type B1. Less than Type B2.	Greater than Type B1. Less than Type B2.
Exhaust System Negative Static Pressure at BSC	Typically 0.1 – 0.5 in H_2O.	Typically 0.1 – 0.5 in H_2O.
Exhaust System Reserve Capacity	Static pressure requirements will not change as the cabinet filters load.	Static pressure requirements will not change as the cabinet filters load.
Cabinet Flexibility	Can be connected or disconnected from exhaust system as needs change.	Can be connected or disconnected from exhaust system as needs change.
Cabinet Cost	Less than Type B	Less than Type B
Installation Cost	Much less than Type B if recirculating; slightly less than Type B if canopy-connected.	Much less than Type B if recirculating; slightly less than Type B if canopy-connected.
Operation Cost		
Electrical Cost (BSC Only)	Equal to Type B1 Slightly greater than Type B2	Equal to Type B1 Slightly greater than Type B2
Tempered Air Loss	If recirculating in lab; none. If canopy-connected, typically 100 CFM/foot of BSC width or less.	If recirculating in lab; none. If canopy-connected, typically 100 CFM/foot of BSC width or less.

Table E.3.1.7 Characteristics of Type B1 and Type B2 BSCs

	Type B1 (figure E4)	Type B2 (figure E5)
Intended Purpose	Type B1 cabinets may be used for routine microbiological work generating minute quantities of chemical vapors required as an adjunct to microbiological studies, if work is done in the directly exhausted portion of the cabinet, or if the vapors will not interfere with the work when recirculated in the downflow air.	Type B2 cabinets may be used for routine microbiological work generating chemical vapors required as an adjunct to microbiological studies.
Airflow Pattern	Room air is drawn in through the sash opening, protecting the operator. HEPA- or ULPA- filtered air flows down through the work area, protecting the product. The room air, and a portion of downflow air in the front of the work area is recirculated through a supply HEPA or ULPA filter before flowing down through the work area. The air in the rear of the work area flows out of the cabinet via an Exhaust HEPA or ULPA filter.	Room air is drawn in through the sash opening, protecting the operator. HEPA- or ULPA-filtered room air flows down through the work area, protecting the product. Both bodies of air are drawn out of the cabinet via an Exhaust HEPA or ULPA filter.
Air Recirculation	Varies by model; typically 50%	None
Inflow	Minimum 100 FPM Average	Minimum 100 FPM Average
Downflow	Varies by model, typically 50-80 FPM average	Varies by model, typically 50-80 FPM average
Biological Containment	All NSF-Listed BSCs must pass the same Biological Containment Tests.	All NSF-Listed BSCs must pass the same Biological Containment Tests.
Exhaust System	Required.	Required.
Exhaust System Type	Should have dedicated ductwork and exhaust blower for each BSC.	Should have dedicated ductwork and exhaust blower for each BSC.
Exhaust System Function	Must pull exhaust air through the Cabinet's Exhaust HEPA or ULPA filter and then through ductwork.	Must pull exhaust air through the Cabinet's Exhaust HEPA or ULPA filter and then through ductwork.
Exhaust System Volume	B1 is approximately 20% less than a Type A.	B2 exhausts 100% or more air than any other Type.
Exhaust System Negative Static Pressure at BSC	Typically 1.5 in H_2O minimum.	Typically 1.5 in H_2O minimum.
Exhaust System Reserve Capacity	Static pressure requirements may increase up to 0.3 in H_2O as exhaust HEPA or ULPA filter loads.	Static pressure requirements may increase up to 0.7 in H_2O as exhaust HEPA or ULPA filter loads.
Cabinet Flexibility	Must be permanently connected to an exhaust system to function properly.	Must be permanently connected to an exhaust system to function properly.
Cabinet Cost	More expensive than Type A	More expensive than Type A
Installation Cost	Similar to a canopy connected Type A.	Most expensive. Higher exhaust volumes may require larger ductwork and higher capacity exhaust fan.
Operation Cost		
Electrical Cost (BSC Only)	Equal to Type A	Less than a Type A
Tempered Air Loss	Less than a canopy connected Type A. Typically 50-100 CFM/foot of BSC width.	Typically 175 CFM/foot of BSC width.

E.3.2 BSC size

Having decided which class and type of BSC is the best, the user should now decide on the size of the unit and its options. Typical workspace widths are 3, 4 and 6 ft. Outside dimensions are approximately 3.5, 4.5, 5.5 and 6.5 ft. In deciding which size is best, the user should mark out an area of benchtop equal to the inside (work area) dimensions of the model they are interested in. The user(s) should perform several "dry runs" of their procedures in this area. If the user can work in this defined space, than the cabinet is the proper size, if not, the user may want to try working in the dimensions of the next larger model. If the user does decide on a larger model, however, be sure that the BSC can be transported to and installed in the laboratory through the existing freight elevators, hallways and doors. It is important to remember that BSC widths typically refer to the internal work area. The external width of the BSC may be significantly wider.

E.3.3 BSC options

E.3.3.1 Service valves

Service valves allow inert gases, air or vacuum lines to be plumbed into the BSC. Many models allow for the easy installation of these valves in the field, however, it is generally less expensive and easier to have the required number of valves installed when the unit is ordered. Although many users connect natural gas to a service valve in the cabinet, this practice should be avoided if possible, because open flames in a Class II BSC disrupts the airflow, and there is the possibility of a buildup of flammable gas in BSCs that recirculate their air.

E.3.3.2 Electrical outlets

Most BSCs have electrical outlets installed in the work area as standard equipment. Specialized outlets, such as Ground Fault Circuit Interrupters (GFCIs) should be installed and tested by the cabinet manufacturer.

Typically the outlets in the work area are limited in their amperage rating. This is due to the amperage requirements of the BSC's motor, lighting, and other electrical components.

Variations in line voltage from the laboratory wall outlet may affect the cabinet airflows. A voltage regulator may need to be installed in order to reduce the potential of variations in airflows.

E.3.3.3 Ultraviolet lighting

Germicidal (or UV) Lamps are often installed as an adjunct to surface disinfection. UV lighting is not recommended in Biosafety cabinetry. While their usefulness is a subject for debate among users and manufacturers, they should be installed and tested by the manufacturer during assembly of the unit.

E.3.3.4 IV bar

Because intravenous (IV) bars or rods have a significant impact on the airflows in the work area, always use the IV bar recommended by the manufacturer.

E.3.3.5 Base stands

Base Stands or supports should also be considered at the time of specification. Some models of cabinets can weigh up to 900 pounds (408 Kg). The BSC must be attached to a manufacturer recommended base stand or a structure rated to support the unit's weight. All base stands have a maximum height specified by the manufacturer to prevent overturning of the BSC; this maximum should never be exceeded.

E.3.3.6 Mobile installations

Mobile Base Stands with and without lift capability have been used when the BSC is operated in multiple locations in the same or adjoining laboratories. Proper cabinet operation should be confirmed by airflow smoke pattern tests at each site of use. If the cabinet is relocated to another facility, or subjected to excessive shock and/or vibration during moving, the BSC should be recertified to ensure it is functioning in a proper manner.

E.4 Prior to the purchase

E.4.1 Consultation

Investigators should consult with a biosafety officer or qualified safety professional and request a risk assessment of the proposed investigation to ensure that an appropriate BSC is used for the work. Purchase of NSF 49 listed Class II biosafety cabinets is recommended, but alternative containment equipment may be suggested for special tasks.

E.4.2 Site assessment

The investigator should thoroughly examine the intended installation site to ensure it will meet the requirements for proper cabinet operation.

E.4.2.1 Location of the BSC

The cabinet should be located away from traffic patterns, doors, fans, ventilation registers, fume hoods and any other air-handling device that could disrupt its airflow patterns. All windows in the room should be closed. Figure E1 shows the preferred location for the cabinet.

E.4.2.2 Clearances

BSCs not connected to an exhaust system should have at least 6 in (15 cm) clearance from the filter face and any overhead obstructions when the cabinet is in its final operating position, to allow for testing of the Exhaust HEPA/ULPA filter. At least 12 in (30 cm) clearance is required if the use of a thermal anemometer exhaust velocity measurement is needed when calculating cabinet inflow velocity. A clearance of at least 6 in (15 cm) should be maintained on both sides of the cabinet, as well as 12 in (30 cm) behind the unit, to allow for service operations if necessary.

E.4.2.3 Exhaust requirements

If the BSC is to be connected to an exhaust system, first examine the location to ensure that it is compatible with the cabinet's exhaust outlet. The area directly above the cabinet's exhaust outlet should be clear of structural elements, water and utility lines, or other fixed obstructions. There should be enough clearance to allow for the passage of a 10 in (25 cm) or 12 in (30 cm) diameter duct. Avoid cabinet locations that require either an elbow directly on top of the cabinet's exhaust connection or an excessive number of elbows to clear other items.

E.4.2.4 Electrical requirements

The electrical outlet that the BSC plugs into should have a dedicated circuit breaker. This will prevent the accidental shutdown of the cabinet, should another device overload the circuit.

Some larger cabinet models, when operated at 115 V, will require a circuit rated for 20 Amp service. As the electrical plugs and sockets for 115 V, 15 and 20 Amp ratings are different configurations; the user should confirm that the site outlet socket matches the BSC plug.

NOTE – Some cabinets do not operate properly when connected to a ground fault circuit interrupter (GFCI). Consult with the BSC manufacturer about compatibility of their model with a GFCI outlet, if one is present.

E.4.2.5 Service line requirements

All service lines to the BSC should meet local building codes, and be equipped with an easily accessible external shut-off valve, should disconnection be required.

E.4.2.5.1 Connecting service valves to flammable materials

NOTE – The use of flammable gases or solvents should be avoided in a BSC. Open flames in the cabinet will disrupt the airflow in the cabinet and may damage the HEPA/ULPA filters. Flammable gases or solvents may reach explosive concentrations in recirculating cabinets or ductwork. If the user feels that their procedure requires the use of an open flame or flammable materials they should contact their institution's safety office.

E.4.2.5.2 Connecting service valves to high pressure service

The use of air or gases under high pressure should be avoided as they may seriously disrupt the airflow patterns in the cabinet.

E.4.2.5.3 Connecting service valves to a central (House) vacuum

If service valves are to be connected to a central (House) vacuum source, appropriate devices, such as disinfectant traps and/or in-line filters should be installed to prevent contamination of the vacuum system.

E.4.2.6 Roof exhaust systems

Roof exhaust systems serving biosafety cabinets should have a stack that extends straight upward at least 10 ft (3 m) above the roof surface or have a stack with a smaller diameter trailing end to produce higher velocity flow to avoid re-entrainment by the building, and should be increased in elevation when necessary to avoid the influence of surrounding structures. Raincaps or any other structure that deflects the straight upward flow of the discharged air should be avoided. No precipitation can enter the stack when air is being exhausted at normal stack velocities. To take care of precipitation during periods when system is shut off, a 1 in (2.5 cm) hole can be drilled in the lowest point of the fan casing and the water allowed to drain onto the roof. It is recommended that roof exhaust fans be energized by direct-connected electric motors to avoid failures caused by slipping and breaking of belts. Another advantage of direct-connected fans is the ability to use the motor non-function to activate an alarm in the laboratory, whereas when a malfunctioning belted fan is employed, the motor can be operating when the fan is idle. A diagram illustrating a recommended roof exhaust facility is shown in Annex E, figure E7.

E.4.3 Pre-purchase checklist

The investigator should notify building management to arrange for feasibility assessment of laboratory alterations and BSC location. The investigator and biosafety officer or qualified safety professional should discuss the following points about the BSC and its delivery:

- ensure all arrangements are planned in advance of the BSCs arrival;

- get a written price quote for the entire package, including the BSC Model number, optional equipment, canopy exhaust connection, etc. Work out the details about shipping and delivery with the manufacturer's representative at the time of purchase;

- determine the costs for shipping and delivery because there may be additional costs depending on the location and level of difficulty of delivery;

– ensure that the sales representative clarifies in writing what is included in "shipping and delivery." Does it include delivery of the BSC to the receiving dock of the building or to the laboratory? Does it include BSC set-up in the work area, and removal of cartoning/crating materials?

– if not covered in the purchase price, the customer will have to get facility personnel, or hire moving contractors to uncrate and move the BSC;

– ensure the corridor pathways are clear for delivery to the laboratory;

– will the BSC fit through door jams?

– will the BSC travel around sharp, narrow corridors and corners?

– will the elevators in the building accommodate the BSC?

– does the BSC have to be brought up steps?

– the moving contractor should be advised that the BSC shall be lifted onto its stand or leg extensions (working position) with a hydraulic lift; and

– responsibility for removal and proper disposal of all packing materials must be established.

E.5 Inspection

E.5.1 When the BSC arrives, inspect it carefully. Compare the invoice with the delivered equipment. Check for any damage or missing materials and report them immediately to the proper carrier and the BSC supplier regardless of how insignificant they may first appear. Be careful of sharp crating material and let the loading dock personnel help check for damage.

E.5.2 Arrange for certification after the BSC is installed. Building operations personnel may be needed to connect the BSC to laboratory plumbing, electrical, and supply/exhaust air ventilation systems.

E.6 Moving a permanently installed biosafety cabinet

E.6.1 It is a common practice to move permanently installed BSCs to other locations within a laboratory or to other laboratories. Despite the apparent simplicity of the job, there are certain conditions that must be met prior to moving this equipment. BSCs should not be moved without consultation with a biosafety officer or qualified safety professional.

E.6.2 Existing BSCs and ancillary equipment, such as canopy connection exhaust ducting, gas, electric and vacuum connections, should be cleared for maintenance by a biosafety officer or qualified safety professional prior to disassembly. Depending on circumstances of the move, (i.e., cabinet use, new location, etc.), BSCs may be required to be space decontaminated. After a BSC is moved, it should be certified according to applicable performance standards.

E.7 Lifespan of BSCs

The current lifespan of a Biosafety Cabinet is approximately 15 years. Use of modern day Biosafety Cabinets (BSC's) began in the early 1970's with BSC's that were manufactured to the NIH-03-112C Standard and subsequently the NSF/ANSI 49. BSC's manufactured in the 70's, 80's and early 90's have provided over 15 years of service. Several considerations should be made of BSCs in this age group.

- Will the BSC need extensive service? (i.e. HEPA/ULPA filter replacement, blower/motor replacement, will the electrical wire harnesses need replacement? etc.)

- Can an older BSC be commissioned after it has been in storage or purchased as a resale?

- Will original test reports be available or will the BSC be commissioned to current NSF Standards?

After 15 years, replacement parts may or may not be available due to electrical or mechanical changes at the factory or industrial part suppliers. For example, magnetic ballasts and T12 fluorescent bulbs will not be available after the year 2010. In addition, today's BSCs have evolved through the years with many improvements in containment, ergonomics, serviceability, and energy efficiency. That should be considered in a repair versus replacement decision.

E.8 Decommissioning process

E.8.1 No biosafety cabinet should be sent to a landfill or a recycling facility as a BSC, it should be disassembled per requirements contained in this section.

E.8.2 Decontamination and PPE

E.8.2.1 After a review of the BSC hazard use, the cabinet may be considered chemically contaminated and requiring special decontamination procedures, not the standard gaseous sterilization. Follow paragraph E.10.2.3.

E.8.2.2 All decommissioned BSCs used with pathogens should be space decontaminated.

E.8.2.3 BSCs to be decommissioned that were used with chemical agents should have a hazard review made to determine whether special decontamination practices and PPE should be followed.

E.8.2.4 For those BSCs used with biological agents that may not be inactivated via formaldehyde, the filters should be incinerated and 10% bleach or other appropriate disinfectant applied to all remaining contaminated surfaces. Obtain prior approval of the Facility Safety Officer.

E.8.2.5.1 PPE should be used as directed by the Facility Safety Officer or the biosafety safety officer.

E.8.3 Metal parts

E.8.3.1 All metal parts of less than 30 pounds (13 kg) per item should be removed from the lab and taken to an appropriate metal recycling container.

E.8.3.2 Metal parts in excess of 30 pounds (13 kg), including the unit chassis, should be taken to a designated area in the facility to be picked up by a commercial recycling vender.

E.8.4 Glass windows

E.8.4.1 All glass safety windows and sashes should be taken to the designated glass container. Remove all parts that are not press fit or glued to the glass edges or surfaces.

E.8.5 Wiring

E.8.5.1 All accessible wiring should be taken to a wiring recycling container.

E.8.6 Electrical ballasts

E.8.6.1 All lamp ballasts should be taken to the ballast collection center at the institution.

E.8.7 Lamps

E.8.7.1 All fluorescent lamps should be taken to the lamp container area at the institution.

E.8.7.2 All ultraviolet lamps should be handled as mercury-containing waste.

E.8.8 Labels

E.8.8.1 All warning, identification and certification labels should be removed and destroyed.

E.8.9 Used HEPA/ULPA filters

E.8.9.1 HEPA/ULPA filters that have been decontaminated are often burned in an incinerator. This disposal method is also effective for HEPA/ULPA filters containing toxic chemicals. Factors to be considered when incinerating filters include, but are not limited to, composition of the waste, feed rate, combustion temperature and dwell time in the primary chamber.

E.8.9.2 HEPA/ULPA filters may be placed in heavy plastic bags, such as those used to bag-out filters from contaminated filter housings. The bagged filters can be chemically decontaminated in situ by cutting small holes in the bag and delivering appropriate disinfectant or neutralizing agent by inserting a garden-type spray through the hole and spraying the filter media. The holes can be sealed with duct tape and shipped to an incinerator or sanitary landfill. This chemical method may be appropriate for filters containing agents (i.e. toxic chemicals) that cannot be inactivated by the usual space decontamination procedures.

E.8.9.3 Decontaminated HEPA/ULPA filters may be safety buried in a sanitary landfill because they no longer pose a hazard.

Location "A" shows the preferred location. Location "B" is an alternate location. The air supply register(s) above or near the cabinet's location should be redirected away from the cabinet face.

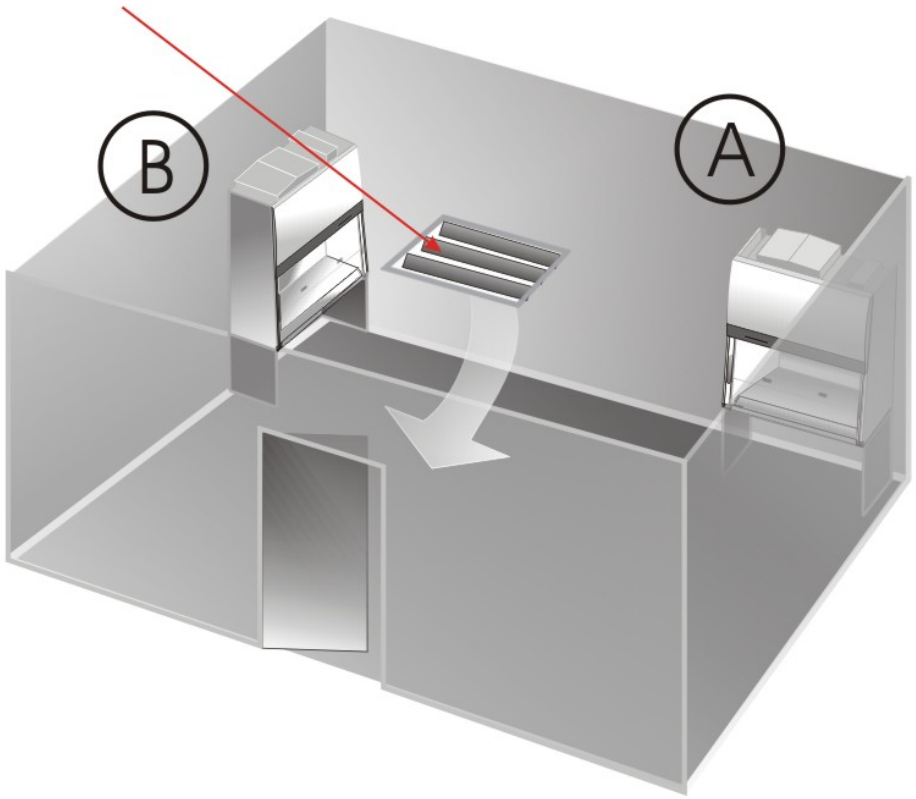

Figure E1 - Suggested Laboratory Locations for Class II Biosafety Cabinets

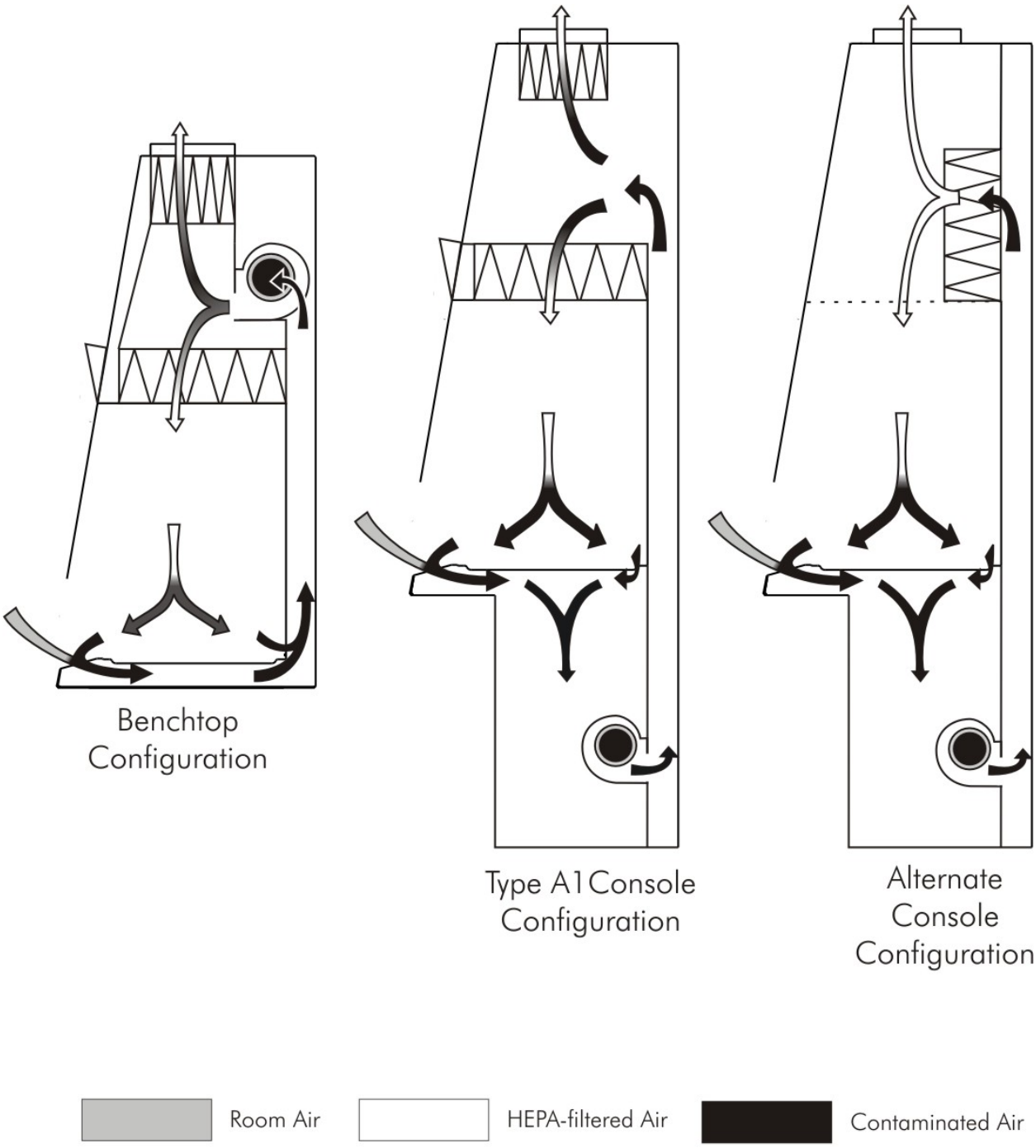

Figure E2 - Airflow Patterns for Class II Type A1 and A2 BSCs

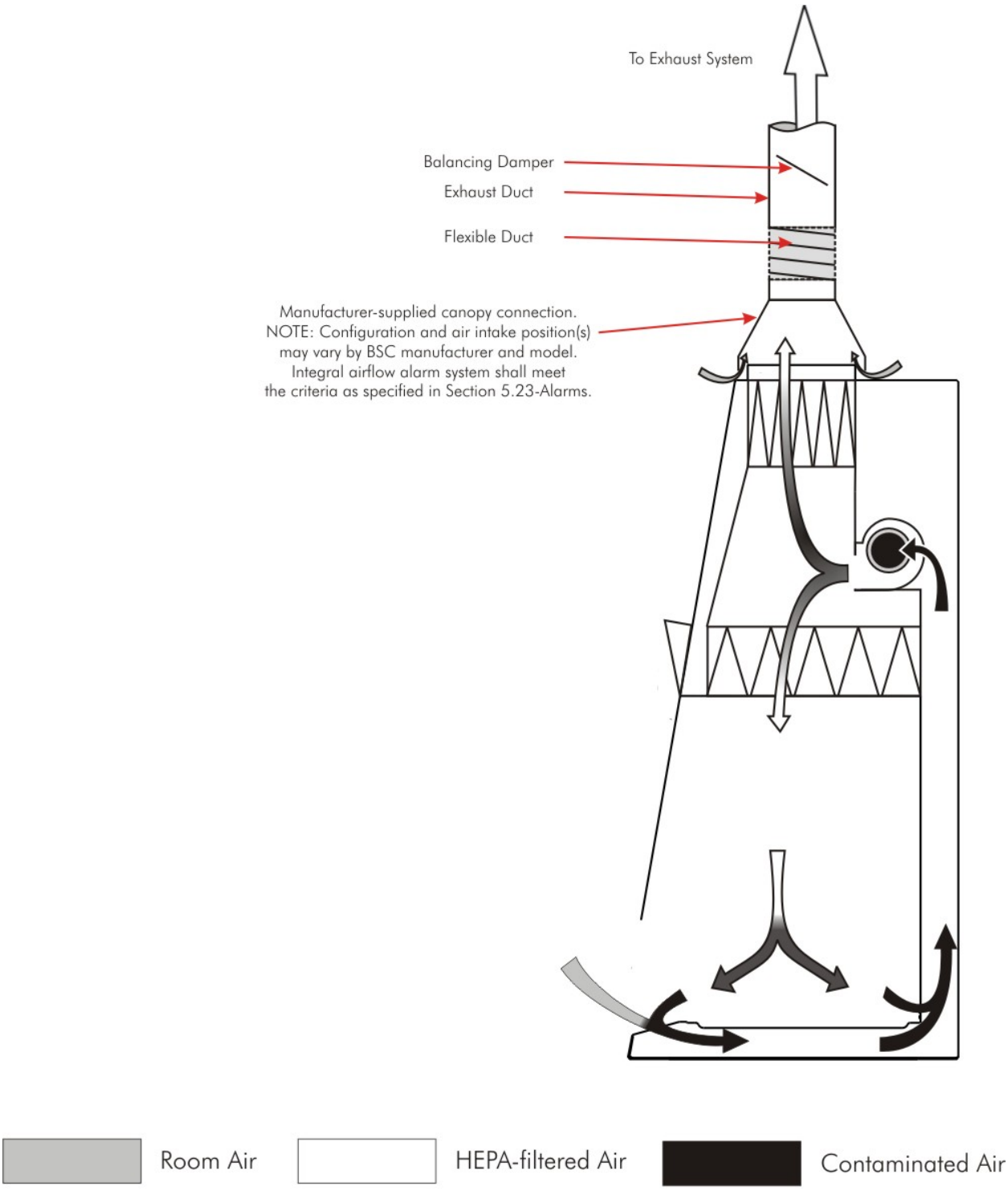

Figure E3 - Suggested Type A exhaust system

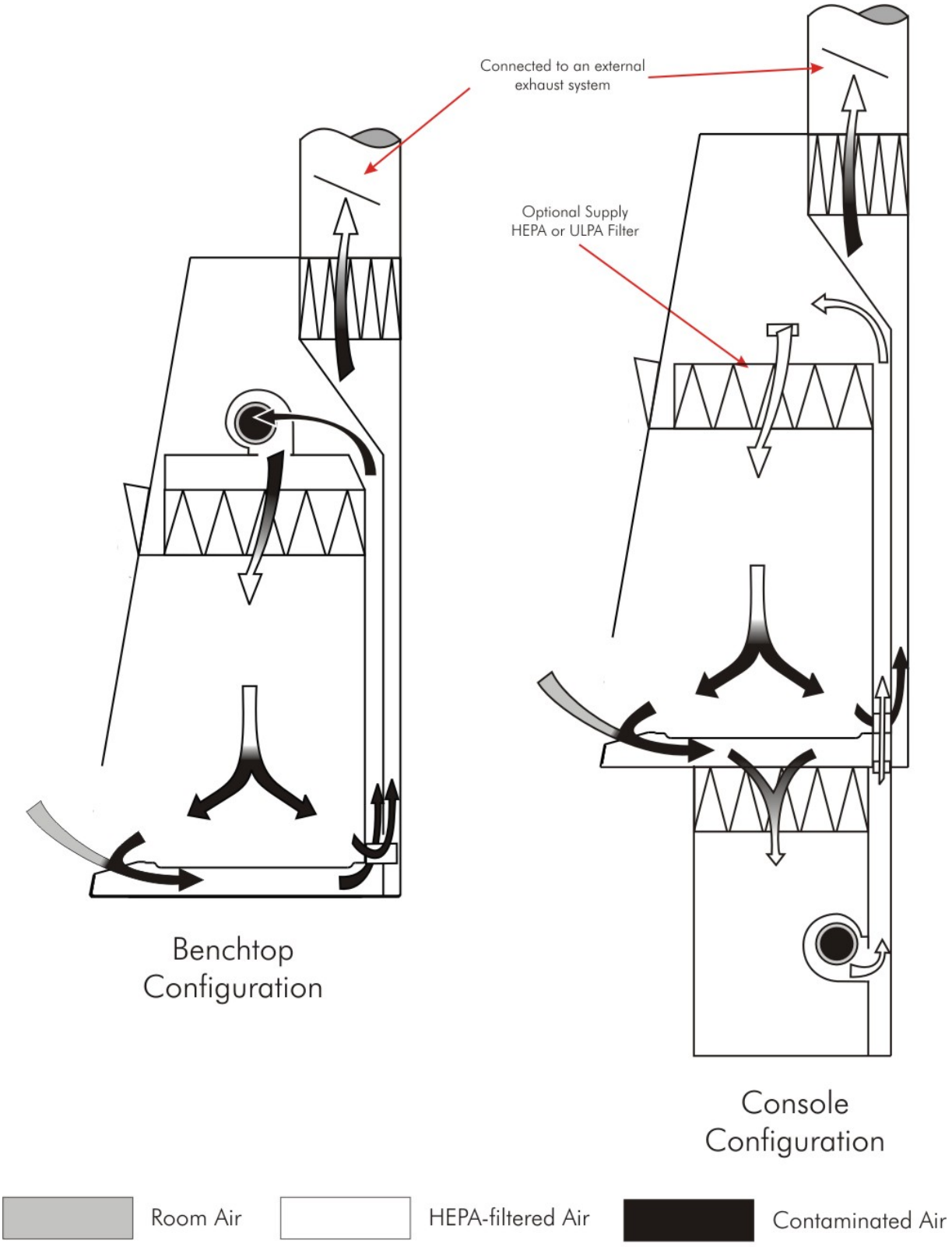

Figure E4 - Airflow Patterns for Class II Type B1BSCs

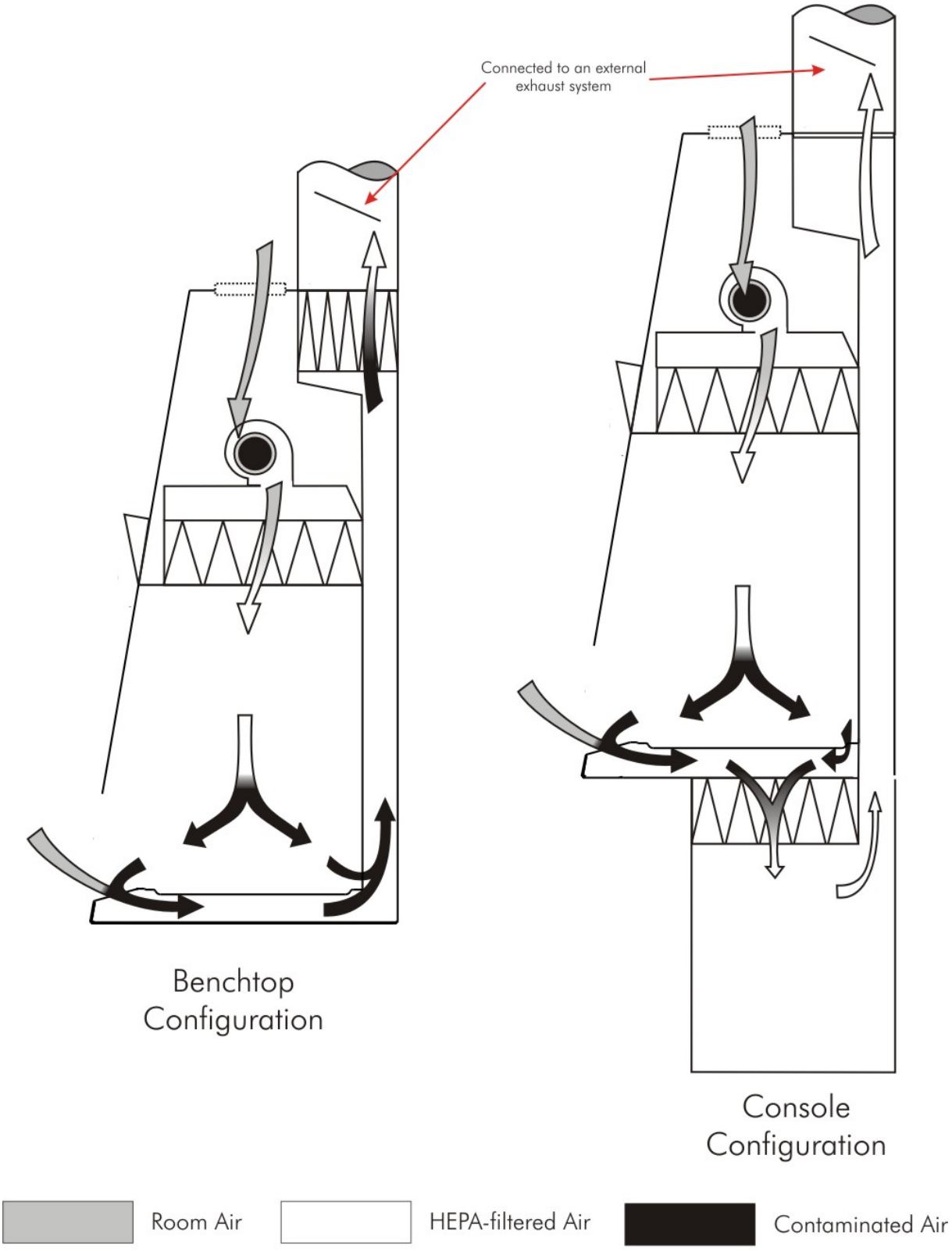

Figure E5 - Airflow Patterns for Class II Type B2 BSCs

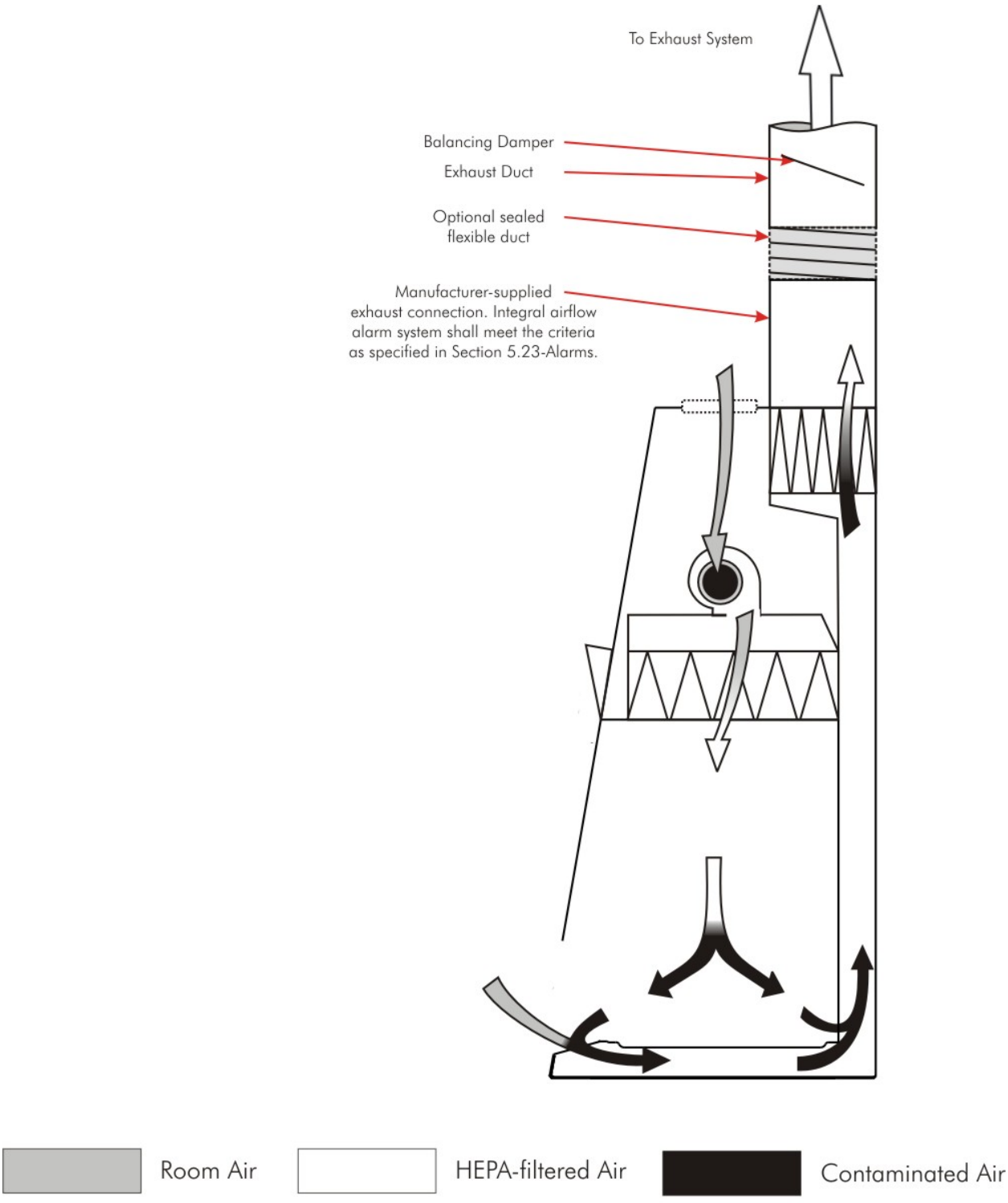

Figure E6 - Suggested Type B exhaust system

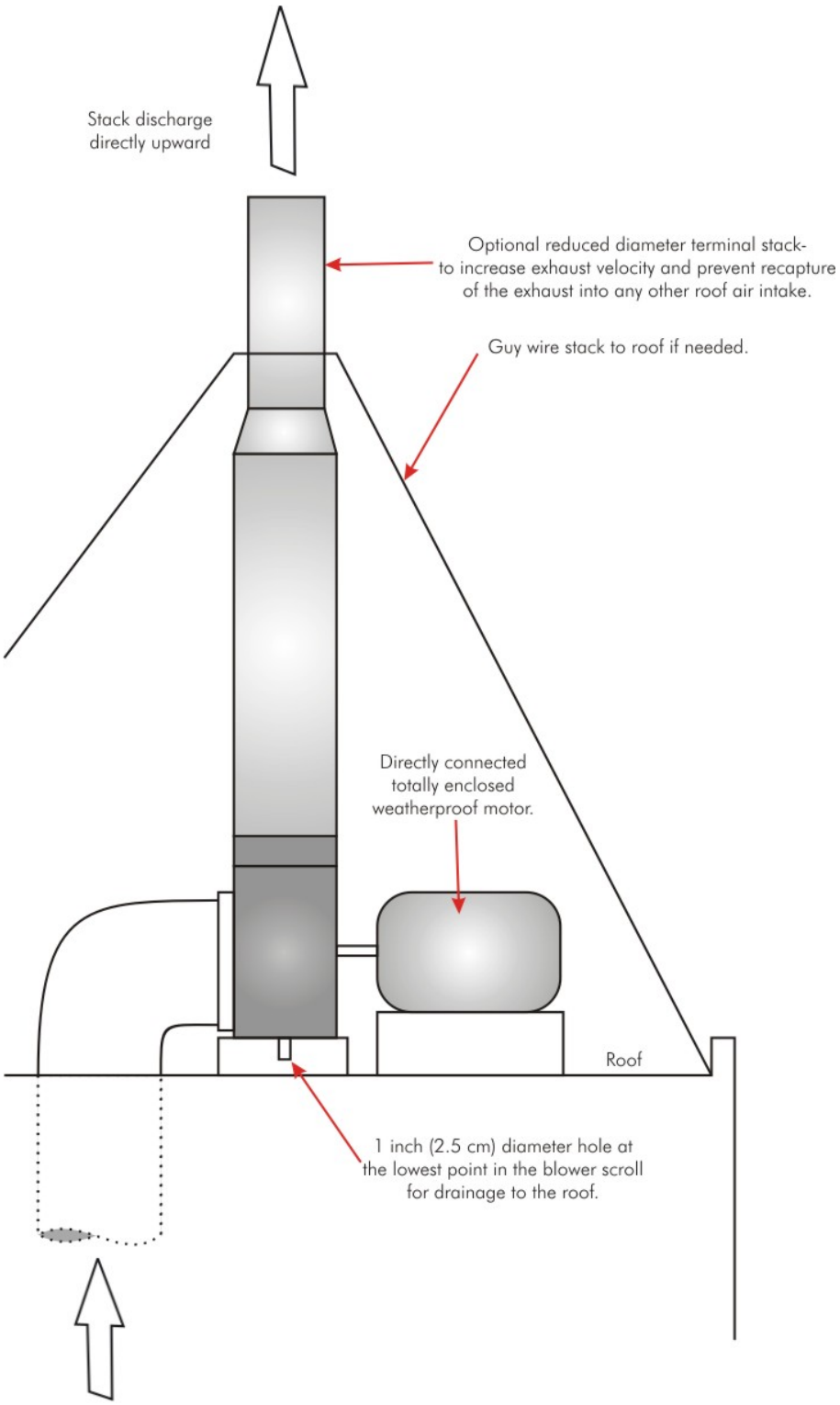

Figure E7 - Exhaust Stack and Blower.

Annex F
(normative)

Field tests

Factory testing shall be done according to Annex A.

F.1 Field certification preconditions and intervals

This annex contains the field tests that define the methods and acceptance criteria that are appropriately applied for determining qualification for field certification of all Class II biosafety cabinets. These field certification procedures are intended to confirm that an installed cabinet evaluated under the current version of the Standard has met all design criteria contained in NSF/ANSI 49 and currently meets all criteria contained in this annex. All cabinets shall be field tested using the procedures described in NSF/ANSI 49, Annex F, with the exception of the downflow velocity test. When the downflow velocity test is performed, the procedure by which the cabinet was certified should be used; however, the acceptance criteria outlined in the 2002 standard shall be applied. Downflow velocity readings shall be taken 4 in (10 cm) above the bottom edge of the sash only when so stated on the manufacturer's data plate label or when the manufacturers' data plate label indicates the cabinet was listed to NSF/ANSI 49-2002 or later.

To ensure that all cabinet operating criteria contained in this annex continue to be met, each cabinet should be field tested at the time of installation and at least annually thereafter. In addition, recertification should be performed whenever HEPA/ULPA filters are changed, maintenance repairs are made to internal parts, or a cabinet is relocated.[40] More frequent recertification should be considered for particularly hazardous or critical applications or workloads. It is customary for the person conducting the designated tests to affix to the cabinet a certificate of satisfactory performance when the cabinet meets all field test criteria.

Field certification of a cabinet is not intended to provide complete verification that the cabinet conforms to all of the requirements of NSF/ANSI 49.

F.1.1 Tests directly related to containment (i.e., personnel and environmental protection) and product protection.

The following physical tests shall be conducted on-site for a certification to be considered for the statement "Field Certified in accordance with NSF/ANSI 49:"

- downflow velocity profile test;
- inflow velocity test;
- airflow smoke patterns test;
- HEPA/ULPA filter leak test;
- site installation assessment tests; and
- cabinet integrity test (positive pressure plenum cabinets only).

For Type A cabinets with exposed biologically contaminated positive pressure plenums, either a Pressure Decay or Soap Bubble Leak Test is mandatory. The tests shall be performed at the time of installation, when positive pressure containment panels are removed, and after relocation of the BSC.

[40] Microbiological equipment that has been used with microorganisms should be decontaminated prior to repair or replacement of components located in contaminated plenums, prior to cabinet relocation, and in some cases prior to recertification. See Annex G, Recommended Microbiological Decontamination Procedure. When equipment has been used with chemical or radioactive agents, appropriate protective clothing and safety procedures should be used during chemical decontamination.

The site installation assessment tests shall include:

- alarm functions as required by this Standard;
- blower interlock; and
- exhaust system performance (proper exhaust duct negative pressure and canopy performance).

F.1.2 Tests related to worker comfort and safety

The following tests are for worker comfort and safety and are performed at the request of the customer or at the discretion of the certification provider:

- lighting intensity;
- vibration;
- noise level; and
- electrical leakage, ground circuit resistance, and polarity tests.

F.2 Downflow velocity

F.2.1 Purpose

This test measures the velocity of air moving through the cabinet workspace 4 in (10 cm) above the bottom edge of the sash and shall be performed on all cabinets accepted under Annex A, section A.6.

F.2.2 Apparatus

A thermal anemometer with an accuracy of ± 3.0 ft/min (± 0.015 m/s) or 3% of the indicated velocity, whichever is larger, shall be used. The device shall be calibrated in accordance with the thermal anemometer manufacturer's instructions or IEST-RP-CC-013 if instructions are not provided. When the conditions vary from sea level by more than 1000 ft (300 m) and/or the temperature varies from 70°F (21°C) by more than 5°F (2°C), an appropriate correction for altitude and/or temperature should be used. The manufacturer's manual for the thermal anemometer or the Industrial Ventilation Manual shall be consulted for the appropriate correction calculation.

F.2.3 Method: setting nominal set point

The removable equipment non-essential to cabinet operation (acceptable option components) shall be removed prior to setting the nominal set points to replicate the as-manufactured conditions tested by the testing organization when required. The air measurement probe shall be held rigidly in a freestanding fixture that permits accurate positioning and does not distort the airflow pattern (ring-stand and clamp).

F.2.3.1 Uniform downflow cabinets

a) The air velocity shall be measured at multiple points across the workspace, using equal points in the horizontal plane 4 in (10 cm) above the bottom edge of the sash, as specified on the data plate.

b) Reported values shall be:

- individual velocity readings in the applicable grid;
- overall average of the velocity readings;
- minimum velocity reading;
- maximum velocity reading;
- acceptance criteria for average airflow velocity;
- acceptance criteria for airflow velocity uniformity; and
- name of test (uniform downflow velocity test).

c) The nominal set point shall be based on the above data in accordance with the manufacturer's instructions.

F.2.3.2 Non-uniform (zoned) downflow cabinets

a) The air velocity shall be measured at multiple points across the work space in zones, as specified on the data plate, 4 in (10 cm) above the bottom edge of the sash.

b) Reported values for each zone shall be:

- individual velocity readings in the applicable grid;
- overall average of the velocity;
- minimum velocity reading;
- maximum velocity reading;
- acceptance criteria for average airflow velocity;
- acceptance criteria for airflow velocity uniformity; and
- name of test (non-uniform (zoned) downflow velocity test).

c) The nominal set point shall be based on the above data in accordance with the manufacturer's instructions.

F.2.4 Acceptance

F.2.4.1 Uniform downflow

A cabinet for which the cabinet manufacturer has specified a uniform downflow velocity shall conform to the following:

- the average downflow velocity shall be within ± 5 ft/min (± 0.025 m/s) of the value specified; and

- the individual point readings shall not vary more than ± 25% or 16 ft/min (0.08 m/s), whichever is greater, from the average downflow velocity.

F.2.4.2 Non-uniform downflow

A cabinet for which the cabinet manufacturer has specified a non-uniform (zoned) downflow velocity shall conform to the following:

- the individual zone average downflow velocities shall be within ± 5 ft/min (± 0.025 m/s) of the values specified by the manufacturer; and

- the individual point readings shall not vary more than ± 25% or 16 ft/min (0.08 m/s), whichever is greater, from the average downflow velocity of each zone.

F.3 Inflow velocity (face velocity) test

F.3.1 Purpose

This test determines the measured and calculated inflow velocity through the work access opening.

F.3.2 Apparatus

The following devices may be used to carry out inflow velocity testing:

- a direct inflow measurement (DIM) instrument with an accuracy of ± 3% of reading ± 7 ft^3/min (± 0.003 m^3/s) or in accordance with Annex B;

- a thermal anemometer with an accuracy of ± 3.0 ft/min (± 0.015 m/s) or 3% of the indicated velocity, whichever is larger;

- a pitot tube constructed according to the dimensions given in the Industrial Ventilation Manual; and

- a freestanding fixture that permits accurate positioning of the thermal anemometer probe that does not distort the airflow pattern (ring-stand and clamp).

F.3.3 Methods

One of these methods was validated per cabinet model and provided by the manufacturer, which was reviewed and approved by the testing organization. Manufacturer validation procedures contained no fewer than ten replicate tests. The testing organization's approval will be based on review of data and successful reproduction of test results. The validated alternate method is on the manufacturer's data plate.

F.3.3.1 General

When the testing organization has determined the nominal set point on a given model and size of cabinet using a DIM device, and an appropriate alternative method has been validated for that cabinet by the testing organization, this alternate method may be used to establish the set point on the same model and size of cabinet in the field.

F.3.3.2 Direct inflow measurement method

a) Seal by taping the device to the center of the front opening of a biosafety cabinet. Seal the open areas on either side of the capture hood portion of the DIM as necessary.

b) All cabinet and exhaust blowers shall be operating. Take at least five non-back pressure compensated readings and average them to determine inflow volume rate. Care should be taken not to restrict the airflow through the instrument intake area.

c) Calculate the average inflow velocity in ft/min (m/s) by dividing the average inflow volume rate in ft^3/min (m^3/s) by the work access opening area in ft^2 (m^2).

d) Include the following in reported data: individual inflow volume rate readings, average inflow volume rate, work access opening dimensions and area, directly measured average inflow velocity, and the methods used to determine them.

e) Reported values shall be:

- individual volume readings;
- overall average of the volume;
- calculated inflow volume;
- work access opening area;
- view screen opening height;
- correction factor used (if applicable);

- acceptance criteria for average airflow volume;
- acceptance criteria for calculated inflow velocity;
- inflow velocity test method; and
- name of test (inflow velocity test).

F.3.3.3 Alternate inflow measurement methods

In addition to the direct inflow method, one of the alternative methods was validated for each cabinet model and was reviewed and approved by the testing organization.

F.3.3.3.1 Method for Type A1 and A2 cabinets that use a thermal anemometer to measure exhaust velocity to determine inflow velocity

a) Take air velocity measurements at multiple points across the exhaust filter face on a grid as specified on the data plate. A clear 12 in (30 cm) of space is required above the exhaust HEPA filter face for valid thermal anemometer measurements.

b) Use the effective open area of the exhaust HEPA/ULPA filter or exhaust port determined by the manufacturer and validated by the testing organization. Measure the effective exhaust area when the manufacturer has not provided it. Cabinets in which the exhaust filter is not accessible or exhaust port flow is non-uniform, such as caused by a damper or exhaust filter housing design, shall be tested as approved by the testing organization.

c) To obtain the exhaust flow volume rate in ft^3/min (m^3/s), multiply the average exhaust air velocity in ft/min (m/s) by the effective exhaust area in ft^2 (m^2).

d) Use the work access opening area as listed by the testing organization. Measure the work access opening area when the manufacturer has not provided it.

e) Calculate the average inflow velocity in ft/min (m/s) by dividing the average exhaust volume rate in ft^3/min (m^3/s) by the work access opening area in ft^2 (m^2).

f) Include the following in reported data: individual exhaust velocity readings, average exhaust velocity, exhaust volume rate, exhaust opening dimensions and area, work access opening dimensions and area, calculated average inflow velocity, and the methods used to determine them.

g) Reported values shall be:

- individual exhaust velocity readings;
- overall average of the exhaust velocity readings;
- calculated exhaust volume;
- calculated inflow velocity;
- exhaust opening dimensions;
- exhaust opening effective area;
- work access opening area and dimensions;
- correction factor used (if applicable);
- acceptance criteria for calculated inflow velocity;
- inflow velocity test method; and
- name of test (inflow velocity test).

F.3.3.3.2 Method for Type A1, A2, and B2 cabinets using a thermal anemometer to measure velocity through a constricted access opening to determine average inflow velocity

a) Restrict the access opening as specified by the testing organization.

b) Take air velocity measurements at multiple points across the restricted opening as specified on the data plate. No fewer than two readings per 1 ft (0.3 m) of access opening width shall be taken.

c) Average the air velocity measurements. Multiply the average by the listed correction factor to obtain the average inflow velocity.

d) Include the following in reported data: height of restriction, individual velocity readings, average velocity, the listed correction factor, calculated inflow velocity, and methods used to determine them.

e) Reported values shall be:

- individual constricted velocity readings;
- overall average of the constricted velocity readings;
- calculated exhaust volume;
- calculated inflow velocity;
- constricted opening dimensions and area;
- work access opening area and dimensions;
- correction factor used (if applicable);
- acceptance criteria for calculated inflow velocity;
- inflow velocity test method; and
- name of test (inflow velocity test).

F.3.3.3.3 Method for Type B1 cabinets using a thermal anemometer to measure velocity through the access opening to determine average inflow velocity

a) Turn off the blower(s) that recirculate air in the cabinet, if tested that way by the testing organization.

b) Set the sash to the height tested by the testing organization.

c) Take air velocity measurements at multiple points across the work access opening on a grid as specified on the data plate.

d) Include individual inflow velocity readings, average inflow velocity, and methods used to determine them in the reported data.

e) Reported values shall be:

- individual inflow velocity readings;
- overall average of the inflow velocity readings;
- calculated inflow volume;
- work access opening dimensions and area;
- correction factor used (if applicable);
- acceptance criteria for average inflow velocity;
- inflow velocity test method; and
- name of test (inflow velocity test).

F.3.3.3.4 Calculated method for Type B2 cabinets using an anemometer and pitot tube if applicable

a) Turn on the cabinet downflow blower and exhaust system blower.

b) Set the sash at the height specified by the testing organization.

c) Measure and calculate the exhaust volume in accordance with the testing organization's verified methodology, or with ASHRAE standards for air velocity measurements in round or rectangular ducts, or with the Industrial Ventilation Manual.

d) Measure the supply air velocity on a grid as specified on the data plate. The air measurement probe shall be held rigidly in a freestanding fixture (ring-stand and clamp) that permits accurate positioning and does not distort the airflow pattern (see Annex A, figure A20). Average the velocity readings and multiply by the area in ft^2 (m^2) of the plane in which the velocities were measured to determine the total filtered supply air volume flow rate in ft^3/min (m^3/s).

e) Subtract the supply air volume rate in ft^3/min (m^3/s) from the total exhaust volume rate in ft^3/min (m^3/s); the difference represents the calculated inflow volume rate in ft^3/min (m^3/s).

f) Divide the calculated inflow volume rate by the area of the access opening in ft^2 (m^2) to determine the average inflow velocity in ft/min (m/s).

g) Include the following in reported data: individual exhaust velocity readings, calculated average exhaust velocity, exhaust duct area, calculated exhaust volume, individual supply velocity readings, average supply velocity, effective supply area, calculated supply air volume, area of the work access opening, calculated inflow air volume, calculated average inflow velocity, and methods used to determine them.

h) Reported values shall be:

- individual duct velocity readings;
- overall average of the duct velocity readings;
- calculated exhaust volume;
- duct size, shape and area;
- work access opening dimensions and area;
- dimensions and area of the supply velocity measurement location (used to determine supply volume);
- individual supply velocity readings (not to be confused with downflow velocities);
- calculated supply air velocity and volume;
- calculated inflow velocity and method used for calculations;
- correction factor used (if applicable);
- acceptance criteria for average inflow velocity;
- inflow velocity test method; and
- name of test (inflow velocity test).

NOTE – Canopy connected A1 and A2 cabinets must be tested with a method that measures the inflow volume at the work access opening.

F.3.4 Acceptance

The average work access opening inflow velocity shall be within ± 5 ft/min (± 0.025 m/s) of the nominal set point verified by the testing organization using the same method.

F.4 Airflow smoke patterns test

F.4.1 Purpose

This test determines that the airflow along the entire perimeter of the work access opening is inward, that airflow within the work area is downward with no dead spots or refluxing, that ambient air does not pass on or over the work surface, and that there is no escape to the outside of the cabinet at the sides and top of the sash.

F.4.2 Apparatus

A visible aerosol or mist that is close to neutrally buoyant in air. The generation process should not create a velocity sufficient to interfere with the air patterns being observed.

NOTE – Titanium tetrachloride is corrosive and should be handled with care.

F.4.3 Method

F.4.3.1 Downflow test

a) Smoke shall be passed from one end of the cabinet to the other, along the centerline of the work surface, at a height of 4 in (10 cm) above the top of the access opening.

b) Reported values shall be:

- name of test (smoke pattern downflow test); and
- pass or fail.

F.4.3.2 View screen retention test

a) Smoke shall be passed from one end of the cabinet to the other, 1.0 in (2.5 cm) behind the view screen, at a height 6.0 in (15 cm) above the top of the access opening.

b) Reported values shall be:

- name of test (view screen retention test); and
- pass or fail.

F.4.3.3 Work opening edge retention test

a) Smoke shall be passed along the entire perimeter of the work opening edges, approximately 1.5 in (3.8 cm) outside the cabinet. Particular attention should be paid to corners and vertical edges.

b) Reported values shall be:

- name of test (work opening edge retention test); and
- pass or fail.

F.4.3.4 Sash seal test

a) Smoke shall be passed up the inside of the sash 2 in (5 cm) from the sides and along the top of the work area.

b) Reported values shall be:

- name of test (sash seal test); and
- pass or fail.

F.4.4 Acceptance

F.4.4.1 Downflow test

The smoke shall show smooth downward flow with no dead spots or reflux (upward flow).

F.4.4.2 View screen retention test

The smoke shall show smooth downward flow with no dead spots or reflux. No smoke shall escape from the cabinet.

F.4.4.3 Work opening edge retention test

No smoke shall be refluxed out of the cabinet once drawn in, nor shall smoke billow over the work surface or penetrate onto it.

F.4.4.4 Sash seal test

There shall be no escape of smoke from the cabinet.

F.5 HEPA/ULPA filter leak test

F.5.1 Purpose

This test determines the integrity of downflow and exhaust HEPA/ULPA filters, filter housings, and filter mounting frames. The cabinet shall be operated within ± 5 ft/min (0.025 m/s) of the nominal set point, with the exception of the downflow HEPA/ULPA filters on B1 cabinets.

F.5.2 Apparatus

F.5.2.1 An aerosol photometer with linear or expanded logarithmic scale shall be used. The instrument shall be capable of indicating 100% upstream concentration with a minimum aerosol concentration of 10 μg/L of polydisperse dioctylphthalate (DOP) particles, or an equivalent fluid that provides the same particle size distribution (e.g., polyalpha olefin [PAO] di[2-ethylhexyl], sebecate, polyethylene glycol, and medicinal-grade light mineral oil)[41] produced by the generator described in Annex A, section A.3.2.2 or equivalent. It shall also be capable of detecting an aerosol concentration in the downstream equal to 10^{-5} of the upstream concentration of the same particles. The sampling rate of air shall be 1 ft^3/min (5×10^{-4} m^3/s) ± 10%. Probe area shall have a maximum open area of 1.7 in^2 (11 cm^2) and a minimum dimension of 0.50 in (1.3 cm). The photometer shall be set up in accordance with the photometer manufacturer's instructions or IEST-RP-CC-013 if instructions are not provided.

[41] Hinds, W., Macher, J., First M. W., "Size Distributions of Aerosols Produced from Substitute Materials by the Laskin Cold DOP Aerosol Generator," presented at the 16th Dept. of Energy Nuclear Air Cleaning Conference; and Yan, X., First, M. W., Rudnick, S. N. "Characteristics of Laskin Nozzle Generated Aerosols," Proc. 21st Nuclear Air Cleaning Conf.,M. W. First, Ed., N. T. I. S., Springfield, VA. Feb. 1991. p.116.

F.5.2.2 An aerosol generator of the Laskin Nozzle type conforming to Annex A, figure A2 or equivalent shall be used to create an aerosol by flowing air through liquid DOP or equivalent substitute. When a Laskin nozzle generator is used, the compressed air supplied to the generator should be adjusted to a minimum of 20 psi (140 kPa), if using DOP or 23 psi (160 kPa) if using PAO, measured at the generator manufacturer's recommended location. The nozzles shall be covered with liquid to a depth not to exceed 1.25 in (31 mm).

F.5.2.3 A pressure gauge for the generator having a maximum range of 0 to 80 psi (0 to 550 kPa) with resolution and accuracy of 1 psi (7 kPa) calibrated by the manufacturer or in accordance with the manufacturer's instructions shall be used.

F.5.3 Method of testing HEPA/ULPA filters

F.5.3.1 Filters that can be scanned

a) Turn on the cabinet blower and lights (types A1 and A2 and B2 downflow filter test). Remove the filter diffusers and protective covers if any are present. Place the generator so the aerosol is introduced into each cabinet fan upstream of the HEPA/ULPA filter(s). When the manufacturer has not identified the aerosol introduction point(s), introduce the aerosol in a manner to ensure thorough mixing in the cabinet airflow. For example, a T-connection can be fitted to the aerosol generator output to enable distribution of challenge into both entrances of a single blower or entrances of multiple blowers. The manufacturer shall determine the aerosol introduction point that provides the most uniform distribution.

b) Turn on the photometer and adjust it in accordance with the manufacturer's instructions.

c) Determine the aerosol concentration upstream of the HEPA/ULPA filter.

– When the challenged airflow is not contaminated, sample the aerosol concentration upstream of the HEPA/ULPA filter.

– When the challenged airflow is contaminated or when measuring the upstream concentration is not practical, the upstream concentration can be calculated. For example, when DOP is used as the challenge aerosol with a Laskin nozzle aerosol generator at 20 psi (140 kPa), the following formula applies:

µg/L = 13,500 x number of nozzles / ft3/min of challenged air

NOTE – Use of DOP substitutes will require modification of this formula, unless the photometer is calibrated with the substitutes to yield results equivalent to those of DOP. Use of DOP substitutes will also require pressures different from 20 psig.

– Use an aerosol concentration that is at least equal to the photometric equivalent of 10 µg/L of DOP.

d) Set up the photometer to the upstream challenge in accordance with the photometer manufacturer's instructions to detect leaks greater than or equal to 0.01% of the upstream concentration.

e) With the nozzle of the probe held not more than 1.0 in (2.5 cm) from the area being tested, scan the entire downstream side of the HEPA/ULPA filter(s) and the perimeter of each filter pack by passing the photometer probe in slightly overlapping strokes at a traverse rate of not more than 2 in/s (5 cm/s). Separate passes shall be made around the entire periphery of the filter, along the bond between the filter pack and frame, and around the seal between the filter and the device.

f) Reported values shall be:

- upstream aerosol challenge concentration;
- method used to report concentration (measured or calculated);
- maximum leak penetration in percent;
- method used (scanned or probe tested); and
- name of test (HEPA/ULPA filter leak test).

F.5.3.2 Filters that cannot be scanned

a) When a cabinet is ducted so that the exhaust filter cannot be scanned, it may be leak tested by drilling a hole approximately 0.3 in (1 cm) in diameter in the duct at a downstream location that will produce a well-mixed aerosol and inserting the photometer sampling probe with rigid extension tubing through the hole.

b) Reported values shall be:

- upstream aerosol challenge concentration;
- method used to report concentration (measured or calculated);
- maximum leak penetration in percent;
- method used (scanned or probe tested); and
- name of test (HEPA/ULPA filter leak test).

F.5.4 Acceptance

F.5.4.1 Filters that can be scanned

Sustained aerosol penetration shall not exceed 0.01% of the upstream concentration at any point.

F.5.4.2 Filters that cannot be scanned

Sustained aerosol penetration shall not exceed 0.005% of the upstream concentration.

F.6 Pressure decay / soap bubble

F.6.1 Pressure decay or soap bubble test

F.6.1.1 Purpose

The pressure decay or soap bubble test is performed to determine whether exterior surfaces of all plenums, welds, gaskets, and plenum penetrations or seals are free of leaks.

F.6.1.2 Apparatus

- manometer, pressure gauge, or pressure transducer system with a minimum range of 0 – 2 in w.g. (0 – 500 Pa) and accurate to ± 0.02 in w.g. (± 5 Pa);
- liquid leak detector;
- plastic sheet (0.02 in extruded high-impact styrene); and
- duct tape.

F.6.1.3 Method (pressure decay)

a) Prepare the cabinet as a closed system, i.e., seal the front sash and exhaust port.

b) Remove decorative panels and other access obstructions, wherever necessary, to expose the plenums to be tested.

c) Attach a manometer, pressure gauge, or pressure transducer system to the test area to indicate the interior pressure (see Annex A, figure A1a).

d) Pressurize the cabinet with air to a reading of 2 in w.g. (500 Pa), turn off the pressurizing air, and measure the pressure after 30 min. A leakage of 10% of the original pressure is allowable. If a cabinet does not hold 2 in w.g. (500 Pa), use the soap bubble method to locate leaks.

e) Reported values shall be:

- pressure range maintained during test;
- pass or fail; and
- name of test (pressure decay test).

F.6.1.4 Method (soap bubble)

a) Prepare the cabinet as a closed system, i.e., seal the front sash and exhaust port.

b) Remove decorative panels and other access obstructions, wherever necessary, to expose the plenums to be tested.

c) Attach a manometer, pressure gauge, or pressure transducer system to the test area to indicate the interior pressure (see Annex A, figure A1a).

d) Pressurize the cabinet with air to ensure a continuous reading of 2 in w.g. (500 Pa) ± 10%.

e) Spray or brush the liquid leak detector along all welds, gaskets, penetrations, and seals on exterior surfaces of cabinet plenums. Small leaks will be indicated by bubbles. Large leaks will occur that blow the detection fluid from the hold without forming bubbles and may be detected by slight feel of airflow or sound.

f) Reported values shall be:

- pressure range maintained during test;
- pass or fail;
- description of leak location(s); and
- name of test (soap bubble leak test).

F.6.1.5 Acceptance

The cabinet shall hold 2 in w.g. (500 Pa) within ± 10% for 30 min or when all welds, gaskets, penetrations, and seals on exterior surfaces of air plenums are free of soap bubbles when at 2 in w.g. (500 Pa) pressure above atmospheric.

F.7 Site installation assessment tests

F.7.1 Purpose

These tests are performed to verify that the biosafety cabinet is integrated properly into the facility.

F.7.2 Apparatus

- owner's manual; and
- a visible source of cold smoke such as titanium tetrachloride.

F.7.3 Method

F.7.3.1 Alarm functions

F.7.3.1.1 Sash alarms

On cabinets equipped with a sliding sash, it shall be raised 1.0 in (25 mm) above and lowered 1.0 in (25 mm) below the manufacturer's recommended height. Signaling of an audible and visual alarm shall be verified for both conditions. For cabinets that have been tested and certified to editions of NSF/ANSI Standard 49 earlier than the 2014 edition, alarm activation is only required when the sash is raised 1.0 in (25 mm) above the manufacturer's recommended height.

F.7.3.2 Exhaust airflow alarms (excluding building automation systems)

Whenever an alarm is present to monitor the exhaust airflow, its operation must be verified. The alarm's operation shall be verified at every certification.

F.7.3.2.1 Exhaust alarm system – Type B1 or B2

Supply fan interlock on B cabinets:

a) Shall be tested at time of alarm verification.

b) Reduce exhaust volume 20% once the cabinet is set or certified in its acceptable airflow range, and verify that audible and visual alarms indicate a loss of exhaust volume within 15 s. The internal cabinet fan(s) shall be interlocked to shut off at the same time the alarms are activated.

NOTE – For direct connected Type B1 or B2 BSCs, measure the static pressure in the duct-work between the cabinet and duct-mounted balancing dampers.

F.7.3.2.2 Exhaust alarm system – Type A1 or A2 canopy connection

The canopy connection on Type A1 or A2 cabinets:

a) Shall be tested at time of alarm verification.

b) Introduce a visible medium source into the canopy air intake(s) while slowly reducing the exhaust volume until there is a loss of capture of the visible medium into the canopy air intake(s). The audible and visual canopy alarms shall respond within 15 s, and the cabinet fan(s) will continue to operate.

NOTE – Direct connected Type A1 or A2 cabinets shall not be considered in compliance with the standard.

F.8 Electrical leakage and ground circuit resistance and polarity tests

All new cabinets shall conform to the requirements of the current edition of any national standard that is based on IEC 61010-1. Cabinets initially qualified under versions of NSF/ANSI 49 prior to the 2009 edition shall conform to UL 61010A-1 or may refer to NSF 49 – 1992 for Electrical Leakage, Ground Circuit Resistance, and Polarity tests if necessary.

F.9 Lighting intensity test

F.9.1 Purpose

This test is performed to measure the light intensity on the work surface of the cabinet in foot-candles (fc [lux]) as an aid in minimizing cabinet operator's fatigue.

F.9.2 Apparatus

A portable photoelectric illuminance meter as described in The Lighting Handbook[42], section 9.8.1. The meter shall be accurate within ± 10%, cosine and color corrected. The illuminance meter shall be calibrated in accordance with the manufacturer's instructions.

F.9.3 Method

a) Measure the background lighting intensity along the side-to-side centerline of the work tray on a uniform linear pattern close to but no greater than 12 in (30 cm) starting 6.0 in (15 cm) from the sidewalls (Annex A, figure A4).

b) Turn on the lights and blower, and take readings at the same points again.

c) Reported values shall be:

- individual background readings;
- individual lighting intensity readings;
- average background intensity;
- average lighting intensity;
- acceptance criteria;
- pass or fail; and
- name of test (lighting intensity test).

F.9.4 Acceptance

Lighting intensities shall average no less than 45 fc (480 lux) greater than background levels, where background light levels average a maximum of 15 fc (160 lux).

F.10 Vibration test

F.10.1 Purpose

This test is performed to determine the amount of vibration in an operating cabinet as a guide to satisfactory mechanical performance, as an aid in minimizing cabinet operator's fatigue, and to prevent damage to delicate tissue culture specimens.

[42] IES, 120 Wall Street, Floor 17, New York, NY 10005 <www.iesna.org>

F.10.2 Apparatus

A vibration analyzer with a minimum reliable reading of 1 x 10^{-4} in (2.5 μm) rms amplitude, or the ability to detect differences of this magnitude, in accordance with manufacturer's instructions.

F.10.3 Method

a) Operate the cabinet with lights on within 5.0 ft/min (0.025 m/s) of the nominal set point velocities.

b) To determine the vibration displacement on the vertical axis, affix the sensing element of the vibration pickup unit firmly onto the geometric center of the work surface(s) by:

- a clamp;
- a bolt; or
- an integral magnet with petroleum jelly film, or a double-faced adhesive tape.

The test position is shown in Annex A, figure A5.

c) Determine the gross vibration amplitude with the cabinet operating.

d) Determine the background vibration amplitude with cabinet blower(s) off, and if applicable, the exhaust blower on.

e) Subtract the background from the gross vibration amplitude to determine the net vibration amplitude attributable to the cabinet.

f) Reported values shall be:

- unit "on" vibration reading;
- background vibration reading;
- net vibration;
- pass or fail; and
- name of test (vibration test).

F.10.4 Acceptance

Net displacement shall not exceed 0.002 in (50 μm) rms amplitude at10 Hz to 7 kHz in the center of the work surface(s) when the cabinet is operating at the manufacturer's recommended airflow velocities.

F.11 Noise level tests

F.11.1 Purpose

This test is performed to measure the noise levels produced by the cabinet as a guide to satisfactory mechanical performance and an aid in minimizing cabinet operator's fatigue. The procedures can be performed in most acoustically ordinary rooms, such as a factory, where walls are neither sound absorbing nor completely sound reflecting.

F.11.2 Apparatus

A type/class 2 sound level meter with a minimum range of at least 50 to 100 db and an "A" weighing scale set up in accordance with the manufacturer's instructions.

F.11.3 Method

a) Operate the cabinet within 5 ft/min (0.025 m/s) of the nominal set point with lights on.

b) Set the instrument to the "A" weighting mode.

c) Measure the noise level 12 in (30 cm) in front of the cabinet (leading front edge of the access opening) and 15 in (38 cm) above the plane of the work surface, in line with the vertical centerline of the cabinet (Annex A, figure A3).

d) To measure the ambient noise level, turn the cabinet blower and lights off, and if applicable, leave the remote exhaust blower on and measure as in c) above.

e) Reported values shall be:

- unit "on" sound level reading;
- background sound level reading;
- net sound level;
- pass or fail; and
- name of test (noise level tests).

F.11.4 Acceptance

Overall noise level in front of the cabinet shall not exceed 70 dbA when measured where the maximum ambient sound level is no greater than 60 dbA. When the ambient sound level is greater than 60 dbA, the reading obtained in Annex F, section F.11.3c) shall be corrected in accordance with curves or tables provided in the instrument operator's manual. If this information is not available, standard correction curves or tables shall be used (see below).

Correction chart for sound level readings

Difference between total and background sound readings in dbA	Number to subtract from total to yield corrected noise level
0-2	reduce background levels
3	3
4-5	2
6-10	1
>10	0

F.12 Record of field certification

A cabinet that has met all the field test criteria listed in Annex F shall have the following information posted on the front of the cabinet in a location readily visible to the user, unless otherwise specified by the user:

F.12.1 Certification label

Biosafety cabinets field tested to this standard shall include the following information:

- date of certification;
- date cabinet should be recertified: no later than ;
- certifier's report number (reference document showing tests performed and results);
- name, address, and telephone number of certifying company; and
- signature of the person who performed the field certification tests.

F.12.2 Certification report

A certification report that will carry the language "certified in accordance with NSF Annex F" or any similar language shall, at a minimum, include the following:

1. BSC model number
2. BSC serial number
3. BSC location
4. BSC venting information
    a. (Ducted or not ducted)
        i. Type of connection (canopy, direct or none)
5. Type of BSC
6. Test equipment used for each test:
    a. Manufacturer
    b. model
    c. serial number
    d. calibration date
7. Specific test data as detailed in Annex F
8. Acceptance criteria for each test
9. Printed name of certification technician
10. Test date
11. Retest date

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Annex G[43]
(informative)

G.1 Recommended microbiological decontamination procedure[44]

G.1.1 Microbiological decontamination

Space decontamination is mandatory when maintenance work, filter changes, and performance tests require access to any contaminated portion of the cabinet. All work surfaces and exposed surfaces should be decontaminated with a suitable surface disinfectant before certification tests are performed and before gaseous decontamination takes place. In addition, it may be desirable to perform gaseous decontamination of the entire cabinet before performing certification tests when the cabinet has been used with agents assigned to biosafety level 2, and is recommended when the cabinet has been used with an agent assigned to biosafety level 3. A qualified safety and risk assessment of cabinets potentially contaminated with biological agents should be performed by a biosafety officer or qualified safety professional. Appropriate decontamination (space and/or surface) should be performed before BSCs are moved to another location. Additionally, after spills and splashes of research agents, contaminated surfaces should be suitably decontaminated.

G.1.2 Certification of cabinet decontamination

BSCs must be decontaminated prior to decommissioning and salvage, before physically moving the cabinet and whenever maintenance work or filter changes or performance tests require access to any contaminated portion of the cabinet.

G.1.2.1 Biological decontamination

Surface decontaminate accessible work surfaces with either chlorine dioxide or formaldehyde. Rinse work surfaces with water and then wipe dry. Use formaldehyde gas or an acceptable alternative space decontamination procedure to decontaminate the HEPA/ULPA filters and cabinet interior spaces Remove and discard all HEPA/ULPA filters and any prefilters. Rinse work surfaces with water and wipe dry.

G.1.2.2 Chemical, radiological, oil, or heavy metal decontamination

Surface decontaminate accessible work surfaces with an appropriate disinfectant and/or cleaning agent wipe down. Use formaldehyde gas or an acceptable alternative space decontamination procedure if biological agents may be present. Rinse work surfaces with water and wipe dry. Remove and discard all HEPA/ULPA filters and any prefilters.

[43] The information contained in this annex is not part of this American National Standard (ANS) and has not been processed in accordance with ANSI's requirements for an ANS. As such, this annex may contain material that has not been subjected to public review or a consensus process. In addition, it does not contain requirements necessary for conformance to the Standard.

[44] Taylor, L. A., Barbeito, M. S., Gremillion, G. G., 1969. "*Paraformaldehydes for Surface Sterilization and Detoxification*," Applied Microbiology 17:614-618.

DECONTAMINATION FORM (Sample)

BSC MODEL Number Serial Number

1. Check each type of hazardous material that has been used or is contained in this equipment. If there has been no contamination, check "NONE" for each hazard.

2. List decontamination procedure and product used for decontamination

3. Indicate biosafety level of facility where cabinet was used:

BSL1 BSL2 BSL3 BSL4 Not applicable

4. Complete and sign the certification below,

CONTAINED HAZARD (v')	DECONTAMINATION PROCEDURE	NONE	HAZARD TYPE
			BIOLOGICAL
			CHEMICAL
			RADIOLOGICAL
			OIL, HEAVY METAL (e.g. lead, mercury, or other hazardous material.

I hereby certify that this equipment has been decontaminated and thoroughly cleaned in accordance with the appropriate procedures (or that the equipment has not been used with any of the materials listed above).

Signature of last user or biosafety officer Date

Name (PLEASE PRINT) Title

Room Number Phone Number

G.1.3 Decontamination methods

In most instances where space decontamination is necessary, one of the procedures described below utilizing either depolymerized paraformaldehyde or chlorine dioxide gas is used. Prior to decontamination with an alternative method (such as vaporous hydrogen peroxide [VHP]), cycle parameters and validation of those parameters must be developed for each model and size of BSC. Material compatibility in terms of degradation and absorption of an alternative decontaminant are critical for maintaining cabinet integrity and the time required for decontamination, respectively. Alternate methods are required in certain instances, e.g., slow disease viruses. The decontamination method should be determined by consultation between user and certification agency. When paraformaldehyde is used for gas decontamination, follow OSHA Regulations Code of Federal Regulations, Title 29, Formaldehyde-1910-1048, which addresses monitoring; posting of regulated areas; respirator selection, protection and fit testing; medical surveillance; hazard communication and training; and recordkeeping. Automatic formaldehyde gas decontamination/neutralization may be used as a substitute to the formaldehyde procedure given below if the manufacturer's instructions have been followed. When using chlorine dioxide gas, similar precautions as used for formaldehyde should be followed. Similarly, automated chlorine dioxide gas systems are available which may be used if the manufacturer's instructions are followed.

G.1.3.1 Paraformaldehyde

CAUTION – All sources of hydrogen chloride must be removed from the cabinet before decontamination. Hydrogen chloride in the presence of formaldehyde, at ambient air conditions, will form the carcinogen Bis(chloromethyl)ether (BCME)[45].

a) Calculate the total volume of the cabinet by multiplying the height, width, and depth.

b) Multiply the total volume of the cabinet by 0.30 g/ft^3 (11 g/m^3) of space to determine the gram weight of paraformaldehyde required [CHECK CONCENTRATION]. Determine the stoichiometric amount of NH_4HCO_3 or alternative to neutralize the resulting formaldehyde gas with ammonia gas. The ammonium carbonate should be weighed out so that it is 10% greater than the weight of paraformaldehyde used for the decontamination to ensure completion of the reaction.

c) If the cabinet is equipped with an exhaust duct, this duct must be gas tight. This may be accomplished at the terminal end of the duct, or if present, at the damper located near the cabinet. If the exhaust duct is more than 10 ft (3 m) long, additional paraformaldehyde may be needed to compensate for the increased volume. If the cabinet exhausts into a recirculating building exhaust system, disconnect the cabinet from the building system and form a gas tight seal (plastic film and tape may be used).

d) If the cabinet exhaust air is discharged into the room, tape a plastic cover over and completely seal the exhaust port.

e) To provide for emergency evacuation of the formaldehyde and to allow removal of the neutralized formaldehyde following the decontamination and neutralization, a flexible hose can be pre-positioned close to the cabinet. This hose must be attached to a chemical fume hood or other exhaust suitable for the evacuation of toxic fumes.

f) Place a heating device, such as a commercially available electric frying pan or a remote formaldehyde generator/neutralizer, with the thermostat set at 450 to 475 °F (232 to 246 °C), on the work tray. The paraformaldehyde is spread evenly over the heating surface of the heating device.

NOTE – The auto-ignition temperature of paraformaldehyde is 572°F (300°C).

[45] NIOSH, Department of Health and Human Services (DHHS), *Hazard Review of Bis(chloromethyl)ether (BCME).*

g) Place an additional heating device on the work tray for the neutralizing agent. The neutralizing agent (NH_4HCO_3 or equivalent) should be separated from the air in the cabinet until needed. Below are two examples of how this separation could be achieved.

- Example 1: The NH_4HCO_3 or equivalent alternative is spread evenly over the heating surface of the heating device. The top of the device is covered with aluminum foil in such a way as to prevent the NH_4HCO_3 or alternative from reacting with the formaldehyde during the decontamination. The aluminum foil can be placed to allow the escape of ammonia gas when heated, or provision can be made to remove the aluminum foil remotely at the start of the neutralization phase. The removal technique must not allow unsafe levels of formaldehyde to escape the cabinet.

- Example 2: The cabinet is sealed using plastic with gloves as an integral part of the sheet of plastic. The NH_4HCO_3 or equivalent alternative is placed in a sealed container inside the cabinet. At the neutralization phase, the person performing the decontamination reaches into the cabinet without breaking the seal by using the gloves. The NH_4HCO_3 or equivalent alternative is removed from the sealed container and spread evenly over the heating surface of the heating device. The heating device is energized and the NH_4HCO_3 or equivalent alternative is heated and releases ammonia.

h) Place a hot plate, a beaker of water, and temperature and humidity indicators on the cabinet work tray. Do not connect electrical cords to the internal cabinet electric supply.

i) Close the opening to the work area with heavy gauge plastic film and tape. Close all possible leak areas, such as the exit of electrical cords, around the sash and the junction of the plastic film and cabinet.

j) Determine the temperature and humidity inside the cabinet.

k) The temperature should be 70 °F (21 °C) or higher, and humidity should be 60 to 85%. Use the hot plate to heat the beaker of water until the desired temperature and humidity are achieved.

l) Prior to depolymerizing the formaldehyde, access to the area or room around the cabinet must be restricted in accordance with applicable federal and state regulation and prudent safety practice. OSHA's Standard on Occupational Exposure to Formaldehyde**Error! Bookmark not defined.** requires that areas where the airborne concentration of formaldehyde exceeds the Permissible Exposure Limits be established as a regulated area with signs and labels marking the area and access restricted to properly trained personnel. Applicable regulations must be reviewed and complied with.

m) Plug the cord of the heating device into an outlet not installed on the cabinet.

n) After 25% of the paraformaldehyde has depolymerized, turn on the cabinet blower(s) for 10 to 15 s. Repeat after 50%, 75%, and 100% of the paraformaldehyde has depolymerized[46]. In cases where the cabinet blower is inoperative, circulation of air within the cabinet should be promoted with additional blowers or fans, or the time of decontamination should be extended beyond the times suggested in p) below.

o) Disconnect the hot plate and heating device used for the paraformaldehyde from the electrical outlets.

[46] Modification by Kruse, R. H., Puckett, W. H. and Richardson, J. H., 1991 "Biological Safety Cabinetry" Clinical Microbiological Review 4:207-241.

p) Allow the cabinet to stand for a minimum of 6 h, preferably overnight (12 h).

q) Prepare the neutralizing agent as previously established in step g) and energize the heating device containing the NH_4HCO_3 and the cabinet blower until the NH_4HCO_3 has dissipated. As with the paraformaldehyde, after 25% of the NH_4HCO_3 has depolymerized, turn on the cabinet blower(s) for 10 to 15 s. In cases where the cabinet blower is inoperative, circulation of air within the cabinet should be promoted with additional blowers or fans or the time of neutralization should be extended to a minimum of 6 h.

r) Let the cabinet stand for at least 1 h before opening seals.

s) If a flexible hose has been provided for the evacuation of the neutralized formaldehyde, slit the plastic covering the exhaust opening of the cabinet and seal the flexible hose to the opening. If the hose is working properly, the plastic covering the front opening of the cabinet should be sucked in. One or two small openings (approximately 6 x 6 in [15 x 15 cm]) are cut into the plastic covering the front opening of the cabinet to allow fresh air to enter the cabinet while the neutralized formaldehyde is being drawn out of the hose at the exhaust opening of the cabinet.

NOTE – Alternate removal procedures are acceptable if they allow for safe and effective removal of the formaldehyde gas.[47]

G.1.3.2 Chlorine Dioxide (CD)

G.1.3.2.1 Method 1 – Fixed amount of CD

a) Calculate the total volume (in ft^3 or m^3) of the cabinet by multiplying the height, width, and depth.

b) Calculate the amount of CD- generating chemical required for the decontamination. Multiply the total volume of the cabinet by 0.13 g/ft^3 (4.7 g/m^3) to determine the mass of CD required to be generated. Multiply this value by the value of mass of CD per unit mass of generating chemicals, as given by the supplier of the generating chemicals.

c) If the cabinet is equipped with an external duct, fully close the exhaust decontamination damper, while leaving balancing, backdraft, EVAV or other dampers in their original position. This duct and the exhaust decontamination damper must be of a “gas tight” design. Sealing may also be accomplished at the terminal end of the duct. If the exhaust duct is more than 10 ft (3 m) long, additional CD-generating chemical may be needed to compensate for the increased volume. If in the unlikely event the cabinet exhausts into a recirculating building exhaust system or does not have a fully functioning gas-tight decontamination damper, disconnect the cabinet from the building system and form a gas tight seal (plastic film and tape may be used).

d) If the cabinet exhaust air is discharged into the room, tape a plastic cover over and completely seal the exhaust HEPA/ULPA filter or port.

e) Place the chlorine dioxide generator within the BSC, (figure G1b) or attach the external CD gas generator delivery system to the BSC (figure G1a). In either case, a means of recirculation to ensure adequate distribution of CD and humidity within the BSC, including above the exhaust filter, will be provided. (The recirculation loop may include the CD generator within the loop.) The inlet tube will preferably be connected into or beneath the workspace and the return tube shall be connected to a location above the exhaust HEPA/ULPA filter.

f) Provide a means either within or external to the BSC, by which the air within the BSC may be humidified and the relative humidity (RH) monitored and maintained within a range of 60 - 85% RH

[47] Fink, D., Israeli, E., Liberman, D., Lupo, D., Murphy, K., 1988. “Biological Safety Cabinets, Decontamination or Sterilization with Paraformaldehyde” Am. Ind. Hyg. Assoc. J. 49 (6): 277-279.

throughout the decontamination process. A hot plate, beaker of water, and temperature and humidity indicators on the cabinet work tray may be used. If using a hot plate within the cabinet, do not connect its electrical cords to the internal cabinet electric supply, as these devices do not generally provide adequate current.

g) Provide a means either within or external to the BSC, by which the CD gas within the cabinet may be subsequently removed. Such a system might involve either the use of activated carbon granules or pellets or a chemical scrubbing system, through which the air within the cabinet can be circulated.

h) Close the opening to the work area with heavy gauge plastic film and tape. Seal all possible leak areas, such as the exit of electrical cords, around inlet and outlet hoses for the CD gas and/or its recirculation, around the sash, and at the junction of the plastic film and cabinet.

i) Determine the temperature and humidity inside the cabinet.

j) The temperature should be 60°F (15°C) or higher, and the humidity should be 60 – 85% RH. Use the hot plate with beaker of water or other means of humidity generation until the desired humidity level is attained. The cabinet blower and/or recirculation blower shall be operating during the entire humidification process.

k) Prior to the generation of CD gas, access to the area or room around the cabinet should be restricted in accordance with applicable federal and state regulation and prudent safety practice. It is recommended that a regulated area of radius of 20 ft be established about the cabinet to be decontaminated with CD, to be so indicated with signs and labels marking the area and access restricted to properly trained personnel. It is recommended that the room or area surrounding the cabinet be under negative relative pressure to prevent gas drifting in the event of leakage.

l) Begin generation and injection of CD gas into cabinet. Use the amount of CD – generating chemical as determined in step (b) above.

m) The cabinet blower (if available) and CD recirculation blower shall be operating during the entire CD gas generation period. Following the completion of CD gas generation, the cabinet blower and/or CD recirculation blower should be energized for at least 1 min during every 15 min of contact time.

n) Allow the cabinet to stand a minimum of 85 min from the initiation of CD gas generation with the assumption that the duration until peak concentration will be under 10 minutes.

o) Activate the system (scrubber) for removal of CD gas from the cabinet. Have the cabinet blower (if available) and CD recirculation blower energized during this period.

p) Allow sufficient time for the CD level within the cabinet to decrease to its STEL, the Short-Term Permissible Exposure Limit (0.3 ppm). This time depends upon the scrubbing system, but will generally require at least 30 min.

G.1.3.2.2 Method 2 – Fixed concentration of CD

a) If the cabinet is equipped with an external duct, fully close the exhaust decontamination damper, while leaving balancing, backdraft, EVAV, or other dampers in their original position. This duct and the exhaust decontamination damper must be of a “gas tight” design. Sealing may also be accomplished at the terminal end of the duct. If the exhaust duct is more than 10 ft (3 m) long, additional CD-generating chemical may be needed to compensate for the increased volume. If in the unlikely event the cabinet exhausts into a recirculating building exhaust system or does not have a fully functioning gas-tight decontamination damper, disconnect the cabinet from the building system and form a gas tight seal (plastic film and tape may be used).

b) If the cabinet exhaust air is discharged into the room, tape a plastic cover over and completely seal the exhaust HEPA/ULPA filter or port.

c) Place the chlorine dioxide generator within the BSC (figure G1b), or attach the external chlorine dioxide gas (CD) generator delivery system to the BSC (figure G1a). For an external generator, the inlet and outlet tubes/hoses to the BSC, may be connected to or beneath the workspace. For all B-type cabinets and for A-type cabinets with an inoperable internal blower, a means of recirculation to ensure adequate distribution of CD and relative humidity within the BSC, including above the exhaust filter, will be provided. (The recirculation loop may include the CD generator within the loop.) The inlet tube will preferably be connected into or beneath the workspace and the return tube will preferably be connected to a location above the exhaust HEPA/ULPA filter.

d) Provide a means either within or external to the BSC, by which the air within the BSC may be humidified and the relative humidity (RH) monitored and maintained within a range of 60 - 85% RH throughout the decontamination process. A hot plate, beaker of water, and temperature and humidity indicators on the cabinet work tray may be used. If using a hot plate within the cabinet, do not connect its electrical cords to the internal cabinet electric supply, as these devices do not generally provide adequate current.

e) Provide a means either within or external to the BSC, by which the CD gas within the cabinet may be subsequently removed. Such a system might involve either the use of activated carbon granules or pellets or a chemical scrubbing system, through which the air within the cabinet can be circulated.

f) Provide a means to monitor the concentration of CD gas during the decontamination. Gas sampling is to be extracted from within the BSC at a distance of at least 1 ft from the CD gas inlet.

g) Close the opening to the work area with heavy gauge plastic film and tape. Seal all possible leak areas, such as the exit of electrical cords, around inlet and outlet hoses for the CD gas and/or its recirculation, around the sash, and at the junction of the plastic film and cabinet.

h) Determine the temperature and humidity inside the cabinet.

i) The temperature should be 60°F (15°C) or higher, and the humidity should be 60 – 75% RH. Use the hot plate with beaker of water or other means of humidity generation until the desired humidity level is attained. The cabinet blower and/or recirculation blower shall be operating during the entire humidification process.

j) Prior to the generation of CD gas, access to the area or room around the cabinet should be restricted in accordance with applicable federal and state regulation and prudent safety practice. It is recommended that a regulated area of radius of 20 ft be established about the cabinet to be decontaminated with CD, to be so indicated with signs and labels marking the area and access restricted to properly trained personnel. It is recommended that the room or area surrounding the cabinet be under negative relative pressure to prevent gas drifting in the event of leakage.

k) Begin generation and injection of CD gas into cabinet. Monitoring the CD concentration within the cabinet, cease generation when the concentration has at least achieved the targeted CD concentration (3.0 or 5.0 mg/L).

l) The cabinet blower (if available) and CD recirculation blower (if present) shall be operating during the entire CD gas generation period. Following the completion of CD gas generation, the cabinet blower and/or CD recirculation blower should be energized for at least 1 min during every 15 min of contact time.

m) Continuously monitor the CD gas concentration during decontamination. Whenever the CD concentration decreases below the targeted concentration level, (3.0 or 5.0 mg/L) generate and inject more CD gas until the CD concentration has at least attained the targeted concentration level.

n) Continue the decontamination for a duration of 60 min for a targeted concentration of 3.0 mg/L or 45 min for a targeted concentration of 5.0 mg/L, measured from the time that the targeted concentration was first achieved.

o) Activate the system (scrubber) for removal of CD gas from the cabinet. Have the cabinet blower (if available) and CD recirculation blower energized during this period.

p) Allow sufficient time for the CD level within the cabinet to decrease to its STEL, the Short-Term Permissible Exposure Limit (0.3 ppm). This time depends upon the scrubbing system, but will generally require at least 30 min.

G.2 Recommended HEPA/ULPA filter disposal procedures

G.2.1 HEPA/ULPA filters that have been decontaminated are often burned in an incinerator. This disposal method is also effective for HEPA/ULPA filters containing toxic chemicals. Factors to be considered when incinerating filters include, but are not limited to, composition of the waste to be burned, feed rate, combustion temperature and dwell time in the primary chamber.

G.2.2 HEPA/ULPA filters may be placed in heavy plastic bags, such as those used to bag-out filters from contaminated filter housings. The bagged filters can be chemically decontaminated in situ by cutting small holes in the bag and delivering disinfectant by inserting a garden-type spray through the hole and spraying the filter media. The holes can be sealed with duct tape and shipped to an incinerator or sanitary landfill. This chemical method may be appropriate for filters containing agents (i.e. toxic chemicals or prions) that cannot be inactivated by the usual space decontamination procedures.

G.2.3 Decontaminated HEPA/ULPA filters may be safety buried in a sanitary landfill because they no longer pose a hazard.

Annex H[48]
(informative)

Recommended materials, finishes, and construction

H.1 Sheet metal and finishes

H.1.1 All cabinet interior work surfaces, including the drain pan assembly, should be fabricated with corrosion-resistant steel conforming to Federal Specification QQ-S-766 (Class 304, Number 3 Finish).

H.1.2 If carbon steel sheet is used in cabinet fabrication, it should be prime grade, stretcher, or roller leveled, conforming to Federal Specification QQ-S-698 (Cold Rolled Sheets, Condition Number 3 Regular Finish).

H.1.3 Before painting, carbon steel surfaces should be free of dirt, oil, and grease. The carbon steel should be given a phosphate coating treatment in accordance with Federal Specifications TT-C-490. Prime and finish coats can be applied by spraying or dipping and should be baked after each coat for a minimum of 15 min at 300°F (148.9°C). The finish should be uniform, with a minimum thickness of 1 mm. Concealed surfaces or hollow metal sections should be protected by the finish, applied by a suitable method after welding and before assembly. Epoxy coatings may be used to coat all carbon steel surfaces and should conform to Federal Specification TT-C-001224. The finish should be uniform. Polyurethane coating may be used to coat all carbon steel surfaces and should conform to Federal Specification TT-C-001227. The finish should be uniform.

H.2 Glass

H.2.1 If safety glass is used for the sash/window, it should be nominally 0.25 in (6.3 mm) laminated safety plate glass.

H.2.2 If tempered glass is used for the sash/window, it should be nominally 0.25 in (6.3 mm) tempered glass conforming to American Society for Testing and Materials C 1048 or equivalent.

H.3 HEPA/ULPA filter gasket materials

HEPA/ULPA filter gasket materials should be cellular sheet or molded rubber or closed cell expanded neoprene gasket materials. Unless otherwise specified, the gasket should be fastened to the influent face of the filter frame. The gasket should be 0.25 ± 0.031 in (6.3 ± 0.8 mm) thick by 0.75 ± 0.031 in (19 ± 0.8 mm) wide and flush with the outer edges of the frame. The gasket should be either molded in continuous, unbroken form, or made from four strips joined at the corners by interlocking means, so that no gaps are visible, and the joint should be airtight. The gasket should be continuously cemented to the face of the filter frame to prevent any air leakage between the gasket and frame.[49]

[48] The information contained in this annex is not part of this American National Standard (ANS) and has not been processed in accordance with ANSI's requirements for an ANS. As such, this annex may contain material that has not been subjected to public review or a consensus process. In addition, it does not contain requirements necessary for conformance to the Standard.

[49] Specifications taken from Military Specification MIL-F-51068 (Cancelled), Gasket Assembly <www.defenselink.mil/pubs/>.

H.4 HEPA/ULPA filter case – Type IC

HEPA/ULPA Filter Case – Type IC (wood type fire retardant treated particle board) is acceptable for the case of the HEPA/ULPA filter. Military Specification MIL-F-51068[50] lists other acceptable materials.

H.5 Specifications

The specifications require filter-mounting tolerances for openings up to 20 in (51 cm), ± 0.063 in (± 1.6 mm); and openings over 20 in (51 cm), to be ± 0.13 in (± 3.2 mm). The squareness of filter mountings should have diagonals within 0.063 in (1.6 mm) total allowance. Flatness at the filter gasket seal surface should be ± 0.015 in (± 0.4 mm) within any 10 in (25 cm) run.[51]

H.6 Sealants

H.6.1 Biosafety cabinet sealants

Two-part accelerated synthetic rubber (polysulfide type), temperature resistance, high adhesion aircraft specification grade, SAE AMS-S-8802, or equivalent, is acceptable. One part silicon base sealant compound, such as Dow Corning RTV 732 Adhesive Sealant, Dow Corning RTV 781 Building Sealant, Dow Corning RTV 734 or RTV 112 Self-leveling Sealants,[52] or equivalent, is acceptable when used in accordance with the manufacturer's recommendations.

H.6.2 HEPA/ULPA filter sealants and adhesives for repairs

Adhesives or sealants may be used to splice the medium or repair the filter, fasten the gasket to the filter frame and seal the filter media pack within the frame. Some recommended, but not limited to materials include polyurethane, epoxy, silicone, acrylics. Other adhesives and sealants may be used if recommended and agreed upon by the customer and the supplier and appropriate to the application, either prior to or after installation. In addition, the medium (media) used within HEPA or ULPA filters may be repaired with either a medium (media) of the same efficiency used or a combination of the filter medium and an approved adhesive. All sealants should be recommended and approved by the manufacturer of the cabinetry and compatible within the operational conditions at the facilities of the end user, their application of the cabinetry and their process guidelines.

H.6.3 The medium within HEPA or ULPA filter units used within the BSC may be patched with either medium of the same efficiency used in the filter or an adhesive. Some available sealants and adhesives that may also be used to splice the filter medium or repair the filter, attach the gasket to the frame or seal the pack to the frame include polyurethane, epoxy, silicone or acrylic. Others may be used as agreed upon by customer and supplier.

H.7 Fans

Fan(s) should be direct connected and conform to Air Movement and Control Association (AMCA)[53] standards.

[50] U. S. Department of Defense, Navy Publishing and Printing Service Office, 700 Robins Ave., Philadelphia, PA 19111-5094 <www.defenselink.mil/pubs/>.

[51] Specifications taken from Military specification MIL-F-51068 (cancelled) <www.defenselink.mil/pubs/>.

[52] The Dow Chemical Company, 2030 Dow Center, Midland, MI 48642 <www.dow.com>.

[53] Air Movement and Control Association (AMCA), 30 West University Dr., Arlington Heights, IL 60004 <www.amca.org>.

H.8 Components and wiring

All electrical components and wiring should conform to the latest edition of the National Electrical Code, National Electrical Manufacturer's Association (NEMA)[54], or Underwriters Laboratories (UL), whichever is applicable and provides the highest standard.

[54] NEMA, 1300 North 17th St., Suite 1847, Rosslyn, VA 22209 <www.nema.org>.

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Annex I[55]

Reference standards and specifications pertinent to Class II biosafety cabinetry[56]

I.1 Miscellaneous publications

I.1.1 Air Moving and Conditioning Association (AMCA)

- AMCA 99 – Standards Handbook
- AMCA 210-67 – Test Code for Air Moving Devices
- AMCA AS 2406 – Fans, Designation of Direction of Rotation and Discharge
- AMCA 211 – Fans, Labeling Requirements

I.1.2 American National Standards Institute, Inc. (ANSI)

- S1.4 – 1984 – Specification for Acoustical Calibrators

- S2.2 – 1959 (R1982) – Methods for the Calibration of Shock and Vibration Pick-ups

- Z26.1983 – Safety Glazing Materials for Glazing Motor Vehicles Operating on Land Highways, Safety Code for

- Z97.1 – 1984 – Performance Specifications and Methods of Test for Safety Glazing Material Used in Buildings

I.1.3 Illuminating Engineering Society (IES)

- The Lighting Handbook: Reference and Application

I.1.4 National Electrical Code

I.1.5 National Electrical Manufacturers' Association (NEMA)

I.1.6 Underwriters Laboratories, Inc.

- UL-62-1965 – Flexible Cord and Fixture Wire
- UL-94-1985 – Test for Flammability of Plastic Materials for Parts in Devices and Appliances
- UL-181 – Factory-Made Air Duct Materials and Air Duct Connectors
- UL-586-1985 – Test Performance of High Efficiency Particulate Air Filter Units
- UL-817-1987 – Cord Sets and Power Supply Cords
- UL-1262-1984 – Laboratory Equipment

I.1.7 U.S. Department of Energy

- ERDA 76-11 – Nuclear Air Cleaning Handbook (March 1976)

[55] The information contained in this annex is not part of this American National Standard (ANS) and has not been processed in accordance with ANSI's requirements for an ANS. As such, this annex may contain material that has not been subjected to public review or a consensus process. In addition, it does not contain requirements necessary for conformance to the Standard.

[56] Latest edition in effect at the time of manufacture.

I.1.8 U.S. Department of Labor

– Occupational Safety and Health Administration (OSHA) Safety and Health Standards for Respiratory Protection – 29CFR* 1910.134

I.1.9 U.S. Department of Health and Human Services

– Centers for Disease Control, National Institute of Occupational Safety and Health, Requirements for Respirator, 30CFR* Part II

*Code of Federal Regulations

I.1.10 American Conference of Governmental Industrial Hygienists

– Industrial Ventilation, A Manual of Recommended Practices, Twentieth Edition, 1989 or Later Edition (this publication is updated every two years)

I.1.11 U.S. Naval Research Laboratory

– Report 5959 (July 1963)

I.1.12 American Society for Testing and Materials (ASTM)

– C 1048 – Specification for Heat Treated Flat Glass, Kind HS, Kind FT Coated and Uncoated Glass

I.2 Federal specifications

– J#C-145 – Cable, Power, Electrical and Wire, Electrical; (Weather Resistant)

– W-C-00596 – Connector, Plug, Electrical; Connector Receptacle, Electrical

– W-S-00896 – Switch, Toggle

– W-S-893 – Switch, Toggle, and Mounting Strap (Interchangeable)

– CC-M-636 – Motor, Alternating-Current (Fractional Horsepower)

– QQ-S-698 – Steel, Sheet and Strip, Low-Carbon

– QQ-S-776 – Steel Plates, Sheets, and Strip-Corrosion Resisting

– TT-C-490 – Cleaning Methods and Pretreatment of Ferrous Surfaces for Organic Coatings

– TT-C-535 – Coating, Epoxy, Two-Component, for Interior and Exterior Use of Metal, Concrete and Masonry

– TT-C-001224 – Coating System, Epoxy, Glaze for Interior Surfaces

– TT-C-001227 – Coating System, Polyurethane Glaze for Interior Surfaces

- PPP-B-601 – Boxes, Wood, Cleated-Plywood
- PPP-B-621 – Boxes, Wood, Nailed and Lock-Corner
- PPP-B-640 – Boxes, Fiberboard, Corrugated, Triple-Wall
- PPP-C-650 – Crates, Wood, Open and Covered
- PPP-C-843 – Cushioning Material, Cellulosic
- PPP-T-60 – Tape, Packaging, Waterproof

I.3 Federal standards

- Federal Standard No. 102 – Preservation, Packaging and Packing Levels
- Federal Standard No. 123 – Marking for Domestic Shipment

I.4 Military specifications

- MIL-C-104 – Motor, Alternating Current (Fractional Horsepower)
- MIL-C-132 – Crates, Wood, Open; Maximum Capacity 2,500 pounds
- MIL-C-3774 – Crates Wood, Open; 12,000 and 16,000 Pound Capacity
- MIL-L-10547 – Liners, Case and Sheet Overwrap, Water-Vaporproof or Waterproof, Flexible
- MIL-P-116 – Preservation, Methods of
- MIL-R-3065 – Rubber, Fabricated Products-Gaskets, Synthetic Rubber
- MIL-S-8802 – Sealing Compound, Temperature-Resistant Aircraft High Adhesion
- MIL-F-51079B – Filters, Particulate, High Efficiency, Fire Resistant, Biological Use

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Annex J[57]
(informative)

J.1 Helium leak test

J.1.1 Purpose

This test on all biologically contaminated air plenums under positive pressure to the room determines whether exterior joints made by welding, gasketing, or sealing with sealants are free of leaks that might release potentially hazardous materials into the atmosphere.

J.1.2 Apparatus

The helium leak detector shall be calibrated in accordance with the manufacturer's instructions using a calibrated leak standard.

J.1.3 Method

a) The room where testing will be performed shall be free of test gases, and air movements shall be kept to a minimum. Where levels are detected, they shall be below the acceptable leak rate for the test or, alternatively, corrected for by the leak detector instrument. No smoking should take place in the test area.

b) Prepare the cabinet as a sealed system (see Annex A, section J.1.1).

c) Pressurize the cabinet with air to 2 in w.g. (500 Pa). If the cabinet holds this pressure without more than ± 10% loss for 30 min, then release pressure. If the cabinet does not hold this pressure, examine for gross leaks with liquid leak detector (see Annex J, section J.1.1), repair, and retest.

d) Helium leak: Flow pure helium through the cabinet until the well-mixed helium concentration at the exhaust point reads 15% helium, and then pressurize the cabinet to 2 in w.g. (500 Pa). Alternatively, use an inflated bladder inside the cabinet to displace 15% of the internal gas volume and inject helium into the cabinet volume while venting the bladder outside the cabinet volume. Then pressurize to 2 in w.g. (500 Pa).

e) Turn on the cabinet blower for 30 s to circulate gas.

f) Adjust the helium leak detector to a sensitivity setting of 1×10^{-5} cc/s, in accordance with the manufacturer's instructions.

g) Move the detector probe over seams, joints, utility penetrations, panel gaskets, and other areas of possible leakage. Hold the detector probe at the surface of cabinet, being careful not to jar the instrument. Move the detector probe over the surface at a rate of approximately 1.0 in/s (2.5 cm/s), keeping the probe 0.25 to 0.50 in (6.3 to 13 mm) away from the surface (see Annex J, figure J1).

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J.1.4 Acceptance

Measured leakage from any point in the cabinet shall not exceed a leak rate of 1 x 10^{-5} cc/s when pressurized to 2 in w.g. (500 Pa) with at least 15% concentration of helium.

J.2 Sulfur hexafluoride (SF_6) leak test

J.2.1 Purpose

This test on all biologically contaminated air plenums under positive pressure to the room determines whether exterior joints made by welding, gasketing, or sealing with sealants are free of leaks that might release potentially hazardous materials into the atmosphere.

J.2.2 Apparatus

– an industrial-type SF_6 leak detector (Ion Track Inc. [ITI] Leakmeter, or equivalent capable of detecting a halide leak of 1 x 10^{-7} cc/s); and

– the SF_6 leak detector (shall be calibrated in accordance with the manufacturer's instructions using a calibrated leak standard).

J.2.3 Method

a) The room where testing will be performed shall be free of test gases, and air movements shall be kept to a minimum. Where levels are detected, they shall be below the acceptable leak rate for the test or, alternatively, corrected for by the leak detector instrument. No smoking should take place in the test area.

b) Prepare the cabinet as a sealed system (see Annex J, section J.1.1).

c) Pressurize the cabinet with air to 2 in w.g. (500 Pa). If the cabinet holds this pressure without more than ± 10% loss for 30 min, release pressure. If the cabinet does not hold this pressure, examine for gross leaks with liquid leak detector (see Annex J, section J.1.1), repair, and retest.

d) Pressurize the air filled cabinet at atmospheric pressure to 2 in w.g. (500 Pa) with SF_6 gas.

e) Turn on the cabinet blower for 30 s to circulate gas.

f) Adjust the SF_6 leak detector to a sensitivity setting of 5 x 10^{-7} cc/s, in accordance with the manufacturer’s instructions.

g) Move the detector probe over seams, joints, utility penetrations, panel gaskets, and other areas of possible leakage. Hold the detector probe at the surface of cabinet, being careful not to jar the instrument. Move the detector probe over the surface at a rate of approximately 1 in/s (2.5 cm/s), keeping the probe 0.25 to 0.50 in (6.3 to 13 mm) away from the surface (Annex J, figure J1).

J.2.4 Acceptance

Measured leakage from any point in the cabinet shall not exceed a leak rate of 5 x 10^{-7} cc/s to compensate for the dilution of halide gas.

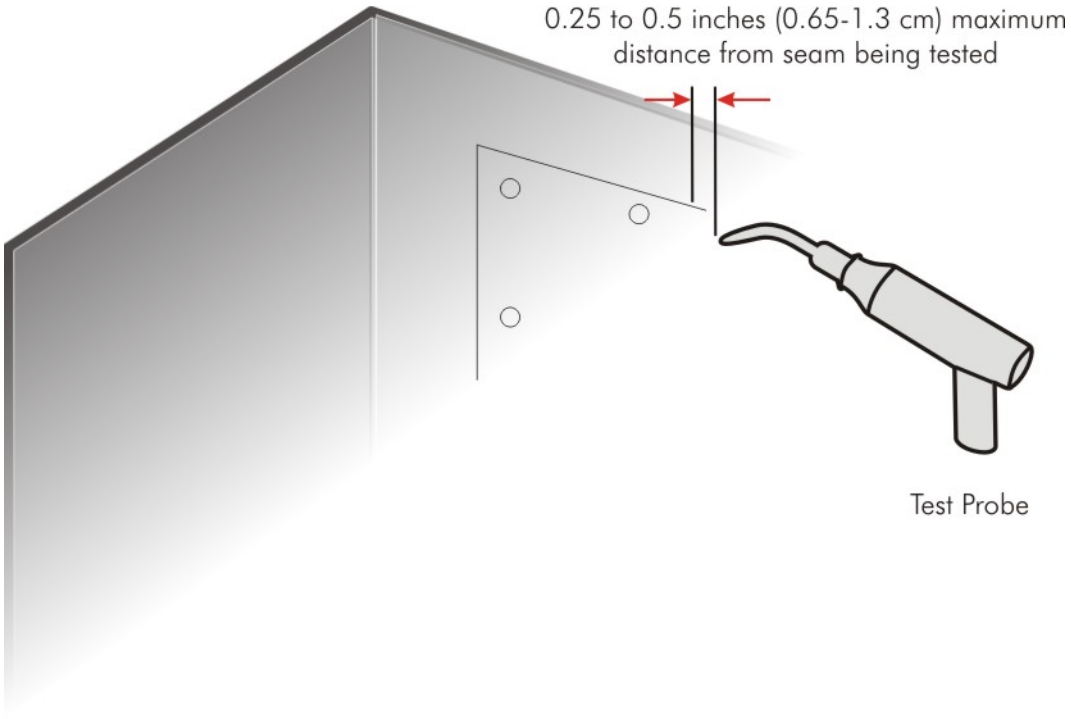

Figure J1 - Scanning for tracer gas leaks.

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Annex K[58]
(informative)

Protocol for the Validation of Alternate Biosafety Cabinet Decontaminating Methods and Agents

K.1 Introduction

Up until the end of 2008 the use of formaldehyde gas had been the only process considered validated for gas decontamination of biological safety cabinets. Among other advantages, formaldehyde gas has been shown to meet two critical standards. First, being a gas at standard laboratory conditions, it is fairly easy to demonstrate that it can be circulated to all regions within sealed cabinets. Secondly, it demonstrates the ability to kill or make inactive bacterial spores when used under conditions as specified within NSF/ANSI 49 – 2007. More specifically, it has been generally shown to produce a 6-log reduction of the spore species Bacillus atrophaeus (formerly referred to as *B. subtilis* var. niger), typically used as a biological indicator for formaldehyde.

There are some disadvantages to the use of formaldehyde gas for this procedure. Formaldehyde is considered a carcinogen or potential carcinogen within much of the technical community. It is not currently registered as a gas-phase decontaminant by the US Environmental Protection Agency. A significant inconvenience is that typical formaldehyde use in a BSC leaves a residue consisting largely of paraformaldehyde on surfaces within the cabinet, which while removable from accessible surfaces, cannot be fully removed following the decontamination. As a result of such issues, alternative decontamination methodologies have been sought.

The NSF has decided that validation of an alternative decontamination system to formaldehyde gas should demonstrate that it is at least as effective as formaldehyde gas. Unfortunately, while formaldehyde has been the standard for decontamination for decades, no formerly recorded “full” validation study of this gas exists by which new methods may be compared. The following protocol was designed to fill this need. To demonstrate efficacy, it relies on the use of the same bacterial endospore, B. atrophaeus, as a biological indicator, targeting demonstration of a 6-log viable population reduction. To demonstrate appropriate penetrability to all interior parts of all types of Class II BSCs, the studies involve placement of BIs within the most challenging parts of the cabinet, and both type A and B cabinets are included in the study.

K.2 Protocol

1. Cabinet preparation

a) The study shall include at least two different makes each of Class II Type cabinet, type A2 (both bench and console (console can be type A1) models), type B1 and type B2 biological safety cabinets. Each cabinet shall be decontaminated by the following procedures a minimum of three times.

b) HEPA/ULPA filters within the tested cabinets will have been previously “loaded” to an increase of greater than 0.3 in w.g. (50%) of their starting clean value.

c) Typically biological indicators consisting of ~ 10^6 *B. atrophaeus* endospores will be used for the validation study. Alternative indicator types might be used with approval of the NSF. The bacteria

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species, substrate and order of magnitude of spore population shall be specified. The material of the BI envelope, if any, shall also be specified.

d) Place a minimum of six pairs of appropriate biological indicators within the biological safety cabinet (BSC). Locations include where possible:

i. One pair of BIs is placed between the pleats on the downstream (clean) side of the exhaust HEPA/ULPA filter near the center. Two more pairs of BIs are at opposite corners of the filter, placed between the pleats no more than 3 in from the nearest outside corner of the exhaust HEPA/ULPA filter.

ii. One pair of BIs is placed within a potentially contaminated positive pressure plenum.

iii. One pair of BIs is placed beneath the work surface in the plenum below the cabinet work area.

iv. One pair of BIs is placed between the pleats near the center of the upstream (dirty) side of the down flow HEPA/ULPA filter.

e) Prepare the BSC for the decontamination process.

f) Provide a means, either within or external to the BSC, by which the air within the BSC may be environmentally monitored throughout the decontamination process.

g) Seal the BSC at the opening to the workspace and at or above the exhaust port. Verify the adequacy of the seal.

2. Decontamination procedure

a) The methodology for the decontamination procedure during the validation study must be clearly specified prior to the study. Several points should be taken in consideration.

i. If possible, the procedure should not designate the use of equipment provided by unique manufacturers.

ii. As the finally approved procedure may have a safety margin applied, it would be useful to have the validation procedure designed at lower chemical exposure (concentration and/or time) than what is intended for ultimate field usage.

iii. Generally, a decontamination method might involve either the introduction of a calculated mass of decontaminant, dependent upon the volume of the BSC, or the introduction of a gas whose concentration is monitored and maintained during the decontamination. The protocol should clearly state if either or both of these methods are being validated.

iv. The decontamination method may have requirements of permitted humidity and/or temperature range within and outside of the BSC. The protocol should clearly specify these and if there is a requirement that such condition exists for a specific duration prior to the introduction of decontaminant.

v. The method should state clearly what events are to designate the commencement and conclusion of the exposure period.

b) If at any time during the decontamination process the decontaminant is detected in the environment exterior to the BSC by instrument or odor at a concentration approaching its TEV level,

stop generation of the decontaminate and do not resume until the leak source(s) has been corrected and it is safe to do so.

c) In order to ensure a uniform concentration of the decontaminant throughout the BSC it may be advantageous to periodically operate the BSC's internal blower (bump the BSC).

d) Monitor the environmental conditions at regular intervals during the decontamination process. Automatic continuous monitoring may be employed.

3. Scrubbing/venting

a) Determine if any scrubbing or venting is necessary to remove or render harmless the decontaminant used.

b) In order to ensure a full removal of the decontaminant from throughout the BSC it may be advantageous to periodically operate the BSC's internal blower, a blower supplied with the scrubber, or both.

c) When the concentration of the decontaminant throughout the BSC is at the corresponding NIOSH STEL limit, and preferably below that, the BSC may be unsealed and vented.

d) Ensure minimal operation of the BSC blower(s) during the venting procedure to minimize the opportunity for contaminating the spore strips with airborne contaminants.

4. Analysis

a) Collect biological indicators

b) Have go/no-go analysis (3-7 days) performed for surviving spores on strips, with the use of positive controls. Effort should be made to reduce potential decontaminant residuals from BIs prior to placing them within growth media.

c) The result at a site of a single trial will be deemed successful if either 1 or 2 BI's from that site test negative (no turbidity in the incubated media tube.) If both strips test positive, that site test will be deemed a failure.

d) For a single cabinet trial, the trial would be considered successful (a pass) if all 6 site tests are successful by the criteria given above. It would be considered unsuccessful (a failure) if the site tests failed at more than 1 location. The trial would be considered a conditional pass if there was a failure at only one site.

e) A cabinet study is considered to have passed if all three trials passed. A cabinet study will also have been considered to pass if there had been one or more trials with conditional passes, as long as there has not been more than one failure for a given site.

f) A cabinet trial may be repeated if there is a clear understanding of the reason of a trial failure that is not based upon the intended target decontamination conditions. As examples, such reasons may include unexpected cabinet leakage, incorrect humidity levels or errors in BI handling.

5. Material compatibility

a) Prior or in conjunction with the biological validation of the alternative decontaminating chemical, a study shall be performed indicating that the chemical presents no or limited adverse effects to the typical materials that are exposed within a BSC. Such materials would include stainless steel, typical

gasketing material, internal paint, HEPA/ULPA filter material (including the filter media, sealing and frame materials), and materials involved with the BSC blower(s).

b) Such a study should demonstrate that at least 10 decontamination cycles performed on a cabinet leads to no deleterious effects on the equipment's functioning and at most limited cosmetic issues.

Standards[59]

The following standards established and adopted by NSF as minimum voluntary consensus standards are used internationally:

2 Food equipment
3 Commercial warewashing equipment
4 Commercial cooking, rethermalization, and powered hot food holding and transport equipment
5 Water heaters, hot water supply boilers, and heat recovery equipment
6 Dispensing freezers
7 Commercial refrigerators and freezers
8 Commercial powered food preparation equipment
12 Automatic ice making equipment
13 Refuse processors and processing systems
14 Plastics piping system components and related materials
18 Manual food and beverage dispensing equipment
20 Commercial bulk milk dispensing equipment
21 Thermoplastic refuse containers
24 Plumbing system components for recreational vehicles
25 Vending machines for food and beverages
29 Detergent and chemical feeders for commercial spray-type dishwashing machines
35 High pressure decorative laminates (HPDL) for surfacing food service equipment
36 Dinnerware
37 Air curtains for entranceways in food and food service establishments
40 Residential wastewater treatment systems
41 Non-liquid saturated treatment systems
42 Drinking water treatment units – Aesthetic effects
44 Residential cation exchange water softeners
46 Evaluation of components and devices used in wastewater treatment systems
49 Biosafety cabinetry: Design, construction, performance, and field certification
50 Equipment for swimming pools, spas, hot tubs, and other recreational water facilities
51 Food equipment materials
52 Supplemental flooring
53 Drinking water treatment units – Health effects
55 Ultraviolet microbiological water treatment systems
58 Reverse osmosis drinking water treatment systems
59 Mobile food carts
60 Drinking water treatment chemicals – Health effects
61 Drinking water system components – Health effects
62 Drinking water distillation systems
140 Sustainable carpet assessment
169 Special purpose food equipment and devices
170 Glossary of food equipment terminology
173 Dietary supplements
177 Shower filtration systems – Aesthetic effects
184 Residential dishwashers
222 Ozone generators
223 Conformity assessment requirements for certification bodies that certify products pursuant to NSF/ANSI 60: Drinking water treatment chemicals – health effects
240 Drainfield trench product sizing for gravity dispersal onsite wastewater treatment and dispersal systems
245 Wastewater treatment systems - nitrogen reduction
305 Personal care products containing organic ingredients
321 Goldenseal root (Hydrasitis canadensis)
330 Glossary of drinking water treatment unit terminology
332 Sustainability assessment for resilient floor coverings
336 Sustainability assessment for commercial furnishings fabric
342 Sustainability assessment for wallcovering products
347 Sustainability assessment for single ply roofing membranes
350 Onsite residential and commercial water reuse treatment systems
350-1 Onsite residential and commercial graywater treatment systems for subsurface discharge
355 Greener chemicals and processes information
358-1 Polyethylene pipe and fittings for water-based ground-source “geothermal” heat pump systems
358-2 Polypropylene pipe and fittings for water-based ground-source “geothermal” heat pump systems
359 Valves for crosslinked polyethylene (PEX) water distribution tubing systems
360 Wastewater treatment systems – Field performance verification
363 Good Manufacturing Practices (GMP) for Pharmaceutical Excipients
372 Drinking water treatment system components – Lead content
401 Drinking water treatment units - Emerging compounds / incidental contaminants
416 Sustainability Assessment for Water Treatment Chemical Products
418 Residential wastewater effluent filters longevity testing
419 Public Drinking Water Equipment Performance – Filtration
14159-1 Hygiene requirements for the design of meat and poultry processing equipment
14159-2 Hygiene requirements for the design of hand held tools used in meat and poultry processing equipment
14159-3 Hygiene requirements for the design of mechanical belt conveyors used in meat and poultry processing equipment

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***THE HOPE OF MANKIND** rests in the ability of man to define and seek out the environment which will permit him to live with fellow creatures of the earth, in health, in peace, and in mutual respect.*