TO: Joint Committee on Biosafety Cabinetry

FROM: Robert W. Powitz, Chairperson of the Joint Committee

DATE: November 4, 2020

SUBJECT: Proposed revision to NSF/ANSI 49 – Biosafety Cabinetry: Design, Construction, Performance and Field Certification (49i141r4)

Revision 4 of NSF/ANSI 49 issue 141 is being forwarded to the Joint Committee for balloting. Please review the changes proposed to this standard and submit your ballot by November 25, 2019 via the NSF Online Workspace <www.standards.nsf.org>.

When adding comments, please identify the section number/name for your comment and add all comments under one comment number where possible. If you need additional space, please upload a word or pdf version of your comments online via the browse function.

**Purpose**
The purpose of this ballot is to affirm revised language regarding the definitions in Section 3 of Standard 49.

**Background**
Issue paper BSC-2019-06 highlighted the need to update the definitions in Section 3 of Standard 49. The proponent contends it has been some time since these have been evaluated and a quick scan of the upcoming BMBL 6th edition indicates several areas where Standard 49 might be improved for consistency.

This issue was presented to the full JC during the 2019 Face-to-Face meeting, where it was suggested the language be sent to the TG on Definitions for consideration. Revision 1 was sent to the Task Group where it received unanimous affirmative voting. Language was thereafter sent to the JC as an approval ballot where it received a vote of 14 : 2 : 1 (Affirmative : Negative : Abstain) and six related comments.

Using the comments, the issue proponent updated the language to Revision 2 approval ballot which then received a vote of 16 : 2 : 0 (Affirmative : Negative : Abstain) and four related comments. Using these revision 2 ballot comments, the issue proponent further revised and executed a Revision 3 approval ballot yielding a vote of 13 : 4 : 2 (Affirmative : Negative : Abstain) and 7 additional comments.

Taking all revisions and comments into consideration, the issue proponent further updated the proposal into this Revision 4 ballot which is now being sent to this Joint Committee for consideration.

**Public Health Impact**
The proposed changes have no negative impact on public health.
If you have any questions about the technical content of the ballot, you may contact me in care of:

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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 air curtain: an artificially created stream of air that moves across a threshold or other opening to create a barrier.

3.2.2 downflow velocity: The velocity of HEPA/ULPA - filtered air as it flows downward through the work area, providing product and cross contamination protection. The velocity is measured in a plane 4 inches (100 mm) above the bottom edge of the sash, when it is in its normal operating height.

3.2.3 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 two or more adjoining areas (zoned) with averages calculated for each zone.

3.2.4 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.

3.2.5 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.

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

3.2.7 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 humans, or animals, or plants, or any combination thereof 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 inches (840 mm). Cabinets with an interior sidewall to sidewall width of 33 inches (840 mm) or less are classified to the nearest 6 inches (150 mm). 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, or test connection, or both, 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, Section A.1. Its installation is durable, not typically requiring service, or replacement, or both, and its function is to allow the certifier to make the necessary adjustments or test measurements without releasing contaminants.

3.5.2 user-modified pass through 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 pass through structure may be permanently attached to the work area of the cabinet, not typically requiring service, or replacement, or both, 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, or replacement, or both. Its functions are to immobilize the items passing through it, and it, and to provide a seal meeting the requirements of Annex A, Section 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 There are four biocontainment levels designated for evaluation of laboratory facilities. Biosafety Levels (BSL) 1-4 are assigned to lab space based on a risk assessment which evaluates infrastructure, engineering controls, equipment, practices and procedures.
3.7 Biosafety Level (BSL):^1^ The essential elements of the four BSLs for activities involving infectious microorganisms and laboratory animals are summarized in *Biosafety in Microbiological and Biomedical Laboratories.*^2^ 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 (BSL-1): BSL-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 (BSL-2): BSL-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 BSCs or other physical containment equipment.

3.7.3 Biosafety Level 3 (BSL-3): BSL-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 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 (BSL-4): BSL-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 redesignate 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.

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^1^ Previously referred to as risk levels (low, moderate, and high).

There are two models for BSL-4 laboratories:

— a cabinet laboratory where all handling of agents must be performed in a Class III BSC; and
— 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.

Rationale: the summary located in Appendix G, Physical Containment of the NIH Guidelines covers this topic in great detail and needn’t be recreated within Standard 49.

3.8 cabinet classification: Although this Standard covers only Class II BSCs, Class I and Class III BSCs are currently defined and known to be commercially available. BSCs can be used for work with biological agents assigned to BSLs 1 through 4, depending on the facility design as described in Biosafety in Microbiological and Biomedical Laboratories. Special note should be taken that Risk Group 4 BSL-4 agents should only be used in Maximum Containment BSL-4 Laboratories and that Class I and Class II BSCs 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 ifpm (0.38 m/s)\(^1\) 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, C1, 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 are 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, A2, and C1 BSCs) or discharged from the building via a canopy connection (Type A1, A2, and C1 BSCs). Exhaust air from Types B1 and B2 BSCs must be discharged to the outdoors via a dedicated, sealed connection.

All Class II cabinets are designed for work involving procedures assigned to BSL 1-3 facilities. Class II BSCs may be used with procedures requiring BSL-4 facilities 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...
Immediately below is the replacement for the current footnote located on page 5 of Standard 49-2019


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; containment may fail when people walk by the work 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.

If working with volatile chemicals, the unit shall be canopy-connected to external exhaust system if permitted by a chemical risk assessment (refer to Section E.3.1.3).

NOTE — Type A1 BSCs manufactured prior to 2010 are not suitable for work with volatile chemicals due to the contaminated positive pressured plenums that are not surrounded by negative pressure plenums.

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.
If working with volatile chemicals, the unit shall be canopy-connected to external exhaust system if permitted by a chemical risk assessment (Refer to Section E.3.1.3).

### 3.8.2.3 Class II Type B1 cabinets

- 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 contaminated downflow air from a region of the work area via an internal dedicated exhaust plenum and through HEPA/ULPA filter(s) to an a dedicated external exhaust system for BSCs with a direct connection and exhausted to the atmosphere; and
- recirculate the balance of the downflow and inflow air through a supply HEPA/ULPA filter(s); 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 if permitted by a chemical risk assessment (Refer to Section E.3.1.3).

### 3.8.2.4 Class II, Type B2 cabinets

- 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 a dedicated external exhaust system for BSCs 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 (non-recirculated nonrecirculated through the work area) negative pressure ducts and plenums.

Type B2 cabinets may be used for work with volatile chemicals if permitted by a chemical risk assessment (Refer to Section E.3.1.3).

### 3.8.2.5 Class II, Type C1 cabinets

- 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 contaminated downflow air from a region of the work area via an internal dedicated exhaust plenum and blower, and then through HEPA/ULPA filter(s);

— recirculate the balance of the downflow and inflow air through a supply HEPA/ULPA filter(s);

— have all biologically contaminated ducts and plenums under negative pressure or surrounded by negative pressure ducts and plenums; and

— may exhaust HEPA/ULPA filtered air either back into the laboratory or via a canopy connection to an external system that exhausts to the atmosphere.

If working with volatile chemicals, the unit must be connected to an external exhaust system. Type C1 cabinets may be used for work with volatile chemicals if permitted by a chemical risk assessment (Refer to Section E.3.1.3).

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 $1 \times 10^{-7}$ cc/s with 1% test gas at 3-inch pressure water gauge) enclosure with a viewing window that is secured with locks, or requires the use of tools to open, or both. 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 and. 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.

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 inch (0.79 mm).

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 to an acceptable level.

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 BSCs):

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 dioctylphthalate (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 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 inch 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/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 Section 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 **readily viewable**: Visible without using tools but may require manual selection.

3.26 **readily visible**: Visible without using tools or manual selection.

3.27 **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”]).

3.28 **sash**: A fixed or sliding window located at the front of the BSC, that forms a barrier between the operator and the work area.

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

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

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

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

3.31 **surfaces**: (See Figure 1).

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

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

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

   3.31.4 **exterior surfaces**: All exposed surfaces not defined as interior.

3.32 **toxic**: Having an adverse physiological effect on biological systems.

3.33 **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.

3.34 **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.

3.35 **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 inches (50 mm) above the lower level of the sash.

3.36 **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.

**Rationale:** It has been some time since the definitions for Standard 49 have been evaluated for updating. A quick scan of the upcoming BMBL 6th edition indicates several areas for which 49 should be calibrated against.