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

NSF International Standard / American National Standard
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 NSF International Standard / American National Standard
for Biosafety Cabinetry –

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

Standard Developer
NSF International

Designated as an ANSI Standard
August 1, 2019
American National Standards Institute
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1 The information contained in this Disclaimer 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 Disclaimer 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.
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Contents

1 General .................................................................................................................................................. 1
  1.1 Scope ............................................................................................................................................. 1
  1.2 Minimum requirements ................................................................................................................. 1
  1.3 Variations in design and construction ....................................................................................... 1

2 Normative references ............................................................................................................................. 2

3 Definitions ............................................................................................................................................. 3

4 Materials ............................................................................................................................................... 11
  4.1 General .......................................................................................................................................... 11
  4.2 Interior work surfaces ..................................................................................................................... 11
  4.3 Exposed interior surfaces .............................................................................................................. 11
  4.4 Other interior and exterior surfaces ............................................................................................. 11
  4.5 Materials and finishes ................................................................................................................. 12

5 Design and construction .......................................................................................................................... 13
  5.1 General .......................................................................................................................................... 13
  5.2 Cleanability .................................................................................................................................. 13
  5.3 Decontamination ............................................................................................................................ 13
  5.4 Canopy exhaust connection ......................................................................................................... 14
  5.5 Direct exhaust connection ............................................................................................................ 14
  5.6 Duct and plenum design ................................................................................................................ 14
  5.7 Internal corners and angles .......................................................................................................... 14
  5.8 External corners and angles ......................................................................................................... 15
  5.9 Joints and seams ............................................................................................................................ 15
  5.10 Fastening methods ........................................................................................................................ 15
  5.11 Welds ......................................................................................................................................... 15
  5.12 Solder ....................................................................................................................................... 15
  5.13 Removable panels ....................................................................................................................... 15
  5.14 Stability ..................................................................................................................................... 16
  5.15 Provision for mounting ................................................................................................................ 16
  5.16 Legs and feet ............................................................................................................................... 16
  5.17 Reinforcing and framing ............................................................................................................. 16
  5.18 Fixed panels ............................................................................................................................... 16
  5.19 Doors and covers .......................................................................................................................... 16
  5.20 Louvers and openings ................................................................................................................. 17
  5.21 Tracks and guides ........................................................................................................................ 17
  5.22 Filters ....................................................................................................................................... 17
  5.23 Gaskets and sealants ................................................................................................................... 18
  5.24 Stopcocks and service outlets ....................................................................................................... 18
  5.25 Alarms ..................................................................................................................................... 18
  5.26 Electrical components ............................................................................................................... 20
  5.27 Lighting .................................................................................................................................... 20
  5.28 Gauges ..................................................................................................................................... 20
  5.29 Drain spillage trough ................................................................................................................... 21
  5.30 Diffuser placement ....................................................................................................................... 21
  5.31 Work area components placement ............................................................................................ 21
  5.32 Data plate(s) ............................................................................................................................. 21
  5.33 Routine maintenance adjustment fixtures .................................................................................. 21
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Performance</td>
<td>28</td>
</tr>
<tr>
<td>6.1</td>
<td>General</td>
<td>28</td>
</tr>
<tr>
<td>6.2</td>
<td>Pressure decay / soap bubble / tracer gas leak</td>
<td>28</td>
</tr>
<tr>
<td>6.3</td>
<td>HEPA/ULPA filter leak</td>
<td>28</td>
</tr>
<tr>
<td>6.4</td>
<td>Noise level</td>
<td>28</td>
</tr>
<tr>
<td>6.5</td>
<td>Lighting intensity</td>
<td>28</td>
</tr>
<tr>
<td>6.6</td>
<td>Vibration</td>
<td>28</td>
</tr>
<tr>
<td>6.7</td>
<td>Personnel, product, and cross-contamination protection</td>
<td>28</td>
</tr>
<tr>
<td>6.8</td>
<td>Stability</td>
<td>29</td>
</tr>
<tr>
<td>6.9</td>
<td>Downflow and inflow velocities</td>
<td>29</td>
</tr>
<tr>
<td>6.10</td>
<td>Inflow velocity</td>
<td>30</td>
</tr>
<tr>
<td>6.11</td>
<td>Airflow smoke patterns</td>
<td>30</td>
</tr>
<tr>
<td>6.12</td>
<td>Drain spillage trough leakage</td>
<td>30</td>
</tr>
<tr>
<td>6.13</td>
<td>Motor / blower performance</td>
<td>30</td>
</tr>
<tr>
<td>6.14</td>
<td>Electrical safety</td>
<td>31</td>
</tr>
<tr>
<td>6.15</td>
<td>Performance data</td>
<td>31</td>
</tr>
<tr>
<td>6.16</td>
<td>Record maintenance</td>
<td>31</td>
</tr>
<tr>
<td>6.17</td>
<td>Air velocity stability</td>
<td>31</td>
</tr>
</tbody>
</table>

**Normative Annex 1** Performance tests

<table>
<thead>
<tr>
<th>Test</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-1.1</td>
<td>33</td>
</tr>
<tr>
<td>N-1.2</td>
<td>33</td>
</tr>
<tr>
<td>N-1.3</td>
<td>34</td>
</tr>
<tr>
<td>N-1.4</td>
<td>36</td>
</tr>
<tr>
<td>N-1.5</td>
<td>37</td>
</tr>
<tr>
<td>N-1.6</td>
<td>38</td>
</tr>
<tr>
<td>N-1.7</td>
<td>49</td>
</tr>
<tr>
<td>N-1.8</td>
<td>50</td>
</tr>
<tr>
<td>N-1.9</td>
<td>52</td>
</tr>
<tr>
<td>N-1.10</td>
<td>55</td>
</tr>
<tr>
<td>N-1.11</td>
<td>56</td>
</tr>
<tr>
<td>N-1.12</td>
<td>57</td>
</tr>
<tr>
<td>N-1.13</td>
<td>58</td>
</tr>
<tr>
<td>N-1.14</td>
<td>60</td>
</tr>
</tbody>
</table>

**Normative Annex 2**

<table>
<thead>
<tr>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Method to verify fitness for use of potential direct inflow</td>
</tr>
<tr>
<td>measurement devices</td>
</tr>
<tr>
<td>83</td>
</tr>
</tbody>
</table>

**Normative Annex 3** Nebulizer selection and calibration

<table>
<thead>
<tr>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selection</td>
</tr>
<tr>
<td>Calibration</td>
</tr>
<tr>
<td>85</td>
</tr>
<tr>
<td>85</td>
</tr>
</tbody>
</table>

**Normative Annex 4** Evaluation of chemical resistance and abrasion resistance of surfaces

<table>
<thead>
<tr>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical resistance</td>
</tr>
<tr>
<td>Abrasion resistance</td>
</tr>
<tr>
<td>89</td>
</tr>
<tr>
<td>89</td>
</tr>
</tbody>
</table>

**Normative Annex 5** Field tests

<table>
<thead>
<tr>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Field certification</td>
</tr>
<tr>
<td>Preconditions and</td>
</tr>
<tr>
<td>intervals</td>
</tr>
<tr>
<td>91</td>
</tr>
<tr>
<td>91</td>
</tr>
<tr>
<td>Downflow velocity</td>
</tr>
<tr>
<td>92</td>
</tr>
<tr>
<td>Inflow velocity</td>
</tr>
<tr>
<td>93</td>
</tr>
<tr>
<td>Airflow smoke patterns</td>
</tr>
<tr>
<td>98</td>
</tr>
<tr>
<td>HEPA/ULPA filter leak test</td>
</tr>
<tr>
<td>99</td>
</tr>
<tr>
<td>Pressure decay / soap bubble</td>
</tr>
<tr>
<td>101</td>
</tr>
<tr>
<td>Site installation</td>
</tr>
<tr>
<td>assessment tests</td>
</tr>
<tr>
<td>102</td>
</tr>
<tr>
<td>Electrical leakage</td>
</tr>
<tr>
<td>and ground circuit</td>
</tr>
<tr>
<td>resistance and</td>
</tr>
<tr>
<td>polarity tests</td>
</tr>
<tr>
<td>105</td>
</tr>
<tr>
<td>Lighting intensity</td>
</tr>
<tr>
<td>106</td>
</tr>
</tbody>
</table>

vi
Informative Annex 1  Biosafety cabinet selection, installation, use, lifespan, and decommissioning...... 111
I-1.1  Institutional safety consultation........................................... 111
I-1.2  Risk assessment procedure ................................................ 111
I-1.3  BSC Class and Type selection ................................................ 116
I-1.4  Site review before BSC purchase ........................................... 124
I-1.5  BSC arrival and certification .................................................. 126
I-1.6  Cleaning and disinfection of BSC work area ......................... 127
I-1.7  BSC use practices and procedures ........................................ 129
I-1.8  Moving a permanently installed BSC ................................. 133
I-1.9  BSC lifespan ........................................................................ 133
I-1.10 Decommissioning process .................................................... 133
I-1.11 Definitions ........................................................................... 135

Informative Annex 2 .................................................................................................................................. 153
I-2.1  Recommended biosafety cabinet decontamination procedure ..................................................... 153
I-2.2  Recommended HEPA/ULPA filter disposal procedures...................................................................... 169

Informative Annex 3  Recommended materials, finishes, and construction .............................................. 171
I-3.1  Sheet metal and finishes ........................................................ 171
I-3.2  Glass ................................................................................. 171
I-3.3  HEPA/ULPA filter gasket materials ...................................... 171
I-3.4  HEPA/ULPA filter case – Type IC ........................................ 172
I-3.5  Specifications ..................................................................... 172
I-3.6  Sealants ............................................................................. 172
I-3.7  Fans ................................................................................... 172
I-3.8  Components and wiring ....................................................... 173

Informative Annex 4  Reference standards and specifications pertinent to Class II biosafety cabinetry ......................................................... 175
I-4.1  Miscellaneous publications .................................................. 175
I-4.2  Federal specifications ........................................................... 176
I-4.3  Federal standards ................................................................ 177
I-4.4  Military specifications .......................................................... 177

Informative Annex 5 .................................................................................................................................. 179
I-5.1  Helium leak test .................................................................. 179
I-5.2  Sulfur hexafluoride ($SF_6$) leak test .................................. 180

Informative Annex 6  Protocol for the validation of alternate biosafety cabinet decontaminating methods and agents ........................................... 183
I-6.1  Introduction ....................................................................... 183
I-6.2  Protocol ............................................................................... 183

Interpretation Annex .................................................................................................................................. 187
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Foreword

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 includes the following revisions:

Issue 54

This revision affirms new and updated language in Annex N-5 (formerly Annex F) concerning the use of the Secondary method for measuring airflow.

Issue 82

This revision affirms new language in Annex I-1 (formerly Annex E) regarding the term percent recirculation.

Issue 92

This revision affirms new and updated language in Section 3 and Annex N-5 (formerly Annex F) regarding canopy field testing.

Issue 120

This revision affirms new language in Section 3 regarding the addition of the newly proposed term plenum.

Issue 122

This revision affirms new and revised language in Annex N-5 (formerly Annex F) regarding the Certification Label.

Issue 127

This revision affirms revised language regarding the use of the term NOTE.

Issue 130

This revision affirms revised language regarding the definition of the term work area.

Issue 133

This revision affirms revised language in Section 5 regarding the data plate.

Issue 136

This revision affirms revised language in Annex N-1 (formerly Annex A) and Annex N-5 (formerly Annex F) regarding the sash seal smoke test.

Issue 138

This revision affirms revised language regarding the range of measurement for vibration frequency.

2 The information contained in this Foreword 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 Foreword 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.
**Issue 139**

This revision affirms revised language in Annex I-1 (formerly Annex E).

**Issue 140**

This revision affirms revised language in Annex N-1 (formerly Annex A) regarding accuracy requirements for the manometer used for the pressure decay and motor blower performance tests.

**Issue 146**

This revision addresses inconsistencies of incubation times and temperatures during the various biological tests in Annex N-1.

**Issue 147**

This revision affirms revised language in Annex N-1 (formerly Annex A) regarding filter porosity for filtering impinger water.

**Issue 148**

This revision affirms revised language in Annex N-1 (formerly Annex A) regarding the confirmation requirements for the Cross Center test.

**Issue 149**

This revision affirms revised language in Section 2 regarding Normative References.

The Interpretations Annex contains responses to interpretation requests. The responses will be published in each version of the Standard until such time that the interpretation response is no longer applicable.

This revision also includes an editorial update to the names of the Annexes within. The Annexes are being changed from alpha characters to numeric, preceded by a ‘Normative’ or ‘Informative’. The Annexes have also been reordered so the Normative Annexes appear first, followed by the Informative Annexes. The table below shows the previous name of the Annex with the corresponding new name of the Annex:

<table>
<thead>
<tr>
<th>Annexes</th>
<th>Previously known as:</th>
<th>Now known as:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annex A</td>
<td>Normative Annex 1 (N-1)</td>
<td></td>
</tr>
<tr>
<td>Annex B</td>
<td>Normative Annex 2 (N-2)</td>
<td></td>
</tr>
<tr>
<td>Annex C</td>
<td>Normative Annex 3 (N-3)</td>
<td></td>
</tr>
<tr>
<td>Annex D</td>
<td>Normative Annex 4 (N-4)</td>
<td></td>
</tr>
<tr>
<td>Annex E</td>
<td>Informative Annex 1 (I-1)</td>
<td></td>
</tr>
<tr>
<td>Annex F</td>
<td>Normative Annex 5 (N-5)</td>
<td></td>
</tr>
<tr>
<td>Annex G</td>
<td>Informative Annex 2 (I-2)</td>
<td></td>
</tr>
<tr>
<td>Annex H</td>
<td>Informative Annex 3 (I-3)</td>
<td></td>
</tr>
<tr>
<td>Annex I</td>
<td>Informative Annex 4 (I-4)</td>
<td></td>
</tr>
<tr>
<td>Annex J</td>
<td>Informative Annex 5 (I-5)</td>
<td></td>
</tr>
<tr>
<td>Annex K</td>
<td>Informative Annex 6 (I-6)</td>
<td></td>
</tr>
</tbody>
</table>
This Standard was developed by the NSF Joint Committee on Biosafety Cabinetry using the consensus process described by the American National Standards Institute.

This Standard and the accompanying text are intended for voluntary use by certifying organizations, regulatory agencies, and/or manufacturers as a basis of providing assurances that adequate health protection exists for covered products.

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.
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 (BSCs) 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 any of the following changes to the blower / motor(s): location, capacity, quantity, or automatic airflow adjustment; 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; the visibility or audibility of the safety signaling; and built-in accessory equipment (centrifuges, ultraviolet (UV) lighting, supports for intravenous drug container, arm rests, etc.). Major modifications also include changes affecting the safe use of the cabinet including the ability to see, hear, and understand the required alarms. Relocation of utility service equipment (electrical outlets, petcocks, etc.), the visual appearance of the cabinet, or user interface(s), are 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.


ANSI 226.1, *Test No. 17*⁴

ANSI/NFPA 70, *National Electrical Code*, 2020⁵

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

APHA/AWWA/WEF, *Standard Methods for the Examination of Water and Wastewater* (hereinafter referred to as *Standard Methods*), Seventeenth Edition (Standard dilution plate methods)⁷


IEC 61010-1, *Safety Requirements For Electrical Equipment For Measurement, Control, And Laboratory Use – Part 1: General Requirements*⁹


IEST-RP-CC001.5, *HEPA and ULPA Filters*¹³

IEST-RP-CC007.2, *Testing ULPA Filters*¹³

IEST-RP-CC013, *Institute of Environmental Sciences Recommended Practice*, Tentative, August, 1986¹³

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³ American Conference of Governmental Industrial Hygienists. 1330 Kemper Meadow Drive, Cincinnatti, OH 45240. <www.acgih.org>


⁵ National Fire Protection Association. 1 Batterymarch Park, Quincy, MA 02169-7471. <www.nfpa.org>

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


⁸ American Society of Heating, Refrigeration, and Air-Conditioning Engineers, Inc. 1791 Tullie Circle, NE, Atlanta, GA 30329. <www.ashrae.org>

⁹ International Electrotechnical Commission. 3 rue de Varambè, 1st floor, PO Box 131, CH-1211 Geneva 20, Switzerland. <www.iec.ch>

¹⁰ Institute of Electrical and Electronics Engineers, Inc. 3 Park Avenue, New York, NY 10016 <www.ieee.org>

¹¹ ASTM International. 100 Barr Harbor Drive, PO Box C700, West Conshohocken, PA 19428-2959. <www.astm.org>

¹² Illuminating Engineering Society. 120 Wall Street, Floor 17, New York, NY 10005. <www.ies.org>

¹³ Institute of Environmental Sciences and Technology. 5005 Newport Drive, Suite 506, Rolling Meadows, IL 60008-1699. <www.iest.org>
IEST-RP-CC021.3, *Testing HEPA/ULPA Filter Media*

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

NIOSH Pocket Guide: *bis(chloromethyl)ether*

OSHA, CFR 29 § 1910.100, *Bloodborne Pathogens*

UL 94, *Standard for Tests for Flammability of Plastic Materials for Parts in Devices and Appliances*

### 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 inches (100 mm) 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 two 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 nonuniform (zoned) downflow:** A downflow velocity profile comprised of several contiguous zones. The average downflow velocities vary from zone to zone.

**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 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.

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15 Centers for Disease Control and Prevention, The National Institute for Occupational Safety and Health. 1600 Clifton Road, Atlanta, GA 30329. <www.cdc.gov/niosh/>

16 US Department of Labor, Occupational Safety and Health Administration. 200 Constitution Avenue NW, Washington, DC 20210. <www.osha.gov>

17 UL LLC. 33 Pfingsten Road, Northbrook, IL 60062. <www.ul.com>
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. Cabinets with an interior sidewall to sidewall width of 33 inches 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, personnel protection slit sampler positioning, and cross contamination test requirements.

3.5 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.

3.6 biosafety cabinet shell penetrations / cable ports

3.6.1 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 to provide a seal meeting the requirements of Section N-1.1.

3.6.2 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 Section N-1.1. 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.

3.6.3 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, 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.

3.7 Biosafety Level (BSL): The essential elements of the four BSLs for activities involving infectious microorganisms and laboratory animals are summarized in Biosafety in Microbiological and Biomedical Laboratories. 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.

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

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.

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.

3.8 **cabinet classification:** Although this Standard covers only Class II BSCs, Class I and Class III cabinets 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 BSL-4 agents should only be used in Maximum Containment 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 ft/min (0.38 m/s)\(^{20}\) 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.

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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 BSLs 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.

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.

If working with volatile chemicals, the unit must be connected to an external exhaust system. Type A1 cabinets may be used for work with volatile chemicals if permitted by a chemical risk assessment (refer to Section I-1.3.1.3).

**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 must be connected to an external exhaust system. Type A2 cabinets may be used for work with volatile chemicals if permitted by a chemical risk assessment (refer to Section I-1.3.1.3).

### 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 contaminated downflow air from a region of the work area via an internal dedicated exhaust plenum and through HEPA/ULPA filter(s) to an external exhaust system with a direct connection and exhausted to the atmosphere;
- 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 I-1.3.1.3).

### 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 if permitted by a chemical risk assessment (refer to Section I-1.3.1.3).

### 3.8.2.5 Class II Type C1 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 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 I-1.3.1.3).

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 $1 \times 10^{-7}$ mL/s with 1% test gas at 3 inches (750 Pa) 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. 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 gloves / 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 N-1 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 N-5 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 inches (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

\[21\text{ National Institutes of Health, Laboratory Safety Monograph: A Supplement to the NIH Guidelines for Recombinant}
\text{DNA Research January 1979 <www.nih.gov>}.\]
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 ft/min (0.015 m/s). 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 ft³/min (12 m³/h). 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 inches w.g. (75 Pa) shall be added for Type B1 cabinets and 0.7 inches w.g. (170 Pa) shall be added for Type B2 cabinets. The resulting values may be used for design and balance exhaust or 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 N-2.

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-CC00713 (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-CC00713 (Type K);
— maximum pressure drop of 1 inch 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-CC001.4.13 Filter media shall be tested in accordance with the methods of IEST-RP-CC02113 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 throwaway, 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;13
— 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 or 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-CC001.4,13 HEPA/ULPA filters.

3.20 **leak tight**: Free of leaks at 2 inches w.g. (500 Pa) of air pressure as described in Annex N-1.

3.21 **plenum**: an air-filled space within or on the biosafety cabinet meant for the distribution of air. It can be positively or negatively pressurized with contaminated or uncontaminated air.

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 **modified canopy installation**: Installation of any canopy other than a designated acceptable option for a NSF Listed Biosafety Cabinet.

3.24 **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 Section N-1.7.

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

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

3.27 **readily viewable**: Visible without using tools but may require manual selection.

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

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

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

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

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

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

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

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

3.33.1 **exposed interior surfaces**: Exposed interior surfaces, other than work surfaces, that are subject to splash, spillage, or airborne contamination during normal use.
3.33.2 **exterior surfaces**: All exposed surfaces not defined as interior.

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

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

3.34 **total work area**: The area inside the cabinet between the sidewalls, rear wall, inside of the sash, bottom of the downflow diffuser, and top of the work tray. The total work area definition is applicable only for purposes of design and construction of the biosafety cabinet and for testing the biosafety cabinet.

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

3.36 **usable work area**: The space within the total work area where the user can perform work, identified by the manufacturer as appropriate for user activities to maintain personal, product and cross-contamination protection.

3.37 **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.

3.38 **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.

3.39 **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.\(^\text{22}\)

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.\(^\text{17}\) Nonrigid diffuser materials shall conform to Class 94 HBF; rigid diffuser materials shall conform to Class 94 HB.

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.

\(^\text{22}\) See Annex I-3 for material selection guidance.
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 9417 and conform to Class 94 HB.

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 Section 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 N-4:

- 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 N-4, there shall be no visible effect on the finish other than a slight change of:

- gloss; or
- discoloration; or
- temporary softening of the finish; or
- any combination of the above; and
- no loss of adhesion or film protection.

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 N-4:

- maximum weight loss: 100 mg; and
- minimum wear value: 500 cycles.
4.5.3 Plastics

Plastics shall meet the applicable requirements of Sections 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 Sections 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 halogen and are nonhardening, nontoxic, stable, odor free, not detrimentally absorbent, and unaffected by exposure to gases, liquids, cleaning compounds, and decontamination agents listed in Section 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. Nonhardening 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

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.

23 See Annex I-2.
5.4 Canopy exhaust connection

If Type A1, A2, and C1 cabinets are connected to an exhaust system, it shall only be done so via a canopy connection; direct connections are not acceptable. They are exhausted with the assistance of a remote fan to the atmosphere. In normal operation, the volume of room air drawn into the canopy connection’s openings or gaps shall be sufficient to ensure the capture of all of the BSC’s HEPA filtered exhaust, as verified by a visible medium. The flow of room air into the canopy connection through openings, or gaps, or both, provides assurance of consistent BSC performance during fluctuations in exhaust system flow rate, or room pressure, or both.

For Types A1, A2, and C1 with a canopy connection, during an exhaust system failure:

— the canopy shall provide properly sized openings or gaps to allow for recirculation of HEPA filtered exhaust into the room,

— the BSC shall maintain an inflow velocity above the lowest value verified by the NSF/ANSI 49 biological challenge testing, and

Alternatively, the Type C1 canopy can direct the HEPA filtered exhaust into the exhaust duct during an exhaust system failure provided:

— the BSC shall maintain an inflow velocity above the lowest value verified by the NSF/ANSI 49 biological challenge testing, if the unit is programmed to operate longer than 15 seconds after an exhaust system failure.

5.5 Direct exhaust connection

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 inch (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 inch (6.4 mm) for a vertical or horizontal intersection. The alternate intersections shall have a minimum continuous and smooth radius of 0.13 inch (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 Section 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 Section 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 Section N-1.7.

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 inches (100 mm) of unobstructed clearance beneath the unit. A 2 inch (50 mm) 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 inches (50 mm) 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 inch (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, or 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.

5.20  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/ULPA filters shall be required for the downflow and exhaust air systems; and

— HEPA/ULPA filters for downflow and exhaust systems shall conform to the materials, construction, and aerosol efficiency requirements of IEST-RP-CC-001.4\textsuperscript{13} 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\textsuperscript{13} 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/ULPA filters shall be scan tested for a leakage not to exceed 0.01% when tested in accordance with Section N-1.2.

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

— HEPA/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/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 $\frac{1}{4}$ inches (6.4 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/ULPA filters during the...
HEPA/ULPA filter leak test (see Section 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; and

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

— HEPA/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 inches (38 mm).

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 Section N-1.3. 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; and

— 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 inch (25 mm) above or positioned 1 inch (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 cabinet 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 activate within 15 seconds of exhaust volume loss exceeding 20%. The internal cabinet fan(s) shall be interlocked to shut off within 15 seconds of exhaust volume loss exceeding 20%. Type B cabinets shall not initiate cabinet blower startup until sensors determine appropriate exhaust flow.
5.25.4 Type A1 or A2 canopy 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 seconds 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.

5.25.5 Type C1 canopy exhaust alarm

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 seconds a loss of capture of room air using a visible medium to verify at the canopy air intake(s):

— when the Type C1 is connected to a canopy that directs the BSC’s exhaust air into the room during an exhaust system failure, the cabinet fan(s) must remain in operation for a maximum of 5 minutes when the alarm is activated; or

— when the Type C1 is connected to a canopy that directs the BSC’s exhaust air into the exhaust duct during an exhaust system failure:

   — the cabinet downflow and exhaust blowers must shut down within 15 seconds of loss of capture of the visible medium; or

   — the default shut down time of 15 seconds may be lengthened to a maximum of 5 minutes if:

      — a risk assessment indicates the BSC, the work being done in it, and the exhaust system it is connected to is appropriate, as outlined in Section I-1.3; and

      — the BSC is connected to an exhaust duct that has been verified to meet or exceed Seal Class A, (a leakage of less than 3 FT3/MIN per 100 ft2 of duct surface area at 1 inch w.g. (0.091 m3/min per 10 m2 of duct surface area at 250 Pa) as described in HVAC Air Duct Leakage Test Procedures – 2012; and

      — the cabinet provides the user an indication of the remaining time until the BSC blowers shut off.

When Type C1 BSCs are connected to an exhaust system and there is insufficient exhaust volume, the BSCs shall not initiate downflow or exhaust blower startup.

5.25.6 Type A1, A2, or C1 cabinet low inflow alarm

Type A1, A2, or C1 cabinets may contain an inflow alarm system to alert the user of a potential loss of personnel protection. When present, an audible and visual alarm shall be required to indicate within 15 seconds of reaching the manufacturer-specified inflow alarm set point.

When starting the cabinet blowers from a dead stop, the inflow alarm must activate a visual indication until the cabinet either enters into a visually indicated warm up period not to exceed 2 minutes or the appropriate inflow velocity is achieved to ensure proper alarm system function.

If the manufacturer-specified inflow velocity alarm set point is more than 10 ft/min (0.051 m/s) less than the nominal inflow velocity, the test as specified in Section N-1.6.3.1.h will be performed with the inflow velocity at this set point ± 3.0 ft/min (0.015 m/s).

24 Sheet Metal and Air Conditioning Contractors National Association. 4201 Lafayette Center Drive, Chantilly, Virginia 20151-1219. <www.smacna.org>
If the manufacturer-specified inflow velocity alarm set point is no more than 10 ft/min (0.051 m/s) less than the nominal inflow velocity, the inflow alarm point shall be tested as specified in Section N-1.6.3.1.h.

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 Section 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; and

— 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 Section 6.2. Circuit overload protection shall be provided for all receptacles. Switches shall be mounted outside the work area. Cabinet wiring diagram(s), such as assembly or ladder schematic, shall be accessible by downloadable barcode, permanent label or sealed plastic pouch attached to a cabinet panel or surface located outside of air plenums systems. A statement providing starting current, maximum current / full load ampere (FLA) rating, 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 Section 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 UV lighting

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

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.

25 UV irradiation can cause erythema of skin and eye damage.
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 $\frac{3}{8}$ inch NPT (DN 10 or equivalent) 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 gallon (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 **Data plate(s)**

5.32.1 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;
- Type classification;
- input voltage and frequency requirements, as well as rated amps.

5.32.2 The following shall be readily viewable from the front of the cabinet:

- nominal set point for downflow and inflow velocities (DIM and thermal anemometer);
- downflow velocity test grid dimensions (Section N-1.8.3); and
- inflow velocity test grid and method (Section N-1.9.3).

5.33 **Routine maintenance adjustment fixtures**

Adjustments required during routine field 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.
Figure 1
Surfaces
Intersection of three planes (internal corner). Two intersections must have an minimum radius of \( \frac{1}{8} \) inch (3.2 mm), the third must have a minimum of \( \frac{1}{4} \) inch (6.3 mm).

Intersection of two planes. There must be a minimum radius of \( \frac{1}{8} \) inch (3.2 mm), vertical or horizontal.

All external corners or angles are to be sealed and finished smooth.

Figure 2
Internal corners and angles

Figure 3
External corners and angles
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
6 Performance

6.1 General

For qualification by the testing organization, BSCs shall meet the performance requirements listed in Sections 6.2 through 6.15, when tested in accordance with Annex N-1. 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 Section N-1.1).

6.2.1 The cabinet shall hold 2 inches w.g. (500 Pa) within ± 10% for 10 min.

6.2.2 For manufacturer testing only, the soap bubble method may be used when pressure plates fail: all welds, gaskets, penetrations, or seals on exterior surfaces of air plenums shall be free of soap bubbles when at 2 inches 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 Section 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 inches (300 mm) in front of the cabinet and 15 inches (380 mm) 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 $2 \times 10^{-4}$ inch ($5 \times 10^{-3}$ mm) root mean square (RMS) amplitude at frequencies between 10 and 1,000 Hz 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 Sections 6.7.1, 6.7.2, and 6.7.3 and Section N-1.6, when operating with the airflows specified in that Annex.
6.7.1 Personnel protection

The system shall be challenged by $1 \times 10^8$ to $8 \times 10^8$ Bacillus subtilis var. niger (B. subtilis) spores for five minutes. 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 minute 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 $1 \times 10^6$ to $8 \times 10^6$ B. subtilis spores for 5 minutes. 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 $1 \times 10^4$ to $8 \times 10^4$ B. subtilis spores for 5 minutes. Some agar plates within 14 inches (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 inches (360 mm) 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 61010-117 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 0.062 inch (1.6 mm) from the static position when a 250 pounds (1110 N) 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 pounds (23 kg) test load distributed uniformly over an area 10 \times 10 inches (250 \times 250 mm) 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 inch (1.6 mm) when a 250 pound (114 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 (nonuniform downflow) and the calculated and measured average inflow velocities of the cabinet shall be set at the nominal set points \( \pm 3 \text{ ft/min} (0.015 \text{ m/s}) \) 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 \( \pm 5 \text{ ft/min} (\pm 0.025 \text{ m/s}) \) (see Section N-1.9) 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 inches (100 mm) above the bottom edge of the sash in its normal operating position (certified height).

6.9.3 Nonuniform (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 Section 6.7 may also qualify if the average inflow velocity is within ± 5 ft/min (± 0.025 m/s) of the nominal set point velocity.

6.10.1 The minimum average inflow velocity of Type A1 cabinets shall be 75 ft/min (0.38 m/s).

6.10.2 The minimum inflow quantity per 1 foot (0.3 m) of work area width of Type A1 cabinets shall be 45 ft³/min (76 m³/h) (see Sections 6.7 and 6.9).

6.10.3 The minimum average inflow velocity of Type A2, B1, B2 and C1 cabinets shall be 100 ft/min (0.51 m/s).

6.10.4 The minimum inflow quantity per 1 foot (0.3 m) of work area width of Type A2, B1, B2, and C1 cabinets volume rate shall be 65 ft³/min (110 m³/h) (see Sections 6.7 and 6.9).

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 gallon (4 L) of water with no visible leakage after a one hour 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 Nationally 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 NRTL certification, (requires at least annual NRTL audits to maintain cabinet design certification) and 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 N-1 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 N-1 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 ft/min (0.015 m/s).

6.17.1 When the cabinet is subjected to a 10 mm free fall drop on each side, the cabinet inflow velocity and downflow velocity (where applicable) shall not change by more than 5 ft/min (0.025 m/s). There shall be no visible damage to the cabinet following the shock.

6.17.2 When the supply voltage to the cabinet is reduced or increased by 10%, the cabinet inflow velocity and downflow velocity (where applicable) shall not change by more than 5 ft/min (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), or downflow velocity (where applicable), or both, shall not change by more than 3 ft/min (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.
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Normative Annex 1
(formerly Annex A)

Performance tests

Before any performance tests are run, the cabinet shall be properly installed and leveled and airflows adjusted to the nominal set point (± 3 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 Section 1.3 of this Standard. Field tests are provided in Annex N-5.

Until certified under NSF/ANSI 49-2002, all new cabinets shall be factory tested using the procedures described in NSF/ANSI 49-2002, Annex A, 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 N-5 relating to variability in the field (ideal conditions).

N-1.1 Pressure decay / soap bubble

N-1.1.1 Pressure decay or soap bubble test

N-1.1.1.1 Purpose

This test determines the overall seal integrity of the cabinet outer hull, including exterior surfaces of all plenums, welds, gaskets, plenum penetrations, and seals.

N-1.1.1.2 Apparatus

- manometer, pressure gauge, or pressure transducer system with a minimum range of 0 to 2 inches w.g. (0 to 500 Pa) and accurate to ± 2% of reading ± 0.001 in w.g. (0.2 Pa);
- manufacturer-provided pressure plates constructed of steel, aluminum, plastic or other nonpermeable material as needed to seal exhaust, fan inlet, and access openings; and
- liquid leak detector.

N-1.1.1.3 Method (pressure decay)

The pressure decay test may be used during manufacturing to demonstrate compliance with Section N-1.1. It shall always be used during cabinet design certification testing.

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

b) Remove decorative panels and other access obstructions, where necessary, to allow proper sealing of openings.

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 inches w.g. (500 Pa), turn off the pressurizing air, and measure the pressure after 10 minutes.
e) If the cabinet does not hold pressure within 10% after 10 minutes, use the liquid leak detector to check for leaks in the pressure plates used to seal the access opening, exhaust, and fan inlet (where applicable). If leaks are found, make needed repairs if possible and repeat step d.

N-1.1.1.4 Method (soap bubble)

The soap bubble test may be used during manufacturing to demonstrate compliance with Section N-1.1 in place of the pressure decay test. The soap bubble test shall not be used for cabinet design certification testing.

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 inches 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.

N-1.1.1.5 Acceptance

N-1.1.1.5.1 Pressure decay

The cabinet shall hold 2 inches w.g. (500 Pa) ± 10% for 10 minutes. This requirement shall be met for all cabinet design certification testing.

N-1.1.1.5.2 Soap bubble

All welds, gaskets, penetrations, and seals on exterior surfaces of air plenums shall be free of soap bubbles when at 2 inches w.g. (500 Pa) ± 10% pressure above atmospheric. This requirement may be met during manufacturing as an alternative to the pressure decay test.

N-1.2 HEPA/ULPA filter leak test

N-1.2.1 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 ft/min (0.015 m/s) of the nominal set point, with the exception of the downflow HEPA/ULPA filters on B1 cabinets.

N-1.2.2 Apparatus

N-1.2.2.1 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], sebecute, polyethylene glycol, and medicinal-grade light
mineral oil)26 produced by the generator described in Section N-1.2.2.2. It shall also be capable of detecting an aerosol of $1 \times 10^{-3}\%$ of the same particles. The sampling rate of air shall be at least 1 ft$^3$/min (28 m$^3$/min) ± 10%. The probe area shall have a maximum open area of 1.7 in$^2$ (1100 mm$^2$) and a minimum dimension of 0.5 inch (13 mm). The photometer shall be calibrated in accordance with the photometer manufacturer's instructions or with IEST-RP-CC-013 if instructions are not provided.

N-1.2.2.2 An aerosol generator of the Laskin Nozzle type conforming to Figure 11 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 inches (31 mm).

N-1.2.2.3 A pressure gauge for the generator having a maximum range of 0 to 80 psi (0 to 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.

N-1.2.3 Method

N-1.2.3.1 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).27 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 to 100), adjust the instrument to read 100 on the 100% scale; or

— for logarithmic readout photometers, adjust the upstream concentration to $1 \times 10^4$ 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 inch (25 mm) 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 (50 mm/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.


27 HEPA/ULPA and ULPA Filter Leak Tests, Institute of Environmental Sciences and Technology. 940 East Northwest Highway, Mount Prospect, IL 60056. <www.iest.org>
N-1.2.3.2 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 inch (8 mm) 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.

N-1.2.4 Acceptance

N-1.2.4.1 Filters that can be scanned

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

N-1.2.4.2 Filters that cannot be scanned

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

N-1.3 Noise level test

N-1.3.1 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 ft/min (± 0.015 m/s).

N-1.3.2 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.

N-1.3.3 Method

a) Turn on the cabinet blower and lights.

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

c) Measure the noise level 12 inches (0.30 m) in front of the cabinet leading front edge of the access opening and 15 inches (0.38 m) above the plane of the work surface, in line with the vertical centerline of the cabinet (see Figure 12).

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 step c above.

N-1.3.4 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 Section N-1.4.3.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 Table N-1.1).
Table N-1.1
Correction chart for sound level readings

<table>
<thead>
<tr>
<th>Difference between total and background sound readings in dB(A)</th>
<th>Number to subtract from total to yield corrected noise level</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 2</td>
<td>reduce background levels</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4 to 5</td>
<td>2</td>
</tr>
<tr>
<td>6 to 10</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>0</td>
</tr>
</tbody>
</table>

N-1.4 Lighting intensity test

N-1.4.1 Purpose

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

N-1.4.2 Apparatus

A portable photoelectric illuminance meter, as described in *The Lighting Handbook*,12 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.

N-1.4.3 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 inches (300 mm), starting 6 inches (150 mm) from the side walls (see Annex N-1, Figure 13).

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 inches (300 mm), starting 6 inches (150 mm) from the side walls (see Annex N-1, Figure 13).

N-1.4.4 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.

N-1.5 Vibration test

N-1.5.1 Purpose

This test determines the amount of vibration in the operating cabinet. The cabinet shall be operated within ± 3 ft/min (± 0.015 m/s) of the nominal set point velocities.
N-1.5.2 Apparatus

A vibration analyzer with an accuracy of 5% of full scale and a minimum reliable reading of $1 \times 10^{-4}$ inch ($2.5 \times 10^{-6}$ m) root mean square (RMS) amplitude or the ability to detect differences of this magnitude, set up in accordance with manufacturer's instructions. The vibration analyzer shall be capable of measuring vibration in displacement mode within the 10 to 1,000 Hz frequency range.

N-1.5.3 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.

b) The test position is shown in Annex N-1, Figure 14.

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

d) Determine the background vibration amplitude with the 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.

N-1.5.4 Acceptance

Net displacement shall not exceed $2 \times 10^{-4}$ inch (5 µm) RMS amplitude at 10 to 1,000 Hz in the center of the work surface(s).

N-1.6 Personnel, product, and cross-contamination protection (biological) tests

N-1.6.1 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 minutes 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 Sections N-1.8 and N-1.9.

N-1.6.2 Materials

- spores of Bacillus subtilis var. niger (B. subtilis), ATCC 9372, or NCTC No. 10073, and
- sterile diluent prepared as follows:
  a.1) Step 1: concentrated diluent phosphate buffer solution (PBS):
     - dissolve 34 g KH$_2$PO$_4$ in 500 mL distilled water;
     - adjust pH to 7.2 ± 0.5 with 1 N NaOH at 77 °F (25 °C); and

28 American Type Culture Collection. 10801 University Boulevard, Manassas, VA 20110. <www.atcc.org>
29 National Collection Type Culture, London, England. <www.ukncc.co.uk>
— dilute to 1 L with distilled water.

a.2) Step 2: final diluent PBS:

— distilled H\textsubscript{2}O: 1 L;
— stock PBS step 1: 1.25 mL;
— final pH: 7.2 ± 0.5;
— autoclave at 250 °F (121 °C) for 15 min; and
— optional – magnesium sulfate (50 g MgSO\textsubscript{4} · 7H\textsubscript{2}O per L distilled water): 5 mL.

or

b) — distilled water: 1 L;
— adjust pH to 7 ± 0.1 at 77 °F (25 °C);
— autoclave at 250 °F (121 °C) for 15 min;

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 × 15 mm and 150 × 22 mm) containing nutrient agar (NA), trypsin (tryptic) soy agar (TSA),\textsuperscript{30,31} 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.\textsuperscript{32} Catalog Number 7540-10, air sampling impingers, or equivalent;

— two slit-type air samplers operating at a rated flow of 1 ± 0.05 ft\textsuperscript{3}/min (28.3 ± 1.4 L/min);

— refluxing 6-jet modified MRE-type short-form colison nebulizer (available as Model CN-38 Nebulizer [Model NSF CN-31/I] from BGI, Inc.),\textsuperscript{33} or any other nebulizer demonstrated to produce a bacterial aerosol of equivalent characteristics;

— one 2.5 inches (64 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 inches (150 mm) into the room through the work access opening of the cabinet;

— a pressure gauge having a minimum range of 0 to 30 psi (0 to 210 kPa) maximum range of 0 to 50 psi (0 to 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;

\textsuperscript{30} Beckton, Dickinson and Company. 1 Becton Drive, Franklin Lakes, NJ 07417. <www.bd.com>

\textsuperscript{31} VGD, Inc. PO Box 1130, Lawrence, KS 66044. <www.vgdllc.com>

\textsuperscript{32} Ace Glass Incorporated. 1430 NW Boulevard, Vineland, NJ 08360. <www.aceglass.com>

\textsuperscript{33} BGI Inc. 58 Guinan Street, Waltham, MA 02451. (www.bgi.mesalabs.com>
— 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 × 15 mm).

b) Incubate for 48 ± 2 hours 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 hours at 99 ± 2 °F (37 ± 1 °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 ± 0.5 °C) for 15 minutes. If cell debris interferes with nebulizer dissemination, the suspension may be clarified by washing three times in PBS by centrifugation at 2500 rpm for 15 minutes. Resuspend in PBS to the original volume.

g) Determine spore concentration by standard dilution-plate methods using PBS and TSA. Spores prepared as above should yield an average count of 2 × 10⁹ to 4 × 10⁹/mL.

h) Incubate plates for 44 to 48 hours at 97 ± 2 °F (36.1 ± 1 °C).

i) Dilute the spore suspension with PBS to obtain a final spore concentration of 5 × 10⁸ to 8 × 10⁹/mL if the spores are to be used immediately.

j) Store the stock spore suspension (2 × 10⁹ to 4 × 10⁹/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.

— 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 hours at 99 ± 2 °F (37 ± 1 °C).

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

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 minutes. 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 × 10⁹/mL.

f) Incubate the plates for 44 to 48 hours at 97 ± 2 °F (36.1 ± 1 °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 × 10⁸ to 8 × 10⁹/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 Section N-1.7.3.1.

— Method C

  a) Aseptically subculture from a stock culture of *B. atrophaeus* ATCC 9372 displaying characteristic orange pigmentation to tryptic soy broth (TSB).

  b) Incubate for 24 hours at 95 to 99 °F (35 to 37 °C).

  c) Aseptically inoculate Roux flasks containing fortified NA with the TSB culture.

  d) Incubate inverted Roux flasks at 95 to 99 °F (35 to 37 °C). Check progress of sporulation for development of mature spores; a phase contrast microscope will show mature spores as phase bright. For *B. atrophaeus* 9372, 95% mature spores are generally obtained in 5 days.

  e) Harvest spores by adding cold sterile deionized water (CSDW) to each flask and washing the bacteria from the agar surface; transfer harvest to sterile centrifuge bottles.

  f) Wash spores three times in CSDW by centrifugation at 10,000 to 12,000 rpm in a refrigerated super centrifuge at 50 °F (10 °C).

  g) After the final wash, suspend spores in aqueous ethanol.

  h) Determine spore concentration by standard dilution-plate methods using PBS and TSA.

  i) Incubate plates for 44 to 48 hours at 97 ± 2°F (36.1 ± 1°C).

  j) Dilute the spore suspension with PBS to obtain a final spore concentration of 5 × 10⁸ to 8 × 10⁹/mL.

  k) Store the stock spore suspension at 39 °F (4 °C) or divide into aliquots to store in sterile screw-cap 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.

— Method D

  a) Aseptically inoculate (by streak plating technique) characteristic orange pigmented *B. atrophaeus* 9372 culture to several TSA petri plates.

  b) Incubate for 1 to 3 days at 86 to 95 °F (30 to 35 °C).

  c) Remove characteristic (pigmented dark orange) colonies and transfer to fortified NA petri plates.
d) Incubate plates at 86 to 95 °F (30 to 35 °C) until 95% spores have formed. Periodically verify sporulation using phase contrast microscopy. Do not harvest until 95% of cells have formed phase bright spores.

e) Harvest spores by adding sterile deionized (DI) water to each plate and gently washing growth from the agar surface. Transfer spores to sterile centrifuge bottles.

f) Wash spores three times in sterile DI water by centrifugation at 4,000 rpm for 30 minutes.

g) Add 10 to 15 mL of sterile DI water to each tube and vortex to resuspend spores.

h) Heat the stock culture suspension at 167 to 185 °F (75 to 85 °C) for 20 minutes.

i) Sonicate the spore suspension for 10 minutes at 60% intensity.

j) Wash spores three times in sterile DI water by centrifugation at 4,000 rpm for 30 minutes. Resuspend in sterile DI water and add sterile glass beads after final wash.

k) Determine spore concentration by standard dilution-plate methods using PBS and TSA.

l) Incubate the plates for 44 to 48 hours at 97 ± 2 °F (36.1 ± 1 °C).

m) Dilute the spore suspension with PBS to obtain a final spore concentration of 5 × 10^8 to 8 × 10^8/mL.

n) Store the stock spore suspension at 39 °F (4 °C) or divide into aliquots to store in sterile screw-cap 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.

N-1.6.3 Personnel protection test (system challenged with 1 × 10^8 to 8 × 10^8 B. subtilis spores in 5 minutes).

N-1.6.3.1 Method

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

b) A nebulizer containing up to 55 mL of spore suspension (5 × 10^8 to 8 × 10^8/mL) shall be centered between sidewalls of the cabinet. The horizontal spray axis shall be placed 14 inches (360 mm) above the work surface; the opening of the nebulizer shall be 4 inches (100 mm) behind the sash. The spray axis shall be parallel to the work surface and directed toward the sash (see Figure 15).

c) The cylinder shall be placed at the cabinet center. The axis of the cylinder shall be 2.75 inches (70 mm) above the work surface. Around the cylinder, 4 AGI-30s shall be positioned with the sampling inlets 2.5 inches (64 mm) outside the cabinet front. Two AGI-30s shall be placed so that their inlet axes are 6 inches (150 mm) 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 inches (51 mm) apart and lie in a horizontal plane 1 inch (25 mm) 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 inch (13 mm) above or below the front intake grill, to minimize the obstruction of airflow into the grill (see Figures 16 and 17).

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 inches (150 mm) in front of the cabinet and 8 inches (200 mm) from each interior sidewall. When the nominal width of the test cabinet is less than 3 feet, 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 inches (150 mm) in front of the cabinet and 2 inches (51 mm) from each interior sidewall. Two AGI-30 samplers shall be placed so that the horizontal plane of the air inlets is 14 inches (360 mm) above the work surface, the vertical axes are 2 inches (51 mm) outside the front edge of the cabinet, and there are 6 inches (150 mm) on each side of the cabinet centerline (see Figure 17).

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

<table>
<thead>
<tr>
<th>Time remaining (min)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>start slit samplers</td>
</tr>
<tr>
<td>25</td>
<td>start nebulizer</td>
</tr>
<tr>
<td>24</td>
<td>start impingers</td>
</tr>
<tr>
<td>19</td>
<td>stop impingers</td>
</tr>
<tr>
<td>18.5</td>
<td>stop nebulizer</td>
</tr>
<tr>
<td>0</td>
<td>stop slit samplers</td>
</tr>
</tbody>
</table>

Three replicate tests shall be performed.

f) Filter the sampling fluid from all of the AGI-30 samplers through a 47 mm diameter 0.2 to 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 97 ± 2 °F (36.1 ± 1 °C). Read plates at 44 to 48 hours of incubation. If plates are overgrown with a contaminant other than the challenge organism, the test shall be considered invalid and retested.

g) For new and major modification redesign cabinet models, repeat the above steps after setting the cabinet airflow velocities at -10 ± 3 ft/min (-0.051 ± 0.015 m/s) inflow using a direct airflow reading instrument and 10 ± 3 ft/min (0.051 ± 0.015 m/s) downflow above and below the 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; and
— 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 ft/min (-0.051 ± 0.015 m/s) from the nominal set point for both downflow and inflow, except as noted below.

When an inflow alarm is present on a Type A1, A2, or C1 BSC, the appropriate cabinet blower(s) speed shall be reduced (from nominal set point) without damper adjustment (if one is present), until the inflow alarm is activated:

— if the manufacturer-specified inflow velocity alarm set point is more than 10 ft/min (0.051 m/s) less than the nominal inflow velocity, the test as specified in this section will be performed with the inflow velocity at this alarm set point ± 3.0 ft/min (0.015 m/s); or

35 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.
— if the manufacturer-specified inflow velocity alarm set point is no more than 10 ft/min (0.051 m/s) less than the nominal inflow velocity, the inflow alarm point shall be tested as specified in this section.

N-1.6.3.2 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 minute 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*.

The standard recognizes that factors outside of the control of the manufacturer may impact the results of this test. These factors can include plate or impinger handling errors, air currents in the test lab, lab contamination with the test organism, or problems with the test equipment. It is not always possible for the test agency to find these factors following a failing test. When the results of a test exceed the maximum allowed recovery for *B. subtilis*, a confirming test may be completed. When the maximum allowed recovery for *B. subtilis* was from a single replicate, the replicate may be replaced with two passing replicates. When the maximum allowed recovery for *B. subtilis* was from two or three replicates, the test may be replaced with a full test that includes three passing replicates.

N-1.6.4 Product protection test (system challenged by $1 \times 10^6$ to $8 \times 10^6$ *B. subtilis* spores in 5 minutes.)

N-1.6.4.1 Method

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

b) Cover the work surface with open agar plates 100 × 15 mm with the cylinder at the midpoint (see Figure 18).

c) Position the horizontal spray axis of the nebulizer containing 55 mL of $5 \times 10^6$ to $8 \times 10^6$ 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 inches (100 mm) 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 inches (64 mm) 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 inches (150 mm) into the room through the front opening of the cabinet, and the axis of the cylinder is 2.75 inches (70 mm) 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 inches (13 mm) above or below the front intake grill to minimize the obstruction of airflow into the grill (see Figure 19).

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

g) The plates shall be incubated at 97 ± 2 °F (36.1 ± 1 °C) and read at 44 to 48 hours. If plates are overgrown with a contaminant other than the challenge organism, the test shall be considered invalid and retested.

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 ft/min (0.051 ± 0.015 m/s) inflow using a direct airflow reading instrument and -10 ± 3 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; and
— removable equipment not essential to cabinet operation shall be removed to set the downflow velocity.

N-1.6.4.2 Acceptance

The number of \textit{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 \textit{B. subtilis}.

The standard recognizes that factors outside of the control of the manufacturer may impact the results of this test. These factors can include plate handling errors, air currents in the test lab, lab contamination with the test organism, or problems with the test equipment. It is not always possible for the test agency to find these factors following a failing test. When the results of a test exceed the maximum allowed recovery for \textit{B. subtilis}, a confirming test may be completed. When the maximum allowed recovery for \textit{B. subtilis} was from a single replicate, the replicate may be replaced with two passing replicates. When the maximum allowed recovery for \textit{B. subtilis} was from two or three replicates, the test may be replaced with a full test that includes three passing replicates.

N-1.6.5 Cross-contamination test (system challenged by $1 \times 10^4$ to $8 \times 10^4$ \textit{B. subtilis} spores for 5 minutes.)

N-1.6.5.1 Method

N-1.6.5.1.1 Set the cabinet at the nominal set point airflow velocities. Tests are completed from one side wall and the center or from both side walls, depending on BSC nominal width. The center test is completed on cabinets with a BSC nominal width greater than 3 feet. Both side walls are tested on cabinets with a BSC nominal width of 3 feet or less.

N-1.6.5.1.2 For cabinets with a BSC nominal width greater than 3 feet, determine the worst-case side wall using a visible medium (i.e., cold smoke). At the side being tested, position the smoke source on front-to-back centerline of the work surface at the side wall, 6 inches (150 mm) above the work surface, with the smoke directed downward. Slowly move the smoke source towards the geometric center of the work surface. Note the point at which approximately half of the smoke is not directly recaptured at the side wall. Compare this point to the one obtained from the other side wall. The side with the shorter distance from the sidewall to its respective point is deemed the worst case. For cabinets with a BSC nominal width of 3 feet or less, start against the left side wall.

N-1.6.5.1.3 Side wall test

a) Position the horizontal spray axis of the nebulizer containing 55 mL of $5 \times 10^4$ to $8 \times 10^4$ spores/mL 3 to 5 inches (76 to 130 mm) above the work surface, with the back of the nebulizer located against the midpoint of the interior side wall selected in Section N-1.6.5.1.2. The spray axis shall be parallel to the work surface and directed toward the opposite sidewall.

b) Place open agar settling plates (100 × 15 mm) on the work surface in the following manner (see Figure 20):

— 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 inches (360 mm) from the side wall being tested; and
— at least one more row of plates nested beyond the 14 inches (360 mm) row; two rows when there is room.
c) Start the nebulizer. After 5 minutes, stop the nebulizer.

d) After 5 minutes, place the covers on the open agar plates. Incubate the plates at 97 ± 2 °F (36.1 ± 1 °C) and read at 44 to 48 hours. If plates are overgrown with a contaminant other than the challenge organism, the test shall be considered invalid and retested.

e) For cabinets with a BSC nominal width of 3 feet or less, perform the same procedure (steps a to e), but place the nebulizer against the midpoint of the right interior wall.

N-1.6.5.1.4 Center test

a) The center test is completed only on cabinets with a BSC nominal width of greater than 3 feet. Reposition the nebulizer used in Section N-1.6.5.1.3 such that the axis of the reservoir is positioned over the geometric center of the work surface with the nebulizer facing the left side wall. The center of the nebulizer barrel shall be positioned at the same height as the top of the cabinet access opening. Either start with fresh suspension or top off the nebulizer used in Section N-1.6.5.1.3. Top off by adding approximately 5 mL of additional suspension and uniformly mix the suspension in the reservoir. After moving and topping off the nebulizer, perform a thorough surface decontamination of the entire work surface and side wall used for the side wall test. The axis of a 2.5 inch (63 mm) outside diameter cylinder, with closed ends, shall be centered side to side in the work area with the axis of the cylinder 2.75 inches (70 mm) above the work surface. One end shall butt against the back wall of the work area and the other end shall extend at least 6 inches (150 mm) into the room through the front opening of the cabinet.

b) Place open agar settling plates (100 × 15 mm) on the work surface in rows. Center one row under the nebulizer along the cabinet front to rear center line. Place two rows to the left side of the center row of plates. The stand for the nebulizer may interfere with plates in the middle. It is acceptable to leave plates out in the middle where this happens since these are control plates used to demonstrate recovery only. If the manufacturer or test agency is aware that adequate control recovery cannot be demonstrated from these three rows of plates alone, additional plates may be added, as instructed by the manufacturer. Placement of additional positive control plates shall be limited to the area directly above the three rows of control plates and the area under the front intake grille near the center of the cabinet (similar to personnel and product protection control plate placement). Apparatus used to suspend plates higher within this zone shall be installed in a manner that minimizes any disturbances to airflow. Place a row of plates with the edge of the plates 14 inches (360 mm) from the cabinet center line. Place additional rows of plates behind these, as cabinet size will allow, up to a maximum of four rows total. When the size of the cabinet does not allow for four rows on each side, place as many rows as will fit. Each row of plates shall be centered from front to rear on the work surface. Rows of plates shall touch each other but not be nested, as they are for the side wall cross contamination test.

c) Start the nebulizer. After 5 minutes, stop the nebulizer.

d) After 5 minutes, place the covers on the open agar plates. Incubate the plates at 97 ± 2 °F (36.1 ± 1 °C) and examine them at 44 to 48 hours.

e) Three replicate tests shall be completed.

f) Repeat steps a through e but with the nebulizer facing the right sidewall of the cabinet and plates positioned on the right side of the cabinet. After repositioning, top off the nebulizer as in step a, and then perform a thorough surface decontamination of the entire work surface before placing any fresh plates.
N-1.6.5.2 Acceptance

N-1.6.5.2.1 Side wall test

Some agar plates, from the challenge sidewall to 14 inches (360 mm) 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 inches (360 mm) shall not exceed 2 CFU per test.

The standard recognizes that factors outside of the control of the manufacturer may impact the results of this test. These factors can include plate handling errors, air currents in the test lab, lab contamination with the test organism, or problems with the test equipment. It is not always possible for the test agency to find these factors following a failing test. When the results of a test exceed the maximum allowed recovery for *B. subtilis*, a confirming test may be completed. When the maximum allowed recovery for *B. subtilis* was from a single replicate, the replicate may be replaced with two passing replicates with the nebulizer positioned on the same side of the cabinet where the failure occurred. When the maximum allowed recovery for *B. subtilis* was from two or three replicates on the same side of the cabinet, the test may be replaced with three additional replicates completed from that side of the cabinet.

N-1.6.5.2.2 Center test

Some agar plates, from the three rows positioned under the nebulizer, will recover *B. subtilis* CFU and shall be used as positive controls. The total number of CFU recovered on agar plates greater than 14 inches (360 mm) from the cabinet center line shall not exceed 5 CFU per test.

The standard recognizes that factors outside of the control of the manufacturer may impact the results of this test. These factors can include plate handling errors, air currents in the test lab, lab contamination with the test organism, or problems with the test equipment. It is not always possible for the test agency to find these factors following a failing test. When the results of a test exceed the maximum allowed recovery for *B. subtilis*, a confirming test may be completed. When the maximum allowed recovery for *B. subtilis* was from a single replicate, the replicate may be replaced with two passing replicates with the nebulizer facing the same side of the cabinet where the failure occurred. When the maximum allowed recovery for *B. subtilis* was from two or three replicates facing the same side of the cabinet, the test may be replaced with three additional replicates completed facing that side of the cabinet.
positive control plates

pass / fail plates

nebulizer

14 inches (360 mm)

cylinder

Figure 10
N-1.7 Stability tests

N-1.7.1 Purpose

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

- resistance to overturning under applied forces (refer to UL 61010-117 or current edition) cited in Section 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.

N-1.7.2 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 pounds (114 kg) load applied to the leading front edge of the cabinet;
  - 250 pounds (1110 N) force uniformly distributed over an area of 10 × 10 inches (250 × 250 mm);
  - 50 pounds (23 kg) load uniformly distributed over an area 10 × 10 inches (250 × 250 mm); and
  - dial indicator with a minimum accuracy of 0.001 inches (0.02 mm).

N-1.7.3 Resistance to overturning

N-1.7.3.1 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.

N-1.7.3.2 Acceptance

The cabinet shall not initiate overturn when tilted 10° from horizontal in the direction most likely to cause overturn.
N-1.7.4  Resistance to distortion under applied forces

N-1.7.4.1  Method

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

b) Apply a force of 250 pounds (1,110 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 Figures 21 and 22, respectively).

Report the deflection.

N-1.7.4.2  Acceptance

The top front edge and the top of the sides shall not move forward more than 0.062 inch (1.6 mm) from a static position when a 250 pounds (1,110 N) lateral force is applied to the top rear edge and top of the opposite side, respectively.

N-1.7.5  Resistance to deflection of work surface under load

N-1.7.5.1  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 23. The stand shall be positioned on the floor in front of the cabinet.

b) Zero the dial indicator and place the 50 pounds (23 kg) test load at the center of the work tray, distributed over an area 10 × 10 inch (250 × 250 mm). Remove the test load and record the distortion measured by the dial indicator (see Figure 23).

N-1.7.5.2  Acceptance

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

N-1.7.6  Resistance to tipping under load (applicable only to freestanding devices with work surfaces)

N-1.7.6.1  Method

Place the 250 pounds (114 kg) test load centered from right to left of the work area on the leading edge of the cabinet (see Figure 24).

N-1.7.6.2  Acceptance

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

N-1.8  Downflow velocity

N-1.8.1  Purpose

This test measures the velocity of air moving through the cabinet work space 4 inches (100 mm) above the bottom edge of the sash, and is performed on all cabinets accepted under Section N-1.6. 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).
N-1.8.2 Apparatus

A thermal anemometer with an accuracy of ± 3 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.

N-1.8.3 Method: Setting nominal set point

The removable equipment nonessential 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).

N-1.8.3.1 Downflow velocity

Measure the air velocity at multiple points across the workspace or zones defined by the manufacturer in the horizontal plane defined 4 inches (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 inches (100 mm), nor more than 8 inches (200 mm) and containing a minimum of three rows or as defined by the zone(s);
- for cabinets with a nominal width of 3 feet (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 feet (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 inches (150 mm) away from the walls and sash enclosing the work area (see Figure 25). 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 inches (150 mm) away from the walls and sash. However, if the grid spacing of 6 inches (150 mm) from the walls and sash results in not being able to meet the above grid requirements of not less than 4 inches (100 mm) with a minimum of four readings per row or zone, then equidistant spacing shall be used with the minimum of four 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.
N-1.8.4 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 inches (100 mm) above the bottom of the sash of cabinets meeting the requirements of Section N-1.6 shall be the values specified by the manufacturer. Subsequent production cabinets of the initial model and size conforming to Section N-1.6 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 Section N-1.8.3. 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 Section N-1.8.3, when the downflow is specified as nonuniform downflow (zoned) by the manufacturer.

N-1.9 Inflow velocity (face velocity) test

N-1.9.1 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.

N-1.9.2 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 ft³/min (± 12 m³/h) or another acceptable source or in accordance with Annex N-2;
- a thermal anemometer with an accuracy of ± 3 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.³

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).

N-1.9.3 Methods

N-1.9.3.1 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 Section N-1.9.4.
N-1.9.3.2 Direct inflow measurement method (primary method)

a) Seal by taping the device to the center of the front opening of a BSC. 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 nonback 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 ft³/min (m³/s) by the work access opening area in ft² (m²).

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 feet (meters).

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 foot (0.3 m) of work area width, and the methods used to determine them.

N-1.9.3.3 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 ASHRAE standards for air velocity measurements in round or rectangular ducts or with the Industrial Ventilation Manual.³

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.³

d) Include exhaust volume rate in ft³/min (m³/s) and exhaust static pressure in inches water gauge (Pascal) in the reported data.

N-1.9.3.4 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:

N-1.9.3.4.1 Method for Type A1 and A2, and C1 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 × 4 inches (100 × 100 mm), with the grid starting points and height above the filter validated by the testing organization (see Figure 26). A clear 12 inches (300 mm) 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 nonuniform, 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 ft³/min (m³/s), multiply the average exhaust air velocity in ft/min (m/s) by the exhaust area in ft² (m²).

d) Calculate the average inflow velocity in ft/min (m/s) by dividing the average exhaust volume rate in ft³/min (m³/s) by the work access opening area in ft² (m²).

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.

N-1.9.3.4.2 Method for Type A1, A2, B1, B2 and C1 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 foot (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.

N-1.9.3.4.3 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 at 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 Figure 27).

d) Take the indicated velocity measurements every 4 inches (100 mm) across the width of the front work access opening but no closer than 4 inches (100 mm) 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.

N-1.9.3.4.4 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 × 4 inches (100 × 100 mm) grid in a horizontal plane 6 inches (150 mm) below the face of the downflow diffuser, starting 2 inches (50 mm) 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 Figure 28). Average the velocity readings and multiply the average by the area in ft² (m²) of the plane in which the velocities were measured to determine the total filtered air supply in ft³/min (m³/s).

e) Subtract the supply air volume rate in ft³/min (m³/s) from the total exhaust volume rate in ft³/min (m³/s); the difference represents the calculated inflow volume rate in ft³/min (m³/s).

f) Divide the calculated inflow volume rate by the area of the access opening in ft² (m²) 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.

N-1.9.4 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 Section N-1.6 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 ft³/min (76 m³/h) per 1 foot (0.3 m) of work area width (see Sections N-1.6 and N-1.8).

The minimum inflow volume of Type A2, B1, and B2 cabinets shall be 100 ft/min (0.51 m/s). The minimum inflow volume shall be 65 ft³/min (110 m³/h) per 1 foot (0.3 m) of work area width (see Sections N-1.6 and N-1.8).

N-1.10 Airflow smoke patterns test

N-1.10.1 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.

N-1.10.2 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.

N-1.10.3 Method

N-1.10.3.1 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 inches (100 mm) above the top of the access opening.
N-1.10.3.2 Sash retention test

Smoke shall be passed from one end of the cabinet to the other, 1 inch (25 mm) behind the sash, at a height 6 inches (150 mm) above the top of the access opening.

N-1.10.3.3 Work opening edge retention test

Smoke shall be passed along the entire perimeter of the work opening edges, approximately 1.5 inches (40 mm) outside the cabinet. Particular attention should be paid to corners and vertical edges.

N-1.10.3.4 Sash seal test

Smoke shall be passed up the inside of the sash 2 inches (50 mm) from the sides and along the top of the work area, 1 inch (25 mm) behind the sash, starting and ending 6 inches (150 mm) above the bottom edge of the sash.

N-1.10.4 Acceptance

N-1.10.4.1 Downflow test

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

N-1.10.4.2 View screen retention test

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

N-1.10.4.3 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.

N-1.10.4.4 Sash seal test

There shall be no escape of smoke from the cabinet.

N-1.11 Drain spillage trough leakage test

N-1.11.1 Purpose

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

N-1.11.2 Method

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

N-1.11.3 Acceptance

The drain spillage trough shall hold a minimum of 1 gallon (4 L) of water and have no visible leakage after a 1 hour holding period.
N-1.12  Motor / blower performance

N-1.12.1  Purpose

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

N-1.12.2  Apparatus

Instrumentation required in Sections N-1.9 and N-1.10 shall be used. A manometer with an accuracy of at least ± 2% of reading ± 0.001 in w.g. (0.2 Pa) shall be used.

N-1.12.3  Method

a) Set the cabinet at the nominal set point, ± 3 ft/min (± 0.015 m/s).

b) Measure the total airflow volume rate, ft³/min (m³/s), and determine that the cabinet blower is delivering at the nominal set point (see Sections N-1.8 and N-1.9). The cabinet supply air volume shall be determined as in Section N-1.9.3.4.4.b.

c) Locate the testing organization approved positive and negative pressure taps. The manufacturer shall locate the positive pressure tap (see Figure 29) 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 Figure 29). 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 Figure 30). 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 Figure 31). 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, or 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 (ft³/min [m³/s]) the restricted cabinet blower is delivering (see Sections N-1.9 and N-1.9.3.4.4.d).

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

N-1.12.4  Acceptance

The total airflow volume rate, ft³/min (m³/s), shall not decrease more than 10% meeting the requirements of Section 6.13.

36 Manufacturer to supply positive and negative pressure taps (see Figure 31) on units submitted for laboratory cabinet design certification.
N-1.13  Cabinet airflow stability

N-1.13.1  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.

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 six 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.

N-1.13.2  Apparatus

— instrumentation required in Sections N-1.9 and N-1.10 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; and

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

N-1.13.3  Method

N-1.13.3.1  Shock stability

a) Measure the inflow velocity for Type A1 and A2 cabinets. Measure a minimum of six 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 two 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 centimeter 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 °F (2 °C) during the test.

N-1.13.3.2 Input voltage stability

a) Measure the inflow velocity for Type A1 and A2 cabinets. Measure a minimum of six 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 two 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% ± 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 % ± 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 °F (2 °C) during the test.

N-1.13.3.3 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 six 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 two 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 °F (2 °C) during the test.
N-1.13.4  Acceptance

N-1.13.4.1  Shock stability

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

N-1.13.4.2  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 ft/min (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 ft/min (0.025 m/s).

N-1.13.4.3  Power failure stability

The difference between the initial inflow velocity and the final inflow velocity shall not exceed 3 ft/min (0.015 m/s). The difference between the initial downflow velocity and the final downflow velocity shall not exceed 3 ft/min (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.

N-1.14  Canopy connection test

N-1.14.1  Purpose

This test demonstrates the ability of a Type A1, A2 or C1 BSC to maintain inflow velocity during a facility exhaust system failure.

N-1.14.2  Method

a) Connect the BSC to a facility exhaust system via the BSC manufacturer’s canopy connection.

b) Adjust facility exhaust flow according to the BSC manufacturer’s instructions and balance the cabinet inflow and downflow velocities within 3 ft/min (0.015 m/s) of the manufacturer’s nominal set points.

c) Follow the BSC / canopy connection manufacturer’s instructions to calibrate the canopy alarm if needed.

d) Reduce facility exhaust flow until a loss of capture of room air at the canopy intake slots is verified using a visible medium.

e) Measure the amount of time from loss of capture to canopy alarm activation.

f) Restore facility exhaust flow to the previous setting.

g) Measure the inflow velocity using a DIM.

h) Turn off the facility exhaust fan. Do not close any valves in the facility exhaust ductwork.

i) Wait 15 seconds after the canopy exhaust alarm is activated and then measure the inflow velocity again, using a DIM instrument.
N-1.14.3  Acceptance

N-1.14.4  The canopy alarm shall activate within 15 seconds of loss of capture of the visible medium.

N-1.14.5  Inflow velocity shall not be reduced by more than 10 ft/min (0.051 m/s) after turning the facility exhaust off.
3/8 inch (9.5 mm) OD × 0.065 inch (1.7 mm) wall brass tubing

5/8 inch (15.9) OD brass collar silver to tubing

0.04 inch (1 mm) diameter radical holes 90° (1.6 rad) apart; top edge of holes just touching bottom of collar (four required)

approximately 0.5 inch (12.7 mm) above bottom of can

0.08 inch (2 mm) diameter longitudinal holes next to tube in line with radial holes (four required)

length variable to suit installation

5/32 inch (~ 3.9 mm)

3/8 inch (~ 9.5 mm)

3/16 inch (~ 4.8 mm)

brass plug – silver braze in place (full penetration)

Figure 11
Detail of Laskin nozzle
Figure 12
Noise level test
12 inches (300 mm) maximum between test points
6 inches (150 mm) from each side of the wall
all readings taken on the front-to-rear centerline of the work surface

Figure 13
Light level test
Figure 14
Vibration test
Figure 15
Personnel protection test

nebulizer (all dimensions refer to the center of the discharge)

4 inches (100 mm) to inside surface of sash

14 inches (360 mm) to work surface
Figure 16
Personnel protection test

- nebulizer (all dimensions refer to the center of the discharge)
- 6 inches minimum (150 mm)
- 4 inches (100 mm)
- 2.5 inch (64 mm) O.D. Stainless steel cylinder sealed at both ends
- 2.75 inches (70 mm)
- agar plate on support
- alternate agar plate position
Figure 17
Personnel protection test

- nebulizer level with top of cylinder
- slit sampler
- all glass impinger (AGI)
- 1 inch (30 mm)
- 8 inches (200 mm)
- 14 inches (360 mm) above work surface
- 2 inches (50 mm)
- 6 inches (150 mm)
- 12 inches (300 mm)
Figure 18
Product protection test
Figure 19
Product protection test

- nebulizer
- 4 inches (100 mm)
- alternate agar plate position
- 2.75 inches (70 mm)
- agar plate on support
- 6 inches minimum (150 mm)
Figure 20
Cross contamination protection test

discharge 3 to 5 inches (80 to 130 mm) above work surface

14 inches (360 mm)
Figure 21
Resistance to distortion test

dial micrometer, placed at the center of the top

apply 250 pounds (1110 N) force to top center of back

tie down, securing chassis to floor
dial micrometer, placed in the center of the side

apply 250 pounds (1110 N) force to top center of side

tie down, securing chassis to floor

Figure 22
Resistance to distortion test
measured at center front edge of work surface, there is no permanent distortion after the test load is removed.

50 pounds (23 kg) test load, at geometric center of work surface

Figure 23
Resistance to deflection test
Figure 24
Resistance to tipping test

250 pounds (114 kg) load applied to center of leading edge

0.0625 inch (2 mm) maximum deflection
grid or zone spacing shall be not less than 4 inches (100 mm), nor more than 8 inches (200 mm) apart

Figure 25
Velocity profile test
Figure 26
Calculated inflow velocity profile test for Type A cabinets

- 4 inch (100 mm) boundary on the front, rear, and both sides of the filter frame
- 4 inch (100 mm) × 4 inch test grid
- 4 inches (100 mm) above the test grid to the filter face
4 inch (100 mm) minimum boundary on both sides, no more than 4 inches from point to point

25% of sash height
75% of sash height

Figure 27
Another example of inflow velocity test, Type B1
Figure 28
Supply air volume test

- 4 inch (100 mm) × 4 inch test grid
- 2 inch (50 mm) boundary on the front, rear, and both sides
- 6 inches (150 mm) from the bottom of the diffuser
Figure 29
Positive pressure tap placement – Motor or blower performance test

flexible style plenum

rigid style plenum

backward curved fan style plenum

console style plenum

+ = appropriate pressure tap location
Ø = inappropriate pressure tap location
maximum distance between the plane of the blower inlet and the end of the probe equal to \( \frac{1}{2} \) of the blower inlet diameter

maximum distance between the plane of the impeller inlet and the end of the probe equal to \( \frac{1}{2} \) of the blower inlet diameter

forward curved blower

backward curved impeller

Figure 30
Negative pressure tap placement – Motor or blower performance test
Figure 31
Typical pressure taps

- 2 inches (50 mm)
- 1.25 inches (32 mm)
- 0.75 inch (19 mm)
- 0.040 inch (1 mm) diameter thru holes typical 2 places
- 0.25 inch (6.4 mm) outside diameter tube
- \(\frac{1}{8}\) inch NPT × 0.25 inch (6.4 mm) tube connector, soldered to seal
- 4 inches (100 mm)
- 0.187 inch (4.8 mm) outside diameter tube barb
Normative Annex 2
(formerly Annex B)

N-2.1 Method to verify fitness for use of potential direct inflow measurement devices

N-2.1.1 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.

N-2.1.2 Install the device using one of the two following methods:

Method 1 (Figure 32)

a) Seal the device to the front opening of a Class II Type B2 BSC 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 BSC intended to be canopy-connected.

b) Seal a calibrated, NIST traceable flow hood, such as Shortridge 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 × 14 inches [360 × 360 mm] for the Shortridge unit).

N-2.1.3 Using the condition of Section N-2.1.2, Method 1, measure the exhaust flow, nonback 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%.

N-2.1.4 Using the configuration of Section N-2.1.2, Method 2, measure the exhaust flow, nonback 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%.

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37 Shortridge Instruments. 7855 E Redfield Road, Scottsdale, AZ 85260. <www.shortridge.com>
N-2.1.5 The calibration is valid for cabinets of the size used and smaller. It is recommended that 6 feet (2 m) cabinets be used in this procedure.

Figure 32
Verifying fitness for use of potential direct inflow measurement devices
Nebulizer selection and calibration

N-2.1 Selection

N-2.1.1 Criteria

Nebulizers are acceptable when they:

— deliver $1 \times 10^8$ to $8 \times 10^8$ airborne spores of *Bacillus subtilis* var. *niger* (*B. subtilis*) in 5 min;

— deliver 94% ± 6% single cell spores;

— have a spore aerosol discharge velocity of 100 ± 10 ft/min (0.51 ± 0.05 m/s);

NOTE — Tests performed by First\(^{38}\) et al., demonstrated that a stainless steel six-jet collision refluxing nebulizer will deliver the bacterial spore aerosol required in Section 6.7.1 when the following conditions are met.

— the nebulizer is equipped with a glass flask approximately 2 inches (50 mm) in diameter and 3.5 inches (90 mm) high and with a 0.90 inch (23 mm), ID horizontal discharge spout on top;

— the nebulizer is operated at 20 psi (140 kPa);

— 55 mL of a $5 \times 10^8$ to $8 \times 10^9$/mL spore suspension is placed in the flask;

— the bottom of the six-jet spray head is 0.71 inch (18 mm) 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 — The six-jet collision refluxing nebulizer need not be retested for performance before use.

N-2.2 Calibration

N-2.2.1 Purpose

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

N-2.2.2 Site

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

N-2.2.3 Frequency

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

N-2.2.4 Materials

— suspension of $5 \times 10^8$ to $8 \times 10^8$ B. subtilis spores per mL;

— nebulizer to be calibrated;

— one all-glass ACI-30 (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.

N-2.2.5 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 (0.51 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 33.

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 L/min at a pressure drop of 8 psig (55 kPa) or greater (ACE Glass No. 7541 impinger) and (2) an impinger with the tip above the liquid surface and passing 12.5 L/min at a pressure drop of 8 psig (55 kPa) or greater, known as AGI-30, (ACE Glass No. 7540 impinger). Either impinger may be used. When the air delivery rate of the nebulizer is not precisely 6 or 12.5 L/min, 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. Two impingers may be used if needed to be certain that impinger flow equals or exceeds nebulizer flow.

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/min (0.51 m/s) output velocity based on airflow (12.5 L/min for the six-jet collision described in this Annex) and diameter of the discharge spout and the impinger sampler (operating according to manufacturer's instructions). Operate nebulizer for 5 minutes (using the switching timer) and the impinger sample for 5.25 minutes.

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.

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.

N-2.2.6 Calculations

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

b) velocity of air leaving nebulizer = the air volume measured in C.2.5.b in ft³/min (m³/s) divided by nebulizer outlet area in ft²; and

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

N-2.2.7 Acceptance

— the average of five replicate calibration tests shall fall between $1 \times 10^8$ and $8 \times 10^8$ spores per 5 minutes nebulizer operation; and

— the velocity of air leaving the nebulizer shall be $100 \pm 10$ ft/min ($0.51 \pm 0.05$ m/s).
Figure 33
Nebulizer calibration
Normative Annex 4
(formerly Annex D)

Evaluation of chemical resistance and abrasion resistance of surfaces

N-4.1 Chemical resistance

N-4.1.1 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.

N-4.1.2 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 hours, and tests shall be performed so the testing surface is wet throughout the entire test period. After 4 hours, the surface shall withstand scrubbing with a stiff brush and hot water at 160 °F (71 °C). Samples shall be dried before examination. Surface stains of dyes shall be removed with an alcohol wash before examination.

N-4.1.3 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.

N-4.2 Abrasion resistance

N-4.2.1 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 D1044-76 using a CS-IOS wheel, and a 1,000 gram load for 500 cycles.

N-4.2.2 Acceptance

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

Factory testing shall be done according to Annex N-1.

N-5.1 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 BSCs. 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 this Annex, 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 inches (100 mm) 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, field recertification should be performed whenever HEPA/ULPA filters are changed, maintenance repairs are made to internal parts, or a cabinet is relocated.\(^\text{40}\). More frequent field 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.

N-5.1.1 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.

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\(^\text{40}\) 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 field recertification. See Section I-2.1, Recommended biosafety cabinet 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).

N-5.1.2 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 field certification provider:

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

N-5.2 Downflow velocity

N-5.2.1 Purpose

This test measures the velocity of air moving through the cabinet workspace 4 inches (10 cm) above the bottom edge of the sash and shall be performed on all cabinets accepted under Section N-1.6.

N-5.2.2 Apparatus

A thermal anemometer with an accuracy of ± 3 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-01313 if instructions are not provided. When the conditions vary from sea level by more than 1,000 feet (300 m), or the temperature varies from 70 °F (21 °C) by more than 5 °F (2 °C), or both, an appropriate correction for altitude, or temperature, or both, shall be used. The manufacturer's manual for the thermal anemometer or the Industrial Ventilation Manual shall be consulted for the appropriate correction calculation.

N-5.2.3 Method: setting nominal set point

The removable equipment nonessential 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).

N-5.2.3.1 Uniform downflow cabinets

a) The air velocity shall be measured at multiple points across the workspace, using equal points in the horizontal plane 4 inches (100 mm) 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.

N-5.2.3.2 Nonuniform (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 inches (100 mm) 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 (nonuniform (zoned) downflow velocity test).

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

N-5.2.4 Acceptance

N-5.2.4.1 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 (0.081 m/s), whichever is greater, from the average downflow velocity.

N-5.2.4.2 Nonuniform downflow

A cabinet for which the cabinet manufacturer has specified a nonuniform (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 (0.081 m/s), whichever is greater, from the average downflow velocity of each zone.

N-5.3 Inflow velocity (face velocity) test

N-5.3.1 Purpose

This test determines the measured and calculated inflow velocity through the work access opening.
N-5.3.2 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 ft³/min
  (± 12 m³/h) or in accordance with Annex N-2;

— a thermal anemometer with an accuracy of ± 3 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*;³
  and

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

N-5.3.3 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.

N-5.3.3.1 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.

N-5.3.3.2 Direct inflow measurement method

a) Seal by taping the device to the center of the front opening of a BSC. 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 nonback 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
  ft³/min (m³/s) by the work access opening area in ft² (m²).

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
Alternate inflow measurement methods

If the DIM method cannot be used, one of the alternative methods below may be used to determine the inflow velocity, if provided by the manufacturer.

Alternate inflow measurement methods shall only be used for any or all of the following reasons:

- the space between the face of the biosafety cabinet and permanent fixture directly opposite the access opening is less than 42 inches (1.1 m);
- the BSC was certified by the testing organization prior to NSF/ANSI 49-2002, when the DIM method for measuring inflow velocity was added to the standard;
- testing is completed on a biosafety cabinet not located in North America; and
- the owner/operator of the BSC requests use of a secondary method due to DIM instrument cleanability when the BSC is located in sterile area or clean room.

The DIM shall be used in all other circumstances.

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

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 inches (300 mm) 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 nonuniform, 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 ft³/min (m³/s), multiply the average exhaust air velocity in ft/min (m/s) by the effective exhaust area in ft² (m²).

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 ft³/min (m³/s) by the work access opening area in ft² (m²).

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).

N-5.3.3.3.2 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 foot (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).

N-5.3.3.3.3 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).

N-5.3.3.3.4 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.3

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 N-1, Figure 30). Average the velocity readings and multiply by the area in ft² (m²) of the plane in which the velocities were measured to determine the total filtered supply air volume flow rate in ft³/min (m³/s).

e) Subtract the supply air volume rate in ft³/min (m³/s) from the total exhaust volume rate in ft³/min (m³/s); the difference represents the calculated inflow volume rate in ft³/min (m³/s).

f) Divide the calculated inflow volume rate by the area of the access opening in ft² (m²) 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).

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

N-5.3.4 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.
N-5.4 Airflow smoke patterns test

N-5.4.1 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.

N-5.4.2 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.

N-5.4.3 Method

N-5.4.3.1 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 inches (100 mm) above the top of the access opening.

b) Reported values shall be:
   — name of test (smoke pattern downflow test); and
   — pass or fail.

N-5.4.3.2 Sash retention test

a) Smoke shall be passed from one end of the cabinet to the other, 1 inch (25 mm) behind the sash, at a height 6 inches (150 mm) above the top of the access opening.

b) Reported values shall be:
   — name of test (sash retention test); and
   — pass or fail.

N-5.4.3.3 Work opening edge retention test

a) Smoke shall be passed along the entire perimeter of the work opening edges, approximately 1.5 inches (40 mm) 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.

N-5.4.3.4 Sash seal test

a) Smoke shall be passed up the inside of the sash 2 inches (50 mm) from the sides and along the top of the work area, 1 inch (25 mm) behind the sash, starting and ending 6 inches (150 mm) above the bottom edge of the sash.
b) Reported values shall be:
- name of test (sash seal test); and
- pass or fail.

N-5.4.4  Acceptance

N-5.4.4.1  Downflow test

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

N-5.4.4.2  View screen retention test

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

N-5.4.4.3  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.

N-5.4.4.4  Sash seal test

There shall be no escape of smoke from the cabinet.

N-5.5  HEPA/ULPA filter leak test

N-5.5.1  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.

N-5.5.2  Apparatus

N-5.5.2.1  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], sebacate, polyethylene glycol, and medicinal-grade light mineral oil) produced by the generator described in Section N-1.3.2.2 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 ft³/min (28 L/min) ± 10%. Probe area shall have a maximum open area of 1.7 in² (1100 mm²) and a minimum dimension of 0.50 inches (13 mm). The photometer shall be set up in accordance with the photometer manufacturer's instructions or IEST-RP-CC-01313 if instructions are not provided.

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**N-5.5.2.2** An aerosol generator of the Laskin Nozzle type conforming to Annex N-1, Figure 12 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 inches (31 mm).

**N-5.5.2.3** 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.

**N-5.5.3 Method of testing HEPA/ULPA filters**

**N-5.5.3.1 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:

   \[ \mu g/L = 13,500 \times \text{number of nozzles} / \text{ft}^3/\text{min of challenged air} \]

   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 (140 kPa).

   — 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 inch (25 mm) 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 (50 mm/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).

N-5.5.3.2 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 inch (8 mm) 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).

N-5.5.4 Acceptance

N-5.5.4.1 Filters that can be scanned

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

N-5.5.4.2 Filters that cannot be scanned

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

N-5.6 Pressure decay / soap bubble

N-5.6.1 Pressure decay / soap bubble test

N-5.6.1.1 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.

N-5.6.1.2 Apparatus

   — manometer, pressure gauge, or pressure transducer system with a minimum range of 0 to 2 inches w.g. (0 to 500 Pa) and accurate to ± 0.02 inch w.g. (± 5 Pa);

   — liquid leak detector;

   — plastic sheet (0.02 inch (0.5 mm) extruded high-impact styrene); and

   — duct tape.
N-5.6.1.3 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.

d) Pressurize the cabinet with air to a reading of 2 inches w.g. (500 Pa), turn off the pressurizing air, and measure the pressure after 30 minutes. A leakage of 10% of the original pressure is allowable. If a cabinet does not hold 2 inches 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).

N-5.6.1.4 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.

d) Pressurize the cabinet with air to ensure a continuous reading of 2 inches 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).

N-5.6.1.5 Acceptance

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

N-5.7 Site installation assessment tests

N-5.7.1 Purpose

These tests are performed to verify that the biosafety cabinet is integrated properly into the facility.
N-5.7.2 Apparatus

— owner’s manual; and
— a visible source of cold smoke such as titanium tetrachloride.

N-5.7.3 Method

N-5.7.3.1 Alarm functions

N-5.7.3.1.1 Sash alarms

When cabinets are equipped with a sliding sash, an alarm shall be activated when the sash is raised or lowered 1 inch (25 mm) above or below the manufacturer’s specified opening height. For cabinets that have been tested and certified to editions of NSF/ANSI 49 earlier than the 2014 edition, alarm activation is only required when the sash is raised 1 inch (25 mm) above the manufacturer’s recommended height.

Sash alarm on cabinets:

— shall be tested at the time of alarm verification;
— raise and lower sliding window above and below the manufacturer’s recommended height. Audible and visual alarm shall indicate window position above or below ± 1 inch (25 mm); and
— reported values shall be:
  — name of test (sash alarm test); and
  — pass or fail.

N-5.7.3.2 Exhaust airflow alarms and interlocks

Whenever an alarm is present to monitor the exhaust airflow from the cabinet connection to the external system, its operation must be verified. Operation of the cabinet’s alarm and interlock, if present, shall be verified at every field certification.

N-5.7.3.2.1 Exhaust alarm system – Type B1 or B2

— shall be tested at time of alarm verification;
— reduce exhaust volume by at least 20% once the cabinet is set or certified in its acceptable air-flow range, and verify that audible and visual alarms indicate a loss of exhaust volume within 15 seconds. The internal cabinet fan(s) shall be interlocked to shut off within 15 seconds of the exhaust volume loss exceeding 20%; and
— reported values shall be:
  — name of test (exhaust alarm test); and
  — pass or fail.

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.
N-5.7.3.2.2  Exhaust alarm system – Type A1 or A2 canopy connection

N-5.7.3.2.2.1 Maintain inflow velocity using canopy connection on Type A1 or Type A2 cabinets:

   a) When a canopy connection is not included as an acceptable option in listing for the BSC being certified, complete the test in step b. When a canopy connection is included as an acceptable option in listing for the BSC being certified, the test in step b is not required.

   b) After cabinet airflow has been tested to verify it is within the acceptable range, deenergize or block the facility exhaust system from the cabinet. Measure inflow velocity of the cabinet as described in Section N-5.3.3.2. The measured velocity shall be no more than 8 ft/min (0.041 m/s) below the lowest value of the inflow velocity range stated on the cabinet data plate.

N-5.7.3.2.2.2 Containment loss of canopy connection on Type A1 or A2 cabinets:

   — shall be tested at time of alarm verification.

   — 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.

   — reported values shall be:

     — name of test (Type A canopy exhaust alarm test); and
     — pass or fail.

Direct connected Type A1 or A2 cabinets shall not be considered in compliance with the Standard.

N-5.7.3.2.3  Exhaust alarm system – Type C1

The canopy connection on a Type C1 BSC that directs its exhaust into the room:

   — shall be tested at the time of alarm verification, and

   — 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 seconds, and the cabinet fan(s) will continue to operate for a maximum of 5 minutes.

The canopy connection on a Type C1 BSC that directs its exhaust into the exhaust duct:

   — shall be tested at the time of alarm verification, and

   — 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 seconds, and the cabinet fan(s) will shut off.

The cabinet blowers may continue to operate, directing exhaust air into the duct system for a maximum of 5 minutes, provided the following test is successfully completed:

   — measure the inflow velocity using a DIM with the cabinet and facility exhaust system operating normally. Turn off the facility exhaust fan. Do not close any control valves in the ductwork. Wait 15 seconds after the cabinet alarm has activated and measure the inflow velocity again, using a DIM.
When Type C1 BSCs are connected to an exhaust system and there is insufficient exhaust volume, the BSCs shall not initiate downflow or exhaust blower startup.

Reported values shall be:

- name of test (Type C exhaust alarm test); and
- pass or fail.

**N-5.7.3.3 Cabinet alarm systems – Type A1, A2, or C1**

When a Type A1, A2, or C1 BSC has an inflow alarm system, its function shall be verified at every certification (see Section N-5.7.3.3.1).

**N-5.7.3.3.1 Airflow alarm system - Type A1, A2, or C1**

The airflow alarm system on Type A1, A2, or C1 cabinets:

- shall be tested at the time of alarm verification;
- using a DIM to measure inflow, reduce velocity to the manufacturer’s designated low alarm point. Audible and visual alarms shall respond within 15 seconds to indicate low airflow alarm; and
- reported values shall be:
  - name of test (airflow alarm test); and
  - pass or fail.

**N-5.7.3.3.2 Internal supply / exhaust fan interlock alarm – Type A1, A2, or C1**

The supply fan interlock alarm on Type A1, A2, or C1 cabinets:

- shall be tested on Type A1, A2, or C1 cabinets;
- interrupt the operation of the cabinets exhaust fan per manufacturer’s instructions. Audible and visual alarm shall respond within 15 seconds as well as the interruption of the cabinet’s supply fan; and
- reported values shall be:
  - name of test (Type A fan interlock / alarm test); and
  - pass or fail.

**N-5.8 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-17 or may refer to NSF 49-1992 for Electrical Leakage, Ground Circuit Resistance, and Polarity tests if necessary.
N-5.9  Lighting intensity test

N-5.9.1  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.

N-5.9.2  Apparatus

A portable photoelectric illuminance meter as described in *The Lighting Handbook*,¹² 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.

N-5.9.3  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 inches (300 mm) starting 6 inches (150 mm) from the sidewalls (Annex N-1, Figure 14).

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).

N-5.9.4  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).

N-5.10  Vibration test

N-5.10.1  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.

N-5.10.2  Apparatus

A vibration analyzer with a minimum reliable reading of $1 \times 10^{-4}$ inches ($2.5 \times 10^{-6}$ m) rms amplitude, or the ability to detect differences of this magnitude, in accordance with manufacturer's instructions. The vibration analyzer shall be capable of measuring vibration in displacement mode within the 10 to 1,000 Hz frequency range.
N-5.10.3 Method

a) Operate the cabinet with lights on within 5 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 N-1, Figure 15.

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).

N-5.10.4 Acceptance

Net displacement shall not exceed 0.002 inch (5 × 10\(^{-5}\) m) rms amplitude at 10 to 1,000 Hz in the center of the work surface(s) when the cabinet is operating at the manufacturer's recommended airflow velocities.

N-5.11 Noise level tests

N-5.11.1 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.

N-5.11.2 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.

N-5.11.3 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 inches (300 mm) in front of the cabinet (leading front edge of the access opening) and 15 inches (380 mm) above the plane of the work surface, in line with the vertical centerline of the cabinet (Annex N-1, Figure 13).

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 step 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).

N-5.11.4 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 Section N-5.11.3.c) 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 following table).

<table>
<thead>
<tr>
<th>Difference between total and background sound readings in dBa</th>
<th>Number to subtract from total to yield corrected noise level</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 2</td>
<td>reduce background levels</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>4 to 5</td>
<td>2</td>
</tr>
<tr>
<td>6 to 10</td>
<td>1</td>
</tr>
<tr>
<td>&gt; 10</td>
<td>0</td>
</tr>
</tbody>
</table>

N-5.12 Record of field certification

A cabinet that has met all the field test criteria listed in This Annex 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:

N-5.12.1 Field certification label

Biosafety cabinets field tested to this Standard shall include the following information:

   — date of field certification;
   — date cabinet should be field recertified: no later than ____________;
   — certifier's report number (reference document showing tests performed and results);
   — name of certifying company, company website, and telephone number. A street address shall be used if a website is not available;
— unit serial number, certifier's report number (reference document showing tests performed and results); and
— signature of the person who performed the field certification tests.

N-5.12.2 Field certification report

A field certification report that will carry the language "certified in accordance with NSF Annex N-5" or any similar language shall, at a minimum, include the following:

— BSC model number;
— BSC serial number;
— BSC location;
— BSC venting information:
  — ducted or not ducted.
— type of connection (canopy, direct or none);
— type of BSC;
— test equipment used for each field test:
  — manufacturer;
  — model;
  — serial number; and
  — calibration date.
— specific field test data as detailed in Annex N-5;
— acceptance criteria for each field test;
— printed name of field certification technician;
— field test date; and
— field retest date.
Informative Annex 1
(formerly Annex E)

Biosafety cabinet selection, installation, use, lifespan, and decommissioning

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 this Standard.

Contents

I-1.1 Institutional safety consultation
I-1.2 Risk assessment procedure
I-1.3 BSC Class and Type selection
I-1.4 Site review before BSC purchase
I-1.5 BSC arrival inspection and field certification
I-1.6 Cleaning and disinfection of BSC work area
I-1.7 BSC use practices and procedures
I-1.8 Moving a permanently installed BSC
I-1.9 BSC lifespan
I-1.10 BSC decommissioning process
I-1.11 Definitions

I-1.1 Institutional safety consultation

A biosafety professional should be consulted prior to a biosafety cabinet (BSC) purchase. Some institutions have BSC purchases approved by the biosafety professional after consultation with the user, architect and engineer. Biosafety 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 BSCs.

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.

I-1.1.1 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.

I-1.2 Risk assessment procedure

I-1.2.1 Risk assessments encompass four main elements:

— hazard identification;
— exposure assessment;
— dose-response assessment; and
— risk characterization, and risk management (job analysis).42

I-1.2.2 Risk assessment team members may include:

- investigator / scientist;
- laboratory staff;
- animal care staff when appropriate;
- animal veterinarian when appropriate;
- plant pathogen, or plant pest containment expert when appropriate; and
- occupational health and biosafety professionals.

I-1.2.3 Risk assessment hazards considered:

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

I-1.2.4 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.

I-1.2.5 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.

I-1.2.6 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.

I-1.2.7 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.
I-1.2.8  Risk management analysis table

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Assessment level</th>
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<tbody>
<tr>
<td></td>
<td>Decrease &lt;</td>
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<tr>
<td><strong>Pathogen disease potential</strong></td>
<td></td>
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<td>known, classified</td>
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<td><strong>Pathogen aerosol potential</strong></td>
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<td>tissue procedure</td>
<td>&lt;&lt;&lt;</td>
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<td>culture procedure</td>
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<td>concentration procedure</td>
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<tr>
<td>animal / nonshedder</td>
<td>&lt;&lt;&lt;</td>
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<tr>
<td>animal / shedder</td>
<td>&gt;&gt;&gt;&gt;</td>
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<tr>
<td><strong>Pathogen infectious route</strong></td>
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<td>respiratory</td>
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<td>&gt;&gt;&gt;</td>
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<td><strong>Disease severity</strong></td>
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<td><strong>Disease prophylaxis</strong></td>
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<tr>
<td><strong>Other factors</strong></td>
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<tr>
<td>livestock pathogen</td>
<td>&gt;&gt;&gt;</td>
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<tr>
<td>poultry pathogen</td>
<td>&gt;&gt;&gt;</td>
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</tbody>
</table>

I-1.2.9  Risk assessment of BSCs exhaust system pressurization in the event of an exhaust system failure

I-1.2.9.1  Introduction

This Section pertains to Types A1, A2, and C1 BSCs connected to an exhaust system via a canopy connection. In the event of an exhaust system failure, these Types of BSCs will positively pressurize the system. This pressurization will be present as long as the BSC continues to operate in an alarm state.
The purpose of this Section is to review:

— the different BSC Types, and their specific reaction to an exhaust system failure;
— the amount of positive pressure that may be encountered;
— the duration of operation of the BSC in an alarm state; and
— the factors that should be considered in performing a risk assessment of these BSCs and their exhaust system.

Because of the unique nature of individual exhaust systems, and the laboratory devices that are connected to them (i.e., BSCs, fume hoods, other ventilated enclosures, and canopies), no definitive answers as to system layout, or which BSC should be used can be given. It is up to the user and their facility’s Safety Officer(s) to understand how these BSCs behave during a system failure, perform an appropriate risk assessment for their system, and for their facility’s building engineers to establish the compatibility of the BSCs for their particular installation.

**I-1.2.10 Background**

ANSI/AIHA Standard Z9.5-2012 states:

> "5.4.2 Exhaust System Ductwork

> 5.4.2.1 Design

> ....Systems and ductwork shall be designed to maintain negative pressure within all portions of the ductwork inside the building when the system is in operation."\(^{2}\)

While this requirement covers the system when in normal operation, nothing is said about the exhaust system or BSC function during a catastrophic failure. When an exhaust system fails, there are going to be risks involved, no matter which Type of BSC is connected to that system, and how the different Types respond to that failure should be understood and evaluated as part of the risk assessment.

**I-1.2.11 Canopy-connected Type A BSCs**

Canopy-connected Type A BSCs are designed to redirect the cabinet’s exhaust back into the laboratory via opening(s) or relief valve(s) that open during an exhaust system failure. Modern canopy design (particularly low profile / high efficiency models) do not exhaust all of the BSC’s air through the connection’s air gap(s) / relief valves; some air flows into the exhaust system, creating pressure in the duct. This pressure should typically be 0.001 to 0.01 inches w.g. at the canopy’s connection to the exhaust system, depending on the canopy design, BSC exhaust volume, and possible obstructions around the canopy’s openings. Type A BSCs must, by NSF requirements, continue to operate, under an audible and visual alarm state, until the exhaust system recovers, or the BSC’s blower(s) is shut off or loses electrical power. The BSC can be started or restarted, indicating an active canopy alarm, only providing particulate containment, directing any gases and vapors back into the laboratory.

Factors to consider in the risk assessment of the use of canopy-connected Type A BSCs in a common or ganged exhaust system include:

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— are the only devices connected to the exhaust system canopy-connected Type A BSCs, or are other ventilated devices (i.e., fume hoods, other ventilated enclosures, and canopy hoods) connected to the system?

— are there a large number of Type A BSCs connected to a single system connected to other types of devices? More BSCs will displace more air into the exhaust system, increasing the risk of backflow into other devices;

— are BSCs allowed to operate continuously, or unattended? If so, this increases the risk of some cabinets to continue to operate unobserved during an exhaust system failure; and

— do all BSC users understand that under no circumstances are the BSCs to be used when a canopy alarm is displayed, and the unit(s) should be turned off until the exhaust system is restored?

I-1.2.12 Canopy-connected Type C1 BSCs

Canopy-connected Type C1 BSCs can be configured to direct its exhaust during an exhaust system failure either:

— back into the laboratory; or
— into the exhaust system.

I-1.2.12.1 When configured to direct its exhaust back into the laboratory

In this configuration, the Type C1 performs similar to a canopy-connected Type A. As in the Type A, BSC operation during an exhaust alarm will only provide particulate containment, directing any gases and vapors back into the laboratory. Unlike the Type A BSCs, the Type C1 must, by NSF requirements, shut off its blowers within a maximum of 5 minutes, under audible and visual alarm state, unless the exhaust system recovers, or the BSC’s blower(s) are shut off. If shut down, the BSC cannot be restarted until the exhaust system recovers.

The risk assessment of Type C1 BSCs in this configuration, is similar to the canopy-connected Type A, except that the Type C1 will automatically shut its blowers off within 5 minutes of an exhaust system failure, and its blowers cannot be started until the exhaust system recovers.

I-1.2.12.2 When configured to direct its exhaust back into the exhaust system

In this configuration, the Type C1 will continue to operate for a programmed interval of 0 to 5 minutes. During this interval, all of the BSC’s exhaust flows into the exhaust system, creating pressure in the duct. This pressure should typically be 0.01 to 0.1 inches w.g. per 100 feet of duct, depending on the BSC, its exhaust volume, and the design of the exhaust system utilizing a sealed and pressure tested duct.

Factors to consider in the risk assessment of the use of Type C1 BSCs configured to direct their exhaust into a common or ganged exhaust system during a failure of that system include:

— are the only devices connected to the exhaust system canopy-connected Type C1 BSCs, or are other ventilated devices (i.e., fume hoods, other ventilated enclosures, and canopy hoods) connected to the system?

— are there a large number of Type C1 BSCs connected to a single system connected to other types of devices? More BSCs will displace more air into the exhaust system, increasing the risk of backflow into other devices;

— is the programmed interval (15 seconds to 5 minutes) the BSCs operates under an exhaust alarm the minimum required for the users to secure any bio- or chemical hazards?
I-1.3  BSC Class and Type selection

I-1.3.1  Selecting the proper BSC should be done in several stages:

a)  Select the proper Class and Type of BSC required, based on the type of protection needed.

b)  Establish, if possible, all of the different tasks to be done within the BSC.

c)  Establish, if possible, all of the different chemicals that may be used in the BSC.

d)  If the BSC is to be connected to an exhaust system, is it compatible with the BSC’s requirements?

e)  If the exhaust system malfunctions, does the user understand its impact on the BSC’s ability to maintain personnel and environmental protection, i.e., containment?

These five stages are reviewed in the questions that follow (Sections I-1.3.1.1 to I-1.3.1.5), and the various configurations of Class II BSCs are shown in Figures 35, 37, 38 and 40.

I-1.3.1.1  Question one: 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 unidirectional flow clean-air device (Clean Air Bench), which is not a BSC, may be the unit of choice. Clean air devices use high efficiency particulate air (HEPA) or ultra low penetration air (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 BSC enclosure offers a simple and economical solution. Room air sweeps around the operator and through the work area. This contaminated air is then HEPA/ULPA filtered and discharged into the laboratory or exhausted outside of the building via an external mechanical exhaust system. The Class I BSC 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 is most efficiently provided by a Class II BSC. The inflow of air around the operator provides personnel protection, HEPA/ULPA filtered air flowing downward through the work area provides product protection, and the discharge of exhaust HEPA/ULPA filtered air provides environmental protection.

I-1.3.1.2  Question two: What are all of the different types of work to be done in a Class II BSC?

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 air device or Class I BSC for current applications, only to find these devices are unsuitable as their work requirements change.

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45 IEST RP CC002, latest revision.
I-1.3.1.3 Question three: 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/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 direct connected to an external exhaust system in order to operate properly; Types A1, A2, and C1 can be converted to operate in either a canopy ducted or recirculating mode, depending on the users’ requirements. The airflow patterns of Types A1, A2, B1, B2 and C1 BSCs are shown in Figures 35, 37, 38 and 40, respectively.

Class II BSCs typically do not feature explosion-proof electrical components in their work area or internally. Therefore, use of flammable or explosive materials in quantities above their explosive limit are not recommended.

Types of chemicals used in cabinet should be considered as some can destroy the filter medium, housings and gaskets causing loss of containment.

The percentage of air in the work area that is recirculated within the BSC versus exhausted varies, based on the BSC Type, subtype, and in some cases, where the chemicals are released in the work area.\[46\] \[47\] \[48\]

When flammable or explosive chemicals are to be used in a BSC, it is the users’ responsibility to:

- be fully cognizant with the properties of chemical(s) and the hazards associated with them;
- calculate the highest percent of recirculation that may occur in the BSC being used;
- ensure the concentration of chemical(s) released in the work area do not exceed their explosive limit;
- utilize the lowest quantities of the chemical(s) required for the procedure being performed; and
- have appropriate spill / splash cleanup procedures in place before using the chemical(s).

I-1.3.1.4 Question four: If the BSC requires an exhaust system, is there an appropriate location for the cabinet and its ductwork?

If a BSC is going to recirculate its HEPA/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 34.

If a BSC must be connected to an external mechanical exhaust system, their compatibility must be established before the BSC is selected. The exhaust system configurations of Type A, Type B, and Type C1 BSCs are shown in Figures 36, 39 and 41, respectively:

- directly ducting Types A and C1 cabinets is not permitted; they shall only be exhausted through a properly designed and fitted canopy exhaust system;
- canopy-connected Types A and C1 require a consistent, low static pressure. While a dedicated exhaust system is preferred, they may share a common exhaust system with other exhausted laboratory devices, if properly balanced;

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— Type B BSCs require a higher static pressure that must increase as their exhaust filters load. They must be on a dedicated exhaust system, and not be ganged with other Type B BSCs, or other exhausted laboratory devices requiring a lower static pressure (e.g., fume hoods, canopy-connected BSCs);

— it is generally not an accepted practice to allow a BSC to positively pressurize an exhaust duct in normal operation. In some cases, however, it may be acceptable for the Type C1 to displace its exhaust air into a failing exhaust system for an interval of up to 5 minutes. To mitigate potential risks, the following procedures should be performed before configuring such an installation:

a) A risk assessment of the installation should be performed. The risk assessment should include evaluation of any other devices that are connected to the same exhaust system, such as other BSCs or laboratory fume hoods. Pressurizing a duct during an exhaust system failure may add risk with any other devices using the same exhaust system.

b) The duct must be verified to meet or exceed Seal Class A, as described in HVAC Air Duct Leakage Test Procedures – 2012.24

c) If these criterion cannot be met, the Type C1 may be reconfigured to either shut its blowers off within 15 seconds during exhaust system failure or the canopy may be reconfigured to direct exhaust air back into the laboratory during exhaust system failure. Alternatively, the Type C1 may be disconnected from the exhaust system if a risk assessment allows.

— the exhaust duct must be placed so it can penetrate ceilings and floors without disturbing other ventilation or plumbing systems; and

— the exhaust system must minimize excessive lengths and elbows.

I-1.3.1.5 Question five: If the exhaust system malfunctions, does the user understand its impact on the BSC's ability to maintain personnel and environmental protection, i.e., containment?

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 exhaust canopy.

For a Type B BSC, a significant loss of exhaust airflow will result in an alarm, turning off the cabinet blower(s). This stops the flow of air into the front of the BSC (inflow), negating personnel protection, potentially allowing materials in the work area of the BSC to escape into the laboratory.

Type B BSCs have operational and maintenance issues that must be considered:

— these cabinets exhaust as much as 1200 cubic feet per minute of conditioned room air making them relatively expensive to operate; and

— the higher static air pressure required to operate Type B cabinets may also result in additional construction costs associated with heavier gauge ductwork and higher capacity exhaust fan.

For a Type C 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 while its blowers are in operation. Personnel and product protection of the BSC will remain unchanged, and chemical vapors generated in the BSC will be exhausted either back into the room, or into the exhaust system, depending on the configuration of the canopy.

If a Type C1 BSC directs its exhaust into the room during a system failure, the shutdown time of the BSC blowers can be lengthened from 15 seconds to up to 5 minutes.
If a Type C1 BSC directs its exhaust into the external exhaust system during a system failure, the default shutdown time of the BSC blowers can be lengthened from 15 seconds to up to 5 minutes, provided:

— a risk assessment indicates the BSC, the work being done in it, and the exhaust system it is connected to is appropriate; and

— the BSC is connected to an exhaust duct that has been verified to meet or exceed Seal Class A, as described in HVAC Air Duct Leakage Test Procedures – 2012.24

NOTE — air recirculation: An informational calculation comparing the volumes of air that are recirculated internally through the BSC’s supply filter and work area, as opposed to being exhausted from the BSC. Calculated by dividing the volume of air recirculated by the total volume of air directed to both the supply and exhaust HEPA filters, and expressed as a percentage, it provides a relative value for different cabinet types (70% for Type A, 30% for Type B1, 0% for a Type B2, etc.). Originally these relative values were calculated from the airflow specifications of NIH-03-112c: Class II Type 1 Safety Cabinet: 1974, and NCI Specification General Purpose Clean Air Biological Safety Cabinet: 1976.

As BSC design has evolved, inflow and downflow velocities and volumes have changed, thus changing the air (percent) recirculation values; they should not be used as a strict design requirement.

Table I-1.1
Characteristics of Type A1 and A2 BSCs

<table>
<thead>
<tr>
<th></th>
<th>Type A1 (Figure 35)</th>
<th>Type A2 (Figure 36)</th>
</tr>
</thead>
<tbody>
<tr>
<td>intended purpose</td>
<td>Routine microbiological work. Work with volatile organic chemicals on the work surface permitted as an adjunct to microbiological research if the BSC is canopy-connected to external exhaust and permitted by risk analysis.</td>
<td>Routine microbiological work. Work with volatile organic chemicals on the work surface permitted as an adjunct to microbiological research if the BSC is canopy-connected to external exhaust and permitted by risk analysis.</td>
</tr>
<tr>
<td>airflow pattern</td>
<td>Room air is drawn in through the sash opening, protecting the operator. HEPA/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/ULPA filter, and the remainder recirculates through a Supply HEPA/ULPA filter before flowing down through the work area.</td>
<td>Room air is drawn in through the sash opening, protecting the operator. HEPA/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/ULPA filter, and the remainder recirculates through a Supply HEPA/ULPA filter before flowing down through the work area.</td>
</tr>
<tr>
<td>air recirculation</td>
<td>Varies by model.</td>
<td>Varies by model.</td>
</tr>
<tr>
<td>inflow</td>
<td>Minimum 75 ft/min (0.38 m/s) average.</td>
<td>Minimum 100 ft/min (0.51 m/s) average.</td>
</tr>
<tr>
<td>downflow</td>
<td>Varies by model, typically 50 to 80 ft/min (0.25 to 0.40 m/s) average.</td>
<td>Varies by model, typically 50 to 80 ft/min (0.25 to 0.40 m/s) average.</td>
</tr>
<tr>
<td>biological containment</td>
<td>All NSF-Listed BSCs must pass the same biological containment tests.</td>
<td>All NSF-Listed BSCs must pass the same biological containment tests.</td>
</tr>
<tr>
<td>exhaust system</td>
<td>Canopy connection as needed.</td>
<td>Canopy connection as needed.</td>
</tr>
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</table>
### Table I-1.1

#### Characteristics of Type A1 and A2 BSCs

<table>
<thead>
<tr>
<th></th>
<th>Type A1</th>
<th>Type A2</th>
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<tbody>
<tr>
<td><strong>exhaust system type</strong></td>
<td>Canopy-connected Type A BSCs may be ganged into a multiple-cabinet exhaust system, if all BSCs are balanced properly.</td>
<td>Canopy-connected Type A BSCs may be ganged into a multiple-cabinet exhaust system, if all BSCs are balanced properly.</td>
</tr>
<tr>
<td><strong>exhaust system function</strong></td>
<td>To convey the BSC exhaust air, plus an additional volume required by the canopy through the ductwork.</td>
<td>To convey the BSC exhaust air, plus an additional volume required by the canopy through the ductwork.</td>
</tr>
<tr>
<td><strong>exhaust system volume</strong></td>
<td>Greater than Type B1, less than Type B2.</td>
<td>Greater than Type B1, less than Type B2.</td>
</tr>
<tr>
<td><strong>exhaust system negative static pressure at BSC</strong></td>
<td>Typically, 0.25 inches w.g. (62 Pa).</td>
<td>Typically, 0.25 inches w.g. (62 Pa).</td>
</tr>
<tr>
<td><strong>exhaust system reserve capacity</strong></td>
<td>Static pressure requirements will not change as the cabinet filters load.</td>
<td>Static pressure requirements will not change as the cabinet filters load.</td>
</tr>
<tr>
<td><strong>cabinet flexibility</strong></td>
<td>Can be connected or disconnected from exhaust system as needs change.</td>
<td>Can be connected or disconnected from exhaust system as needs change.</td>
</tr>
<tr>
<td><strong>cabinet cost</strong></td>
<td>Less than Type B.</td>
<td>Less than Type B.</td>
</tr>
<tr>
<td><strong>installation cost</strong></td>
<td>Much less than Type B if recirculating; less than Type B if canopy-connected.</td>
<td>Much less than Type B if recirculating; less than Type B if canopy-connected.</td>
</tr>
<tr>
<td><strong>electrical cost (BSC only)</strong></td>
<td>Slightly more than Type B2.</td>
<td>Slightly more than Type B2.</td>
</tr>
<tr>
<td><strong>tempered air loss</strong></td>
<td>If recirculating in lab; none. If canopy-connected, typically 75 CFM/ft (7 m³/m) of BSC width or less.</td>
<td>If recirculating in lab; none. If canopy-connected, typically 100 CFM/ft (9 m³/m) of BSC width or less.</td>
</tr>
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</table>

### Table I-1.2

#### Characteristics of Type B1 and Type B2 BSCs

<table>
<thead>
<tr>
<th></th>
<th>Type B1</th>
<th>Type B2</th>
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<tr>
<td><strong>intended purpose</strong></td>
<td>Type B1 cabinets may be used for routine microbiological work. Work with volatile organic chemicals on the work surface permitted as an adjunct to microbiological research if permitted by risk analysis. A majority of the downflow air is directly exhausted from the rear portion of the cabinet.</td>
<td>Type B2 cabinets may be used for routine microbiological work. Work with volatile organic chemicals on the work surface permitted as an adjunct to microbiological research if permitted by risk analysis. All downflow air is directly exhausted from the work area with no recirculation.</td>
</tr>
<tr>
<td><strong>airflow pattern</strong></td>
<td>Room air is drawn in through the sash opening, protecting the operator. HEPA/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/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/ULPA filter.</td>
<td>Room air is drawn in through the sash opening, protecting the operator. HEPA/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/ULPA filter.</td>
</tr>
<tr>
<td><strong>air recirculation</strong></td>
<td>Varies by model; less than 50%.</td>
<td>None.</td>
</tr>
<tr>
<td></td>
<td>Type B1 (Figure 37)</td>
<td>Type B2 (Figure 38)</td>
</tr>
<tr>
<td>---------------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>inflow</td>
<td>Minimum 100 ft/min (0.51 m/s) average.</td>
<td>Minimum 100 ft/min (0.51 m/s) average.</td>
</tr>
<tr>
<td>downflow</td>
<td>Varies by model, typically 50 to 80 ft/min (0.25 to 0.40 m/s) average.</td>
<td>Varies by model, typically 50 to 80 ft/min (0.25 to 0.40 m/s) average.</td>
</tr>
<tr>
<td>biological containment</td>
<td>All NSF listed BSCs must pass the same biological containment tests.</td>
<td>All NSF listed BSCs must pass the same biological containment tests.</td>
</tr>
<tr>
<td>exhaust system</td>
<td>Required.</td>
<td>Required.</td>
</tr>
<tr>
<td>exhaust system type</td>
<td>Must have dedicated ductwork and exhaust blower for each BSC.</td>
<td>Must have dedicated ductwork and exhaust blower for each BSC.</td>
</tr>
<tr>
<td>exhaust system function</td>
<td>Must pull exhaust air through the Cabinet’s Exhaust HEPA/ULPA filter and then through ductwork.</td>
<td>Must pull exhaust air through the Cabinet’s Exhaust HEPA/ULPA filter and then through ductwork.</td>
</tr>
<tr>
<td>exhaust system volume</td>
<td>B1 is approximately 20% less than a Type A.</td>
<td>B2 exhausts 100% or more air than any other BSC Type.</td>
</tr>
<tr>
<td>exhaust system negative static pressure at BSC</td>
<td>Typically 0.7 inches w.g. H₂O (170 Pa) minimum.</td>
<td>Typically 1 to 2.5 inches w.g. (249 to 622 Pa) H₂O minimum.</td>
</tr>
<tr>
<td>exhaust system reserve capacity</td>
<td>Static pressure requirements may increase up to 0.3 inches w.g. H₂O (74 Pa) H₂O as exhaust HEPA/ULPA filter loads.</td>
<td>Static pressure requirements may increase up to 2.5 inches w.g. (622 Pa) as exhaust HEPA/ULPA filter loads.</td>
</tr>
<tr>
<td>cabinet flexibility</td>
<td>Must be permanently connected to an exhaust system to function properly.</td>
<td>Must be permanently connected to an exhaust system to function properly.</td>
</tr>
<tr>
<td>cabinet cost</td>
<td>More expensive than Type A.</td>
<td>More expensive than Type A.</td>
</tr>
<tr>
<td>installation cost</td>
<td>More expensive than a canopy-connected Type A and require a dedicated exhaust fan.</td>
<td>Most expensive. Higher exhaust volumes require larger ductwork and higher capacity dedicated exhaust fan.</td>
</tr>
<tr>
<td>electrical cost (BSC only)</td>
<td>Slightly more than a Type B2.</td>
<td>Typically lowest of any BSC.</td>
</tr>
<tr>
<td>tempered air loss</td>
<td>Equal to a canopy-connected Type A. Typically 50 to 100 CFM/ft (4.6 to 9.3 m³/m) of BSC width.</td>
<td>Typically 175 CFM/ft (16.3 m³/m) of BSC width.</td>
</tr>
</tbody>
</table>
### Table I-1.3
Characteristics of Type C1 BSCs

<table>
<thead>
<tr>
<th>Description</th>
<th>Type C1 (Figure 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>intended purpose</td>
<td>Type C1 cabinets may be used for routine microbiological work. Work with volatile organic chemicals on the work surface is permitted as an adjunct to microbiological research if the cabinet is connected to an exhaust system, and is acceptable after performing a risk analysis. Typically, a majority of the downflow air is directly exhausted from the center portion of the cabinet.</td>
</tr>
<tr>
<td>airflow pattern</td>
<td>Room air is drawn in through the sash opening, protecting the operator. HEPA/ULPA filtered air flows down through the work area, protecting the product. The room air, and a portion of the downflow air in the work area is recirculated through a supply HEPA/ULPA filter before flowing down through the work area. Typically, the air in the center of the work area flows directly out of the cabinet via an Exhaust HEPA/ULPA filter.</td>
</tr>
<tr>
<td>air recirculation</td>
<td>Varies by model; typically less than 50%.</td>
</tr>
<tr>
<td>inflow</td>
<td>Minimum 100 ft/min (0.51m/s) average.</td>
</tr>
<tr>
<td>downflow</td>
<td>Varies by model, typically 50 to 80 ft/min (0.25 to 0.4 m/s) average.</td>
</tr>
<tr>
<td>biological containment</td>
<td>All NSF listed BSCs must pass the same biological containment tests.</td>
</tr>
<tr>
<td>exhaust system</td>
<td>Canopy connection as needed. If BSC exhaust is to be directed into the exhaust duct during a system failure, the ductwork must be sealed and tested for leakage.</td>
</tr>
<tr>
<td>exhaust system type</td>
<td>Canopy-connected Type C1 BSCs may be ganged into a multiple-cabinet exhaust system, if all BSCs are balanced properly.</td>
</tr>
<tr>
<td>exhaust system function</td>
<td>To convey the BSC exhaust air, plus an additional volume required by the canopy through the ductwork.</td>
</tr>
<tr>
<td>exhaust system volume</td>
<td>Greater than Type B1 Less than Type B2.</td>
</tr>
<tr>
<td>exhaust system negative static pressure at BSC</td>
<td>Typically 0.25 inches w.g. (62 Pa).</td>
</tr>
<tr>
<td>exhaust system reserve capacity</td>
<td>Static pressure requirements will not change as the cabinet filters load.</td>
</tr>
<tr>
<td>cabinet flexibility</td>
<td>Can be connected or disconnected from exhaust system as needs change.</td>
</tr>
<tr>
<td>cabinet cost</td>
<td>More expensive than Type A; similar to Type B.</td>
</tr>
<tr>
<td>installation cost</td>
<td>Much less than Type B if recirculating; less than B1 if connected to a ganged exhaust system; similar to Type B1 if connected to a dedicated system.</td>
</tr>
<tr>
<td>electrical cost (BSC only)</td>
<td>Similar to a Type A2.</td>
</tr>
<tr>
<td>tempered air loss</td>
<td>If recirculating in lab; none. If canopy-connected, typically 75 CFM/ft (7 m³/m) of BSC width or less.</td>
</tr>
</tbody>
</table>
I-1.3.2 BSC width

Having decided which Class and Type of BSC is the best, the user should now decide on the width of the BSC. When deciding the width, 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 width, 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 greater than the work area width. The BSC should be installed with at least 6 inches (150 mm) of unobstructed space on each side. For example, a typical BSC with a 4 foot (1219 mm) workspace may have an outside width of 4.5 feet (1,372 mm). When 6 inches (150 mm) of space is allowed on each side of the BSC for servicing, the width needed to install the BSC is 5.5 feet (1,676 mm).

I-1.3.3 BSC options

I-1.3.3.1 Service values

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.

I-1.3.3.2 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.

I-1.3.3.3 Ultraviolet lighting

Germicidal (or UV) lamps are often installed as an adjunct to surface disinfection. UV lighting is not recommended in BSCs. 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.

I-1.3.3.4 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.

I-1.3.3.5 Base stands

Base Stands or supports shall 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 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.
I-1.3.3.6 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, or vibration, or both, during moving, the BSC should be recertified to ensure it is functioning in a proper manner.

I-1.4 Site review before BSC purchase

I-1.4.1 Consultation

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

I-1.4.2 Site assessment

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

I-1.4.2.1 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 34 shows the preferred location for the cabinet. The BSC should be located at the wall furthest from and facing the entry door. If this is not possible, the BSC should be located on the side wall perpendicular to the hinge side of the door.

I-1.4.2.2 Clearances

BSCs not connected to an exhaust system should have at least (12 inches [300 mm]) 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 inches (300 mm) clearance is required if the use of a thermal anemometer exhaust velocity measurement is needed when calculating cabinet inflow velocity.

All BSCs should be placed in a laboratory at a location that provides a minimum of:

- 6 inches (150 mm) from adjacent walls or columns;
- 6 inches (150 mm) between two BSCs;
- 6 inches (150 mm) space between both sides of the cabinet and 6 inches (150 mm) behind the BSC to allow for service operations;
- 40 inches (1020 mm) of open space in front of the BSC;
- 60 inches (1520 mm) from opposing walls, bench tops and areas of occasional traffic;
- 20 inches (510 mm) between BSC and bench tops along a perpendicular wall;
- 100 inches (2540 mm) between two BSCs facing each other;
- 60 inches (1520 mm) from behind a doorway;
— 40 inches (1020 mm) from an adjacent doorway swing side; and
— 6 inches (150 mm) from an adjacent doorway hinge side.

I-1.4.2.3  Exhaust requirements

If the BSC is to be connected to an external mechanical 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 inches (250 mm) or 12 inches (300 mm) 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.

I-1.4.2.4  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 volts, will require a circuit rated for 20 ampere service. As the electrical plugs and sockets for 115 volts, 15 and 20 ampere 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.

I-1.4.2.5  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.

I-1.4.2.5.1  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.

I-1.4.2.5.2  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.

I-1.4.2.5.3  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, or in-line filters, or both, should be installed to prevent contamination of the vacuum system.

I-1.4.2.6  Roof exhaust systems

Roof exhaust systems serving BSCs should have a stack that extends straight upward at least 10 feet (3 m) above the roof surface or have a stack with a smaller diameter trailing end to produce higher velocity flow to avoid reentrainment by the building, and should be increased in elevation when necessary to avoid the influence of surrounding structures.49 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

49 ASHRAE/AHIA Z9.5
exhausted at normal stack velocities. To take care of precipitation during periods when system is shut off, a 1 inch (25 mm) 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 nonfunction 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 Figure 40 – High velocity fans.44

I-1.4.3 Prepurchase checklist

The investigator should notify building management to arrange for feasibility assessment of laboratory alterations and BSC location. The investigator and biosafety 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.

I-1.5 BSC arrival and certification

I-1.5.1 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.
I-1.5.2 Arrange for field 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.

I-1.6 Cleaning and disinfection of BSC work area

I-1.6.1 Dos and don’ts of disinfectant efficacy

— do evaluate the cleaning processes and frequencies for ability to control organisms separately from the disinfectant efficacy study;

— do design a meaningful study that represents use in the facility;

— do consider additional studies if quality metrics indicate current agents are no longer controlling microorganisms in the area using prescribed disinfection methods;

— don’t expect the disinfectant study to replace routine cleaning, disinfection and monitoring programs;

— don’t rely on disinfectant studies to establish that cleaning frequencies and processes to control organisms are sufficient and acceptable; and

— don’t wait until there is a problem to evaluate the disinfectants being used in the facility.

I-1.6.2 Surface disinfectants

I-1.6.2.1 Halogens (hypochlorous acid [HOCl]) – Active ingredient

— chlorine bleach oxidizing disinfectant that is sporicidal;

— stainless steel is corroded by chlorine bleach. Sodium hypochlorite must be neutralized with sodium thiosulfate or followed by use of a disinfectant or sterile water;

— 1:5 dilution of Clorox™ with water (10,000 ppm) is needed to inactivate Mycobacteria in sputum);

— 1:100 dilution with water (500 ppm) must be made fresh daily and combined with a nonionic detergent;50

— 1:50 dilution stored at room temperature in a closed plastic container will deteriorate to the equivalent of a 1:100 dilution after 1 month (Amer. J. Infect. Control 17:1, 1989);

— bleach mixed with acid cleaner produces chlorine gas – 1 ppm TLV; and

— bleach mixed with ammonia-containing cleaner produces monochloramine and dichloramine irritants.

I-1.6.2.2 Chlorine dioxide

— tuberculocidal, bactericidal, virucidal and fungicidal.

I-1.6.2.3 Quaternary ammonium salts

— each compound exhibits its own antimicrobial characteristics;

— chemical names of quaternary ammonium compounds used in healthcare are alkyl dimethyl benzyl ammonium chloride, alkyl didecyl dimethyl ammonium chloride, and dialkyl dimethyl ammonium chloride;

— newer quaternary ammonium compounds are referred to as twin-chain or dialkyl quaternaries (e.g., didecyl dimethyl ammonium bromide and dioctyl dimethyl ammonium bromide);

— a quaternary detergent cleaner is used in maximum containment (BSL-4) facility showers;

— most common institutional disinfectant sold under hundreds of trade names; and

— use dilution ranges from 0.5 to 3% depending on the compound.

I-1.6.2.4 Phenolics

— EPA registered as tuberculocidal;

— many trade names and concentrations of amylphenol and phenyl phenol;

— allergies, skin absorption; and

— phenolics are not sporicidal.

I-1.6.2.5 Alcohols

— flammable;

— alcohols are not sporicidal and must be used as a sterile solution to prevent spread of fungal spores;

— 70% alcohol sprayed on the work surface of an operating BSC becomes ineffective within seconds; and

— alcohol attacks acrylic, polypropylene, PVC, and polycarbonate plastics over time.

I-1.6.2.6 Iodophores

— often used at a 0.5% concentration;

— Wescodyne™ diluted 1:213 with water is an effective BSC surface disinfectant;

— nonstaining, but will leave a brown residue; nontoxic;

— active against gram negative and gram positive bacteria, viruses, fungi, yeast, M. tuberculosis and many bacterial and fungal spores; and

— used on work surfaces, water baths, and incubators.

I-1.6.2.7 Peroxides (stabilized) hydrogen peroxide 6 to 25%

— often used to sanitize surfaces in the food industry; and

— sporicidal agent recommended by cleanroom industry.

I-1.6.2.8 Peracetic acid

— sporicidal agent recommended by cleanroom industry.
I-1.7  BSC use practices and procedures

I-1.7.1  Ergonomics

I-1.7.1.1 Ergonomics is important for proper BSC use and user health and safety. An evaluation of normal work practices should be performed with each user when working in a BSC.

I-1.7.1.2 Evaluation criteria should be at a minimum: proper user posture, effective work zone layout for work practices, and correct vision or sightlines.

I-1.7.1.3 User accommodations include a six-way articulating chair with seat and back controls for personalized adjustment to assure proper user posture. Feet should rest on the floor, chair foot support or foot rest. Fully support an individual’s back is fully supported with proper chair adjustments.

I-1.7.1.4 Forearm / armrest supports are available on some BSCs to provide forearm support on the work access opening. Periodic mini-breaks during BSC work should be taken by resting your forearm to avoid stress and fatigue.

I-1.7.1.5 Effective work area layouts will minimize reach to avoid neck and shoulder stress and fatigue. Rotating tables are available to minimize reach.

I-1.7.1.6 Always prepare your work procedure to eliminate glare and bright reflections on the sash window and keep the sash sightlines clear to your work zone.

I-1.7.2  BSC practices

— BSC must be properly certified before use;
— BSC must be positioned in an area with no drafts or foot traffic;
— do not bypass any sash closure or position alarms; and
— do not block or restrict any grill(s) or discharge openings.

I-1.7.3 Do not modify a BSC in a way that may compromise its containment unless approved by appropriate safety personnel for the following modifications:

— installing a water faucet;
— connecting a drain to sewage system;
— installing waste cans under or in the work surface;
— installing other devices (i.e., centrifuge) into the work surface; and
— positioning cables, hoses etc., through the work access opening.

I-1.7.4  BSC techniques

— have proper BSC training including proper aseptic technique before use;
— decontaminate BSC work surface before use;
— ensure that sash is at the proper height depending on BSC model;
— do not use an open flame;
— do not use high pressure gases;
— do not use volatile chemicals without a risk assessment by qualified chemical safety personnel system (refer to Section I-1.3.1.3);
— do not place large objects on the BSC work surface;
— do not place fan-cooled devices in the BSC work area;

— minimize room activity;

— activity in the room should be held to a minimum. Unnecessary activity may create disruptive air currents as well as interfere with BSC operation. A person walking past the front of a BSC may cause draft velocities up to 175 ft/min (89 m/s), which can disrupt the air inside the BSC.

— utilize unidirectional air flow;

— keep two important facts in mind: (1) air supplied to the work area through a supply filter from the top, is contaminant free; and (2) airborne contamination generated in the work area is controlled by flow of airstreams in a top-to-bottom direction;

— a solid object placed in the sterile air stream will disrupt the air flow and consequently, the capability to control lateral movement of airborne particulates. A cone of turbulence will extend below the object and uniform flow of the air stream will not be regained until for approximately equal to three to six times the size of the object. Within this cone of disturbed air, particles may be carried laterally by multidirectional eddy currents;

— transfer of viable materials and manipulations which may generate aerosols should not be performed above sterile materials;

— employ aseptic technique;

— do not assume that the BSC will prevent contamination when performing procedures. BSCs will control airborne contamination of viable agents (i.e., microorganisms), but the BSC will not eliminate contact transmission of contamination. Contamination control procedures such as aseptic technique is required to obtain maximum benefit from the BSC;

— open bottles, tubes or flasks should be kept as parallel as possible to the downflow air to minimize capture of particulates. This precaution is merely good aseptic technique;

— equipment in direct contact with agents must remain in the cabinet until bagged or until surface-decontaminated. Trays of discard pipettes must be covered before removal from the BSC;

— when there is a spill or splatter of agent in the work area, all surfaces and items in the BSC should be surface-decontaminated before materials are removed;

— using natural gas to flame flask or tube necks is not recommended. A gas burner flame in BSC also contributes significantly to the heat build-up inside the BSC and also disrupts the sterile supply air stream. If a procedure demands use of a flame, a Bunsen burner with an on-demand ignition is recommended. Do not use a constant flame gas burner. Bunsen burners must be placed near the rear of the workspace where air turbulence will have a minimal effect;

— a plastic-backed absorbent paper should be placed on the work surface during mixing procedures and replaced whenever significant spills occur and when the work is completed;

— vials should be opened with a tool specifically designed for that purpose. Vials should be vented with a filter needle to eliminate internal pressure or vacuum;

— before opening ampoules, make sure that no liquid remains in the tip of the ampoule. A sterile gauze pad should be wrapped around the neck of the ampoule before it is opened; and

— a collection vessel should be available on the work surface for discard of solutions.
I-1.7.5 BSC start up procedure

a) Follow manufacturer’s startup procedure. If cabinet alarm condition is present, investigate root cause before continuing;

b) Inspect air intake grilles for obstructions and foreign materials and remove any obstructions. Remove all items from the work area;

c) Adjust view screen to proper height;

d) Turn on blower and allow 5 minutes to purge the air;

e) Wash hands and arms with mild, nonantimicrobial soap for 30 seconds. Put on a rear-fastening, long-sleeved gown with gathered cuffs. Put on a pair (or two pairs) of appropriate long sleeve (11.5 to 12 inch) gloves (nitrile gloves are recommended for biological work). Consider, when appropriate, disposable sleeve protectors and a second or third pair of appropriate gloves. This will minimize the shedding of skin flora into the work area and also protect hands and arms from viable microbial contamination. Before and after work in a BSC, hands and arms should be washed mild, nonantiseptic soap;

f) Disinfect the interior surfaces of the BSC by wiping down with appropriate disinfectant for an appropriate contact time. 70% alcohol is not considered an appropriate disinfectant because it has no effect on fungal spores;

g) Place a plastic-backed pad on the work surface without covering the air intake / exhaust grills. This will prevent spills from hitting the stainless steel surface and creating aerosols;

h) Put all items for the experiment in the BSC and keep clean items segregated from dirty items by 12 inches (300 mm). Organize the material so that dirty “contaminated” items will not be passed over (cross contaminate) clean items;

i) Exercise care that no items be placed over the front intake grills. Materials should be arranged so that clean materials are separated from dirty (used) virus materials. Passage of contaminated materials over noninoculated cultures or clean glassware should be avoided to prevent contamination. Transfer of viable materials should be performed as deeply into the cabinet (away from open face) as possible;

j) Allow air to stabilize for 5 minutes before starting work. This will rid the area of all "loose" contamination that may have been introduced with the items;

k) Work from "clean" to "dirty" areas and work at least 6 inches (150 mm) back from rear of the front air intake grill;

l) Move arms in and out of the work access opening perpendicular to the front of the BSC in a slow steady motion to minimize disruption of the front air curtain;

m) Minimize penetration of the work opening air curtain;

n) A minimum number of needed items should be placed into the BSC to prevent overloading. Work should be planned to minimize the number of times an operator’s hands and arms must enter and leave the air curtain. Ideally, have everything needed for your procedure placed in the BSC before starting, so that nothing needs to pass in or out through the front air curtain until the procedure is completed. Do not raise your hands inside the BSC above the top level of the sash height. If you raise your hands above the sash height, air may flow up your hands to elbows and possibly out of the BSC;
o) Know your "safe working area". BSC safe working area is the work tray or depressed area. All work should be performed on or above the work tray. The area on or above and within 6 inches (150 mm) from the rear of the front grill is a nonsafe working area;

p) This is a general operational guideline to control airborne contaminants of low to moderate risk. Procedure protocols defined in terms of the barrier or control concepts unique to BSCs must be developed for maximum safety and protection; and

q) For preparation of antineoplastic drugs, the following procedures summarize the OSHA Technical Manual TED 1-0.15A, Section VI, Chapter 2 "Controlling Occupational Exposure to Hazardous Drugs". This document should be reviewed before preparing antineoplastic drugs in a BSC.

I-1.7.6 Terminal purging and wipedown

a) Following completion of work, allow the BSC to run for a 5 minute period without personnel activity to purge air in the work area;

b) Decontamination of the interior surfaces should be repeated after removal of all materials, cultures, apparatus, etc. Check grills and diffuser grids for spilled or splashed materials that may support fungus growth in the workspace; and

c) The interior surfaces of the workspace should next be disinfected with an appropriate disinfectant for an appropriate contact time. Use of chlorine bleach in the BSC will damage the BSC stainless steel work surface. Most surface disinfectants require a specific contact time, depending upon the microbiological agents used within the BSC. Consult appropriate disinfectant documents for proper application and suitability against the material used in the BSC.

I-1.7.7 Use the following procedure to effectively clean or surface disinfect the BSC work zone surfaces:

a) Raise the sliding sash window to a full-open position.

b) Silence the audible alarm during the cleaning process.

c) Wipe all surfaces in parallel strokes from clean to dirty.

I-1.7.8 Paper catch prefilter

Some BSCs have a paper catch filter installed behind the rear divider panel of the work zone. This area forms the return air path to the motor / blower. If the airflow is blocked, performance of the BSC can be compromised. Therefore, the paper catch should be checked and cleaned no less than weekly or daily if paper products are used for procedures. Removed paper must be properly discarded as contaminated hazardous waste.

I-1.7.9 BSC shut down

a) Turn off blowers and lights. Do not use cabinet as a depository for any lab equipment or materials during periods of nonoperation.

b) Close the sash window.

c) If antineoplastic agents are prepared in the BSC, it is recommended that the BSC run 24 hours per day. This lessens the possibility that contaminants may escape.

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I-1.8 Moving a permanently installed BSC

I-1.8.1 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 professional.

I-1.8.2 Existing BSCs and ancillary equipment, such as canopy connection exhaust ducting, gas, electric and vacuum connections, should be cleared for maintenance by a biosafety 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 before the move. After a BSC is moved, it should be certified according to applicable performance standards.

I-1.9 BSC lifespan

The current lifespan of a BSC is approximately 15 years. Use of modern day BSCs began in the early 1970s with BSCs that were manufactured to the NIH-03-112C Standard and subsequently the NSF/ANSI 49. BSCs manufactured in the 1970s, 1980s, and early 1990s 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 may not be available. 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 BSC repair versus replacement decision.

I-1.10 Decommissioning process

I-1.10.1 No BSC should be sent to a landfill or a recycling facility as a BSC, it should be disassembled per requirements contained in this Section.

I-1.10.2 Decontamination and personal protective equipment (PPE)

— 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 Section I-1.10.2.3;

— all decommissioned BSCs used with pathogens should be space decontaminated;

— 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;
— for those BSCs used with biological agents such as prions or biological toxins, that may not be
inactivated via gas decontamination, 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; and

— PPE should be used as directed by the Facility Safety Officer or the biosafety safety professional.

I-1.10.3 Metal parts

— 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; and

— 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 vendor.

I-1.10.4 Glass windows

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.

I-1.10.5 Wiring

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

I-1.10.6 Electrical ballasts

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

I-1.10.7 Lamps

— all fluorescent lamps should be taken to the lamp container area at the institution; and

— all ultraviolet lamps should be handled as mercury-containing waste.

I-1.10.8 Labels

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

I-1.10.9 Used HEPA/ULPA filters

I-1.10.9.1 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.

I-1.10.9.2 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.

I-1.10.9.3 Decontaminated HEPA/ULPA filters may be safety buried in a sanitary landfill because they no
longer pose a hazard.
I-1.11 Definitions

I-1.11.1 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."])

I-1.11.2 airflow

I-1.11.2.1 air curtain: A artificially created stream of moving air that is drawn across a threshold or other opening to create a barrier.

I-1.11.2.2 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 inches (100 mm) above the bottom edge of the sash, when it is in its normal operating height.

I-1.11.2.3 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.

I-1.11.2.4 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.

I-1.11.2.5 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.

I-1.11.2.6 nonuniform (zoned) downflow: A downflow velocity profile comprised of several contiguous zones. The average downflow velocities vary from zone to zone.

I-1.11.2.7 uniform downflow: A downflow velocity profile wherein the individual point velocities are all approximately the same.

I-1.11.3 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.

I-1.11.4 biosafety cabinet nominal width: The interior sidewall to sidewall width. The cabinet nominal width is expressed in 12 inches (300 mm) 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.

I-1.11.5 biosafety cabinet shell penetrations / cable ports

I-1.11.5.1 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 Section N-1.1. 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.

I-1.11.5.2 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, 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.

I-1.11.5.3 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 to provide a seal meeting the requirements of Section N-1.1.

I-1.11.6 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.

I-1.11.7 Biosafety Levels (BSLs): The essential elements of the four BSLs for activities involving infectious microorganisms and laboratory animals are summarized in *Biosafety in Microbiological and Biomedical Laboratories*. 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. BSLs should not be considered the same as microorganism Risk Groups.

I-1.11.7.1 Biosafety Level 1 (BSL-1): Basic BSL-1 laboratory 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.

I-1.11.7.2 Biosafety Level 2 (BSL-2): Containment BSL-2 laboratory 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.

I-1.11.7.3 Biosafety Level 3 (BSL-3): High Containment BSL-3 laboratory 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.

I-1.11.7.4 Biosafety Level 4 (BSL-4): Maximum Containment BSL-4 laboratory 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

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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.

There are two models for BSL-4 laboratories:

- Cabinet Laboratory where all handling of agents must be performed in a Class III BSC; and
- 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.

I-1.11.8 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 BSL-4 agents should only be used in Maximum Containment Laboratories and that Class I and Class II BSCs are only acceptable in Maximum Containment Laboratories with positive pressure containment suits.

I-1.11.8.1 Class I: A Class I BSC provides personnel and environmental protection without product protection. Personnel protection is provided as a minimum velocity of 75 ft/min (0.38 m/s) 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.

I-1.11.8.2 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 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, 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 sealed connection.

All Class II cabinets are designed for work involving procedures assigned to BSLs 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.

I-1.11.8.2.1 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.

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— 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 may be canopy-connected to external exhaust system if permitted by a chemical risk assessment (refer to Section I-1.3.1.3).

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.

I-1.11.8.2.2 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 must be canopy-connected to external exhaust system if permitted by a chemical risk assessment (refer to Section I-1.3.1.3).

I-1.11.8.2.3 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 contaminated downflow air from a region of the work area via an internal dedicated exhaust plenum and through HEPA/ULPA filter(s) to an external exhaust system 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 I-1.3.1.3).
I-1.11.8.2.4 **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 if permitted by a chemical risk assessment (refer to Section I-1.3.1.3).

I-1.11.8.2.5 **Class II, Type C1 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 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 I-1.3.1.3).

I-1.11.8.3 **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. 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.

I-1.11.9 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.

I-1.11.10 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.

I-1.11.11 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 N-1 of this Standard.

I-1.11.12 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 N-5 of this Standard.

I-1.11.13 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.

I-1.11.14 closed: Fabricated with no openings exceeding 0.031 inch (0.79 mm).

I-1.11.15 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 ft/min. 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 ft³/min. 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.

I-1.11.16 decontamination: Inactivation or destruction of infectious agents or neutralization of toxic agents to an acceptable level.

I-1.11.17 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.

I-1.11.18 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 N-2.

I-1.11.19 high efficiency air filters (for use in Class II BSCs):

I-1.11.19.1 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\textsuperscript{13} (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\textsuperscript{13} (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.\textsuperscript{13} Filter media shall be tested in accordance with the methods of IEST-RP-CC021\textsuperscript{13} with performance levels to meet the minimum efficiency requirements as specified above and the pressure drop requirements as required by the specific application.

I-1.11.19.2 **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;\textsuperscript{13}
— 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,\textsuperscript{13} HEPA/ULPA filters.

I-1.11.20 **leak tight**: Free of leaks at 2 in w.g. (500 Pa) of air pressure as described in Annex N-1.

I-1.11.21 **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 Section N-1.7.

I-1.11.22 **polydisperse aerosol**: Aerosol with a light scattering median size of 0.7 µm and a geometric standard deviation of 2.4.

I-1.11.23 **readily accessible**: Fabricated to be exposed for cleaning and visual inspection without using tools.

I-1.11.24 **readily removable**: Capable of being taken away from the main unit without using tools.

I-1.11.25 **removable**: Capable of being taken away from the main unit using simple tools (screwdriver, pliers, open-end wrench, etc. [also see readily removable]).
I-1.11.26  **sash**: A fixed or sliding window located at the front of the BSC, that forms a barrier between the operator and the work area.

I-1.11.27  **sealed**: Fabricated with no openings that will permit entry or leakage of air (leak-tight).

I-1.11.28  **smooth**: A surface free of pits and inclusions, with cleanability equal to or exceeding the following.

I-1.11.28.1 **interior work surfaces and exposed interior surfaces**: Number 3 (100 grit) finish on stainless steel.

I-1.11.28.2 **other interior surfaces and exterior surfaces**: Commercial grade cold-rolled, hot-rolled, or combination cold / hot-rolled steel free of visible scale.

I-1.11.29  **surfaces**

I-1.11.29.1 **interior work surfaces**: Surfaces used when performing a task, operation, or activity.

I-1.11.29.2 **exposed interior surfaces**: Exposed interior surfaces, other than work surfaces, that are subject to splash, spillage, or airborne contamination during normal use.

I-1.11.29.3 **other interior surfaces**: Interior surfaces not exposed to splash or spillage but exposed to vapor, or volatile toxic substances, or both.

I-1.11.29.4 **exterior surfaces**: All exposed surfaces not defined as interior.

I-1.11.30  **toxic**: Having an adverse physiological effect on biological systems.

I-1.11.31 **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.

I-1.11.32  **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.

I-1.11.33 **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 inches (5 mm) above the lower level of the sash.

I-1.11.34 **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.
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 34
Suggested laboratory locations for Class II BSCs
NOTE — The simplified diagram illustrates airflow patterns only. All areas of the BSC containing contaminated air under positive pressure must be jacketed by negative pressure.

Figure 35
Airflow patterns for Class II Type A1 and A2 BSCs
NOTE — The simplified diagram illustrates airflow patterns only. All areas of the BSC containing contaminated air under positive pressure must be jacketed by negative pressure.

Figure 36
Suggested Type A exhaust system
NOTE — The simplified diagram illustrates airflow patterns only. All areas of the BSC containing contaminated air under positive pressure must be jacketed by negative pressure.

Figure 37
Airflow patterns for Class III Type B1 BSCs
NOTE — The simplified diagram illustrates airflow patterns only. All areas of the BSC containing contaminated air under positive pressure must be jacketed by negative pressure.

**Figure 38**

Airflow patterns for Class II Type B2 BSCs
NOTE — The simplified diagram illustrates airflow patterns only. All areas of the BSC containing contaminated air under positive pressure must be jacketed by negative pressure.

Figure 39
Suggested Type B exhaust system
NOTE — The simplified diagram illustrates airflow patterns only. All areas of the BSC containing contaminated air under positive pressure must be jacketed by negative pressure.

Figure 40
Airflow patterns for Class II Type C1 BSCs
NOTE — The simplified diagram illustrates airflow patterns only. All areas of the BSC containing contaminated air under positive pressure must be jacketed by negative pressure.

Figure 41
Suggested Type C exhaust system
Figure 42
Exhaust stack and blower

stack discharge directly upward

optional reduced diameter terminal stack to increase exhaust velocity and prevent recapture of the exhaust into any other roof air intake

guy wire stack to roof, if needed

directly connected, totally enclosed weatherproof motor

1 inch (2.5 cm) diameter hole at the lowest point in the blower scroll for drainage to the roof
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Informative Annex 2
(formerly Annex G)

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 this Standard.

Generation of any gas or vapor used to decontaminate a BSC should be done in a safe manner to limit exposure to personnel and to the environment. A safe manner should include instructions and equipment necessary to safely stop the generation of gas or vapor, and neutralize or remove the gas or vapor to limit exposure.

I-2.1 Recommended biosafety cabinet decontamination procedure

I-2.1.1 Decontamination

Biosafety cabinet decontamination is one of many critical components to the health and safety of lab personnel, the environment and the general public. Before performing any decontamination activity the compatibility of the decontamination process to the BSC, the effectiveness against possible contaminants and the potential health risks to personnel must be determined. Proper personal protective equipment (PPE) should be worn as specified within the safety data sheets (SDSs) or determined by a qualified safety professional. BSCs should be decontaminated before and after each use, prior to relocation, servicing, or decommissioning, or both. Both surface and space decontamination activity should be considered as potential decontamination strategies. Surface decontamination, performed by applying a chemical disinfectant to accessible BSC surfaces should be used in routine practice. Space decontamination, performed using a gas or vapor sterilant, is typically reserved for areas that are unreachable or inaccessible within the BSC such as internal air plenums, motor blowers and HEPA/ULPA Filters. The following describes decontamination strategies that can assist in eliminating the transmission of infectious agents and hazardous substances outside of the biosafety cabinet.

Decontamination is the inactivation or destruction of infectious agents or neutralization of toxic agents as stated in definitions Section 3.16.

The purpose of this Annex, as part of the Standard, is to establish the appropriate minimum requirements for field microbiological decontamination procedures of BSCs that are designed to protect personnel, product, and the environment and to implement the decontamination procedure(s) safely and effectively.

This Standard includes the requirements for the field decontamination procedures attained from the actual decon validation data of BSCs. It will be specific to the decontamination generation devices operational key parameters in conjunction with the generation chemicals that provided the decontamination validation data, incorporating a safety margin, as applied to the class or style of BSC.

Efficacy of the decontamination procedure utilizing a specific chemical(s) shall be evaluated and accepted by the validation data. If no such data is available, efficacy must be determined prior to use. Space decontamination of BSCs using gases or vapors must be validated using appropriate biological indicators with or without supplementary chemical indicators (if available) to meet this Standard.

Material compatibility studies shall also be performed with the validation studies indicating that the decontaminating chemical presents no or limited adverse effects to the typical materials that are exposed within a BSC. Material compatibility testing should include but not be limited to 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). 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.

I-2.1.1.1 Cabinet decontamination for field certification purposes

BSCs shall be appropriately decontaminated (either or both the space or surface) when maintenance work, HEPA/ULPA filter changes, and performance tests require access to any contaminated portion of the cabinet. All work surfaces and exposed surfaces shall be decontaminated with a suitable surface disinfectant before field 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 field certification tests when the cabinet has been used with agents assigned to BSL-2, recommended when the cabinet has been used with an agent assigned to BSL-3, and required when the cabinet has been used with an agent assigned to BSL-4.

I-2.1.1.2 Cabinet decontamination for relocation

BSCs shall be appropriately surface decontaminated prior to moving to another location. Based on risk assessment by qualified personnel, space decontamination may also be required. Mobile BSCs may not require decontamination if kept housed within the facility it is used in. Relocation out of the containment facility will require appropriate decontamination.

I-2.1.1.3 Cabinet decontamination for spills and splashes

BSCs shall be appropriately decontaminated (surface) after spills and splashes of research agents. Contaminated surfaces should be suitably decontaminated. This also is to include all exposed interior surfaces, sashes, grilles, the drain spillage trough and drain valve, and any exposed exterior work surfaces such as arm rests.

I-2.1.1.4 Cabinet decontamination for decommissioning and salvage

BSCs shall be appropriately decontaminated (either or both the space or surface) prior to decommissioning and salvage. See Section I-1.10 Decommissioning process – for all requirements.

I-2.1.2 Decontamination types

I-2.1.2.1 Biological surface decontamination

All surfaces and exposed areas of the cabinet work area should be surface decontaminated with a suitable disinfectant or neutralizing agent. Surface decontamination of the biosafety cabinet should be completed at least before and after work, prior to cabinet field certification and before any space decontamination activity described below. Disinfecting the cabinet work area, work surface and any equipment used before and after every procedure will not only assist in minimizing the transmission of hazardous substances but also prevent a buildup of deposits over time, simplifying future decontamination activities.

Many common surface disinfectants can be used for microbial decontamination, including quaternary-ammonium compounds, iodophors, ethanol, phenol compounds and isopropanol. Hypochlorite (chlorine bleach) in diluted concentrations, e.g., 10%, is commonly used as a surface disinfectant, however caution should be used as chlorine bleach can cause stainless steel to crack, or pit, or both, if not completely removed from the metal surface. If using chlorine bleach as a surface disinfectant always follow up with a sterile water rinse and wipe all surfaces completely dry.

For the removal or detoxification of chemicals used within the BSC, organic solvents such as alcohols, ethers, ketones, aromatics, straight-chain alkanes, and common petroleum products can be used.
Whichever surface disinfectant is chosen, first check the SDS or with a qualified safety professional for proper PPE, compatibility with chemicals, agents and equipment before use.

Additional information can be referenced within this Standard in Section I-1.6.2 Surface disinfectants, as well as in the BMBL, Appendix A – Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets.

I-2.1.2.2 Biological space decontamination

Access to unreachable contaminated areas of the BSC may require a space decontamination using an approved sterilant. Space decontamination using a gas or vapor sterilant may be advised to have complete decontamination of all components of the BSC. Common sterilants include formaldehyde, hydrogen peroxide, and chlorine dioxide. Prior to use a safety and risk assessment of the biosafety cabinet's biological agents and compatibility should be performed by a biosafety officer or qualified safety professional. It may be desirable to perform a space decontamination of the entire cabinet before performing field certification tests, service activities such as HEPA/ULPA filter replacement or troubleshooting if the BSC has been used with agents assigned to Risk Group 2 or higher.

Whichever space decontamination sterilant is chosen, first check the SDS or with a qualified safety professional for proper PPE, compatibility with chemicals, agents and equipment before use.


I-2.1.2.3 Chemical, radiological, oil, or heavy metal decontamination

NOTE — Annex I-2 covers only microbiological decontamination.

Clean all surfaces with an appropriate cleaning agent. Use formaldehyde gas, chlorine dioxide, hydrogen peroxide vapor or an acceptable alternative space decontamination procedure if biological agents may also be present. Not all surfaces may be thoroughly cleaned such as motors, blower impellers, HEPA/ULPA filters, prefilters, diffusers, etc. and will require appropriate disposal.

Additional information of radiological decontaminations can be found in the BMBL, Appendix A – Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets.
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:

   BSL-1   BSL-2   BSL-3   BSL-4   Not applicable

4. Complete and sign the certification below:

<table>
<thead>
<tr>
<th>CONTAINED HAZARD (y)</th>
<th>DECONTAMINATION PROCEDURE</th>
<th>NONE</th>
<th>HAZARD TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>BIOLOGICAL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>CHEMICAL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>RADIOLOGICAL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>OIL, HEAVY METAL (e.g., lead, mercury, or other hazardous material.)</td>
</tr>
</tbody>
</table>

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 ___________________________
I-2.1.3  Decontamination Cabinet design as per cabinet classification

Although this Standard covers only Class II biosafety cabinetry, Class I and Class III cabinets may also require decontamination. All Biosafety cabinets can be used for work with biological agents assigned to BSLs 1 through 4, depending on the facility design as described in BMBL.

Current biosafety cabinet designs covered under this Annex are:

— Class I;
— Class II Type A1;
— Class II Type A2;
— Class II Type B1;
— Class II Type B2;
— Class II Type C1, and
— Class III.

I-2.1.3.1  Key parameters for decontamination as per the validated procedure

Key Parameters covered under this Annex are:

— gas or vapor base generating chemical(s) starting concentrations (percentage) as per manufacturer’s provided chemicals with the specific generation device;
— gas or vapor generating chemical(s) starting amount or quantity (weight) as per manufacturer’s provided chemicals with the specific generation device in correlation with the calculated volume of the BSC under decon;
— temperature range within the BSC prior to the introduction of decontaminant;
— humidity range within the BSC prior to the introduction of decontaminant;
— injection or generation rate and times shall be injected or generated at the manufacturers specified flow rates as per volume of the BSC under decontamination;
— circulation or internal distribution rates and times shall be specified by the manufacturer in order to ensure a uniform concentration of the decontaminant throughout the BSC. It is advantageous to periodically operate the BSCs internal blower (bump the BSC) to assist circulation. The manufacturer’s generation device may also provide a means to assist circulation of the decontaminating agent;
— exposure time shall include commencement and conclusion of the exposure period or specific duration;
— final concentration shall be provided within the BSC, by which the chemical concentration within the BSC was monitored throughout the decontamination validation process; and
— neutralization or aeration, scrubbing / venting rates and times to remove or render harmless the decontaminant used.

I-2.1.3.2  Decontamination cabinet design

Cabinets shall be designed to be decontaminated with an inactivating agent (such as formaldehyde gas, chlorine dioxide gas or hydrogen peroxide vapor) without being moved. Closure to contain decontaminating agents should be limited to gas-tight sealing of air intake(s) and exhaust openings with metal plates, plastic, nonpermeable compatible film and tape, or equivalent.
Gas tight valves or dampers, if provided or field installed, suitable for decontamination shall be located on the clean, noncontaminated side of the HEPA/ULPA filter(s) air intake(s) or exhaust openings.

Gas tight supply, return or recirculation ports to attach to the generation devices, of proper size, if provided or field installed (temporary or permanent), suitable for decontamination shall be located on the clean, noncontaminated side of the HEPA/ULPA filter(s), air intake(s) and front access or exhaust openings.

I-2.1.3.3 Decontamination cabinet preparation

Cabinets shall be prepped in similar techniques to be decontaminated no matter what the inactivating agent (formaldehyde gas, chlorine dioxide gas or hydrogen peroxide vapor) is used, to as gas-tight sealing methodologies as possible.

The direction of the decontaminate introduction in conjunction with the sealing parameters shall be in the same direction of the airflows within an operating BSC. This is to ensure when the BSC is bumped, (sometimes not possible on a B1, B2 or a BSC with the motor not operational) it will assist in movement of the humidification or dehumidification and even distribution of the decontamination chemical. It also ensures that no potentially contaminated air from within the BSC will be pulled into the decontaminating device; it will only be recirculated via the exhaust HEPA filter, as to rely on for personal and surrounding environmental protection.

For cabinets with nonoperational blowers, a means shall be provided to circulate the humidification, de-humidification and the decontaminating gas or vapor to ensure a uniform concentration.

I-2.1.3.3.1 Exhaust preparations

Various classifications require different exhaust sealing configurations. Follow the specific methodology as per stated in the validated procedure. Field ducting special requirements are listed below for ducted BSCs:

- Class I;
- Class II Type A1, A2 (exhausted via thimble or canopy);
- Class II Type B1, B2;
- Class II Type C1 (exhausted via thimble or canopy); and
- Class III.

a) Isolate the facility exhaust system from the decontamination process. This may be as simple as closing a gas-tight valve at the exhaust connection for a Type B BSC. It may require removing the canopy on a Type A BSC or disconnecting the BSC from the ductwork.

b) If the BSC exhaust ducting does not have or has a damaged gas-tight decontamination damper, disconnect the cabinet from the building exhaust system and form a gas tight seal. If the cabinet exhaust air is discharged into the room, tape a plastic cover over and completely seal the exhaust port.

c) A gas tight valve may be located further downstream from the BSC.

d) When possible, leave the back draft, EVAV or other balancing damper(s) in their original position. If used to seal off the ductwork, note the original position for restoration after the work is complete.

e) Ensure that this is indeed a gas tight damper with no by-pass leakage. This shall be checked with a manometer with accuracy of 0.001” w.c. (0.25 Pa) after the BSC has been sealed and all equipment in place. Pressure loss shall be zero or as close to zero as possible.

Temporally by-pass any low flow alarms to allow the recirculation blower to operate in Type B1 cabinets. The generator or additional fans shall be used to recirculate the proper humidity levels and decontamination gases for Type B2 cabinets.
I-2.1.3.3.1.1 Exhaust duct considerations

If the exhaust duct is more than 3 feet (1 m) long from the final sealing location to the exhaust HEPA/ULPA filter, additional gas or vapor will be needed to compensate for the increased volume. The additional volume (calculated from length, widths and or diameter) of ductwork must be added to the gas or vapor concentrations for the BSC under decon. Calculate additional gas or vapor as per manufacturer’s chemical calculations.

I-2.1.3.3.1.2 Intake preparations

Classifications are generally the same, field special requirements are listed below for ducted BSCs:

- Class I, Class II Type A1, A2, B1: BSCs may be sealed in various methods in addition to a technique such as tenting the entire front / sides / back of the BSC; and

- Class II Type B2: Seal the downflow supply intake in addition to the front access opening of the BSC.

I-2.1.3.4 Decontamination risk assessment, cabinet security and safety precautions

BSCs locations shall be safely secured prior to the decontamination procedure no matter what inactivating agent (formaldehyde gas, chlorine dioxide gas or hydrogen peroxide vapor) is used.

Universal precautions shall be strictly adhered to.

I-2.1.3.4.1 Decontamination risk assessment

Prior to beginning any decontamination, service work or related testing, a risk assessment shall be performed by a biosafety officer or the site’s qualified safety representative to determine what safety precautions must be taken to eliminate or reduce exposures or hazards to personnel, product and the environment.

These hazards may include: biological, radiological, volatile or toxic chemicals, oil, heavy metals, cytotoxic agents, hazardous drugs, APIs, toxins, recombinant DNA / genetically modified organisms (GMOs), prions, or other hazardous materials.

Special instructions shall be added to specify procedures if any of these hazards are present. A “risk assessment” form shall be reviewed, completed, and signed by the customer contact or cognizant authority having knowledge of the past uses and materials manipulated within the BSC or other device.

I-2.1.3.4.2 Decontamination cabinet security

Advanced notice of the decontamination shall be given to all personnel that may work in or around the area where the decontamination is to take place. All unrelated laboratory animals, plants, tissue cultures, incubators, cell media and associated test materials should be protected.

Verification is to be provided that the BSC is located within an un-recirculated space with a pressure negative relative to all bordering areas, labs and hallways, etc. Alternatively, a negative pressure secondary containment system may be used to surround the decontamination area.

Secondary containment is highly recommended for all personal and surrounding environmental protection. This method of protection allows the user’s employees to continue working during the decontamination process. Secondary containment involves construction of a tent that surrounds the sealed BSC (primary containment) being decontaminated.
The output of a blower is attached to a scrubber, catalyst, or in-house nonrecirculated exhaust vent, and its input connected by hose into the tented area. With the blower on or a temporary connection to in-house nonrecirculated exhaust point, a negative pressure is developed within the secondary containment barrier. This ensures that any gas or vapor that may leak out of the BSC during decontamination will stay contained within the barrier and be filtered through the scrubber, catalyst, or exhausted to outside ambient. The air exiting the scrubber or catalyst will be free of the decontaminating gas, allowing for personnel to move freely around the area surrounding the decontamination.

If a secondary containment system or barrier is not incorporated, a minimum of a 5 to 20 foot (1.5 to 6.1 m) negative pressure exclusion zone, or basically the entire un-recirculated space with appropriate signage, must be created about the BSC to be decontaminated to limit the possibility of exposing unauthorized personnel to the decontaminating agent.

If requested, the decontamination may be scheduled over weekends or overnight to reduce potential exposure to personnel or others. If the BSC or device is located in a negative pressure secondary containment system or in a un-recirculated negative pressure space with all unassociated decontamination personal and or laboratory animals removed throughout the entire process, the decontamination may be performed during normal working hours.

I-2.1.3.4.3  Decontamination safety precautions

Appropriate signage and “CAUTION” cordon-off tape must be posted as per gas or vapor:

— OSHA signage standard 1910.145 shall be followed, i.e., size, colors, etc.

I-2.1.3.4.3.1  Formaldehyde gas signage:

DANGER
KEEP OUT
FORMALDEHYDE GAS IN USE
IRRITANT AND POTENTIAL CANCER HAZARD IN USE
Authorized Personnel Only
In case of emergency call [operator and site or customer contact]
[starting / ending dates]
After hours call – [operator and site or customer contact]
[starting / ending times]

I-2.1.3.4.3.2  Chlorine dioxide gas signage:

DANGER
KEEP OUT
CHLORINE DIOXIDE GAS IN USE
Hazardous oxidizing gas – Authorized personnel only
In case of emergency call [operator and site or customer contact]
[starting / ending dates]
After hours call – [operator and site or customer contact]
[starting / ending times]
I-2.1.3.4.3 Hydrogen peroxide vapor signage:

DANGER
KEEP OUT
HYDROGEN PEROXIDE VAPOR IN USE
Hazardous oxidizing gas – Authorized personnel only
In case of emergency call [operator and site or customer contact]
[starting / ending dates]
After hours call – [operator and site or customer contact]
[starting / ending times]

The signage shall indicate start and end dates and times by the responsible operator and contact information.

Signs shall remain posted prior to and during the entire decontamination process until confirmation that all gases or vapors have dissipated. As an example, small rooms containing equipment being decontaminated shall be posted with signage and caution tape at all entrance doors. Typically an exclusion area with a minimum of a 5 to 20 foot (1.5 to 6.1 m) radius about the equipment being decontaminated is used.

Once warnings are posted, admission into the decontamination area will be prohibited to all personnel except those directly involved with the decontamination.

The decontamination area will be off limits until the decontaminating gas or vapor concentration within the area is determined to be at a safe level by a portable gas or vapor analyzer, colorimetric indicator tube testing or equivalent.

I-2.1.3.4.4 Decontamination safety precautions, PPE, SDS, exposure limits:

Appropriate PPE must match the hazard. Operating personnel shall be properly trained for use of PPE and tested prior to each use. Don appropriate PPE when handling, measuring or potentially coming in contact with any hazardous chemicals.

The generation chemicals (gas, solid or liquid) used for gas or vapor decontamination are corrosive to skin. When handled, the operator must wear at minimum; safety glasses, a lab coat, and gloves. The chemicals should not be left exposed to open air for unnecessary duration. It is advised that the operator don the appropriate respirator when adding the chemicals to the generator.

Review all the appropriate SDS’s prior.

Review and know all exposure limits:

— OSHA permissible exposure limit (PEL), 8 hour time-weighted average (TWA);
— NIOSH recommended exposure limits (RELs), TWA, short-term exposure limit (STEL);
— ACGIH threshold limit values (TLVs), TWA, STEL; and
— NIOSH – Immediately Dangerous to Life or Health Concentrations (IDLH).

An appropriate respirator, dependent upon the worst case scenario, (preferably a full-face or PAPR type) must be immediately available for any personnel who must be within a decontamination zone. If leaks are detected from the decontamination system or BSC during operation, the gas or vapor generation must be stopped and a respirator donned until the leak has been repaired.

In the event of gross leakage, where sealing is impossible in a timely manner, a contingency plan is to cease the decontamination process, don the appropriate PPE, and initiate neutralization, scrubbing, or venting. Individuals immediately outside the secondary containment area or within room should be asked to vacate the area.
Monitor the environmental conditions at regular intervals during the decontamination process. Automatic continuous monitoring may be employed.

If at any time during the decontamination process the decontaminant is detected in the environment exterior to the BSC or secondary containment by odor or instrument at a concentration approaching TLV level, stop generation of the decontaminant and do not resume until the leak source(s) has been corrected.

I-2.1.3.4.5 Emergency exhaust provisions

To provide for emergency evacuation of the decontamination gas / vapor and to allow removal of the neutralizing agent following the decontamination and neutralization, a flexible hose can be prepositioned close to the cabinet. This hose must be attached to the chemical fume hood or other exhaust suitable for the evacuation of toxic fumes.

I-2.1.3.4.6 Decontamination cabinet piping of utilities, flammable gases

Shut off utility lines piped into the BSC prior to decontamination. All utility gases such as natural gas, oxygen or other flammables shall be turned off and then verified that they are off. Preferably shut off the gas flow to the BSC at the remote shut off valve (every BSC should have one). Ensure the internal valve or petcock is also fully closed. In the event the natural gas cannot be remotely shutoff, verify that there are no leaks in the gas valve or petcock by using the soap bubble leak test solution.

I-2.1.4 Decontamination methodologies

In most instances where space decontamination is necessary, one of the procedures described below using depolymerized paraformaldehyde (formaldehyde gas), chlorine dioxide gas, or hydrogen peroxide vapor is used. Prior to decontamination with an alternative method 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. The decontaminating agent should always be an EPA registered pesticide.

A qualified safety and risk assessment of cabinets potentially contaminated with biological agents or others should be performed by a biosafety officer or qualified safety professional. Alternate methods are required in certain instances, e.g., slow disease viruses or agents mentioned in Section I-2.1.2.2. The decontamination method should be determined by consultation between user and field certification agency or personnel performing the decontamination.

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 shall be followed. Similarly, automated chlorine dioxide gas systems are available which may be used if the manufacturer’s instructions are followed.

When using vaporous hydrogen peroxide, similar precautions as used for formaldehyde shall be followed. Similarly, automated vaporous hydrogen peroxide systems are available which may be used if the manufacturer’s instructions are followed.
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).

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/ft³ (11 g/m³) of space to determine the gram weight of paraformaldehyde required [CHECK CONCENTRATION]. Determine the stoichiometric amount of NH₄HCO₃ 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) Follow Section I-2.1.3.3.1 to seal the cabinet exhaust opening.

d) 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).

e) Place an additional heating device on the work tray for the neutralizing agent. The neutralizing agent (NH₄HCO₃ 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 NH₄HCO₃ 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 NH₄HCO₃ 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 NH₄HCO₃ 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 NH₄HCO₃ 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 NH₄HCO₃ or equivalent alternative is heated and releases ammonia.

f) 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.

g) 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.

h) Determine the temperature and humidity inside the cabinet.

i) 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.

55 NIOSH, Department of Health and Human Services (DHHS), Hazard Review of Bis(chloromethyl)ether (BCME).
j) 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 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.

k) Plug the cord of the heating device into an outlet not installed on the cabinet.

l) After 25% of the paraformaldehyde has depolymerized, turn on the cabinet blower(s) for 10 to 15 seconds. Repeat after 50%, 75%, and 100% of the paraformaldehyde has depolymerized. 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 step p below.

m) Disconnect the hot plate and heating device used for the paraformaldehyde from the electrical outlets.

n) Allow the cabinet to stand for a minimum of 6 hours, preferably overnight (12 hours).

o) Prepare the neutralizing agent as previously established in step g) and energize the heating device containing the NH₄HCO₃ and the cabinet blower until the NH₄HCO₃ has dissipated. As with the paraformaldehyde, after 25% of the NH₄HCO₃ has depolymerized, turn on the cabinet blower(s) for 10 to 15 seconds. 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 hours.

p) Let the cabinet stand for at least 1 hour before opening seals.

q) 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 × 6 in [15 × 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.

I-2.1.4.2 Chlorine dioxide (CD)

I-2.1.4.2.1 Method 1 – Fixed amount of CD

a) Calculate the total volume (in ft³ or m³) 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/ft³ (4.7 g/m³) 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) Follow Section I-2.1.3.3.1 to seal the cabinet exhaust opening.

d) Depending upon the manufacturer, CD gas generation may take place within the BSC or external to the BSC with a dispersion system to circulate the CD gas within the BSC. Following the manufacturer's instructions, place the CD generator and delivery system within the BSC, or attach the external CD generator and delivery system to the BSC. 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.

e) 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 to 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.

f) 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.

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, or its recirculation, or both, 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 to 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, or recirculation blower, or both, 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 feet 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 dispersion of CD gas into or within the cabinet. Use the amount of CD-generating chemical as determined in step b above.

l) 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, or CD recirculation blower, or both, should be energized for at least 1 minute during every 15 minutes of contact time.

m) Allow the cabinet to stand a minimum of 85 minutes from the initiation of CD gas generation with the assumption that the duration until peak concentration will be under 10 minutes.

n) 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.
o) 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 minutes.

I-2.1.4.2.2 Method 2 – Fixed concentration of CD

a) Follow Section I-2.1.3.3.1 to seal the cabinet exhaust opening

b) Depending upon the manufacturer, CD gas generation may take place within the BSC or external to the BSC with a dispersion system to circulate the CD gas within the BSC. Following the manufacturer’s instructions, place the CD generator and delivery system within the BSC, or attach the external CD generator and delivery system to the BSC. 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 inoperative internal blower, a means of recirculation to ensure adequate distribution of CD and RH 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.

c) Provide a means either within or external to the BSC, by which the air within the BSC may be humidified and the RH monitored and maintained within a range of 60 to 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.

d) 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.

e) 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 foot from the CD gas inlet.

f) Close the opening to the work area with heavy gauge plastic film and tape. Seal all possible leak areas, including but not limited to the following:

- the exit of electrical cords;
- around inlet and outlet hoses for the CD gas and its recirculation;
- around the sash; and
- at the junction of the plastic film and cabinet.

g) Determine the temperature and humidity inside the cabinet.

h) The temperature should be 60 °F (15 °C) or higher, and the humidity should be 60 to 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, or recirculation blower, or both, shall be operating during the entire humidification process.

i) 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 feet 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.
j) Begin generation and dispersion of CD gas into or within the cabinet. Monitoring the CD concentration within the cabinet, cease generation when the concentration has at least achieved the targeted CD concentration (3 or 5 mg/L).

k) 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, or recirculation blower, or both, should be energized for at least one minute during every 15 minutes of contact time.

l) Continuously monitor the CD gas concentration during decontamination. Whenever the CD concentration decreases below the targeted concentration level, (3 or 5 mg/L) generate and inject more CD gas until the CD concentration has at least attained the targeted concentration level.

m) Continue the decontamination for a duration of 60 minutes for a targeted concentration of 3 mg/L or 45 minutes for a targeted concentration of 5 mg/L, measured from the time that the targeted concentration was first achieved.

n) 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.

o) 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 minutes.

I-2.1.4.3 Hydrogen peroxide (HP)

I-2.1.4.3.1 Method 1 – Fixed amount of HP with gas vaporizer inside BSC cabinet

a) Calculate the total volume (in ft³ or m³) of the cabinet internal volume area by multiplying the height, width, and, depth.

b) Calculate the amount of HP required for the decontamination, using the HP solution required by the equipment manufacturer.

c) Follow the hydrogen peroxide gas generator manufacturer’s instructions to fill and prepare the instrument.

d) Place the vapor phase hydrogen peroxide gas generator (VU) within the BSC on the work surface and run the power cord outside the BSC under the sash.

e) Follow directions in Sections I-2.1.3.3.1 and I-2.1.3.3.2 to seal the cabinet and seal off the facility exhaust system. Provide a means to circulate the HP if the cabinet blower is not operational.

f) Check that the RH within the BSC is in the range of 10 to 85% RH and the ambient temperature is 60 to 90 °F (16 to 32 °C). If parameters are not within these ranges adjust accordingly.

g) Verify all possible leak areas, such as the exit of electrical cords, around the sash, and at the junction of the plastic film and cabinet are sealed. See Section I-2.1.3.3.1 for leak testing instructions.

h) Follow all security and safety precautions in Sections I-2.1.3.4.3 and I-2.1.3.4.4.

i) Select the appropriate program / settings on the HP generator to deliver the calculated volume of peroxide. Reference appropriate equipment manufacturer’s manual.

j) Start decontamination cycle following manufacturer’s manual and appropriate local, state, and federal safety regulations.
If the cabinet blower is operational, pulse the blower for 10 to 12 seconds, 20 minutes into the gassing cycle, and then every 20 minutes during the gassing cycle. If cabinet blower is not available, use other means to circulate the HP.

Monitor the environment around the BSC with an appropriate HP gas leak detector device during the gassing phase to ensure there are no leaks.

Allow the cycle to run to the completion of gassing and then turn off the HP decontamination unit.

If the BSC is not connected to a facility exhaust system, skip to step o. If the BSC is connected to a facility exhaust system, Open up the manual exhaust damper, of the BSC, slightly and let vent for 5 to 10 minutes, after which slit the plastic sheeting at the sash and open up the manual exhaust damper, on the BSC, to full open. Be sure not to allow the internal pressure of the BSC to become too negative and damage the casing of the BSC.

At the end of the gassing phase, switch to the aeration phase of the cycle (the system may do so automatically). Follow the manufacturer’s instructions for aeration.

Allow sufficient time for the HP level within the cabinet to decrease to its STEL, the short-term permissible exposure limit (1 ppm). This time depends upon the HEPA filter size, condition and size of cabinet but will generally require 25 to 35 minutes. Additional aeration may be required.

Method 2 – Fixed amount of HP with gas vaporizer OUTSIDE the BSC

Calculate the total volume (in ft³ or m³) of the cabinet internal volume area by multiplying the height, width, and, depth.

Calculate the amount of HP required for the decontamination, using the HP solution required by the equipment manufacturer. Refer to decontamination kit Manufacturers User Manual as needed.

Follow directions in Sections I-2.1.3.3.1 and I-2.1.3.3.2 to seal the cabinet and seal off the facility exhaust system, allowing for connection of the external HP generator.

Follow the hydrogen peroxide gas generator manufacturer’s instructions to fill, prepare and connect the instrument to the cabinet.

Check that the RH within the BSC is in the range of 10 to 85% RH and the ambient temperature is 60 to 90 °F (16 to 32 °C). If parameters are not within these ranges adjust accordingly.

Verify all possible leak areas, such as the exit of electrical cords, around the sash, and at the junction of the plastic film and cabinet are sealed. See Section I-2.1.3.3.1 for leak testing instructions.

Follow all security and safety precautions in Sections I-2.1.3.4.3 and I-2.1.3.4.4.

Select the appropriate program / settings on the HP generator to deliver the calculated volume of peroxide.

Start decontamination cycle following manufacturer’s manual and appropriate local, state, and federal safety regulations.

Monitor the environment around the BSC with an appropriate HP gas leak detector device during the gassing phase to ensure there are no leaks.

Allow the cycle to run to the completion of gassing for the required exposure time. Follow the manufacturer’s instructions for aeration. At the end of aeration, end the decontamination cycle and turn off the HP decontamination unit.
l) If the BSC is not connected to a facility exhaust system, skip to step m. If the BSC is connected to a facility exhaust system, open up the manual exhaust damper of the BSC, slightly and let vent for 5 to 10 minutes. After 5 to 10 minutes, slit the plastic sheeting at the sash and open up the manual exhaust damper on the BSC to full open. Be sure not to allow the internal pressure of the BSC to become to negative and damage the casing of the BSC.

m) Allow sufficient time for the HP level within the cabinet to decrease to its STEL, the short-term permissible exposure limit (1 ppm). This time depends upon the HEPA filter size, condition and size of cabinet but will generally require 25 to 35 minutes. Additional aeration may be required.

I-2.2 Recommended HEPA/ULPA filter disposal procedures

I-2.2.1 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.

I-2.2.2 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.

I-2.2.3 Decontaminated HEPA/ULPA filters may be safely buried in a sanitary landfill because they no longer pose a hazard.
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Informative Annex 3
(formerly Annex H)

Recommended materials, finishes, and construction

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 this Standard.

I-3.1 Sheet metal and finishes

I-3.1.1 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).

I-3.1.2 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).

I-3.1.3 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 minutes at 300 °F (149 °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.

I-3.2 Glass

I-3.2.1 If safety glass is used for the sash / window, it should be nominally 0.25 inch (6.4 mm) laminated safety plate glass.

I-3.2.2 If tempered glass is used for the sash / window, it should be nominally 0.25 inch (6.4 mm) tempered glass conforming to American Society for Testing and Materials C 1048 or equivalent.

I-3.3 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 inch (6.4 ± 0.8 mm) thick by 0.75 ± 0.031 inch (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.\(^{58}\)

\(^{58}\) Specifications taken from Military Specification MIL-F-51068 (Cancelled), Gasket Assembly. <www.defenselink.mil/pubs/>
I-3.4 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 lists other acceptable materials.

I-3.5 Specifications

The specifications require filter-mounting tolerances for openings up to 20 inches (51 cm) ± 0.063 inch (1.6 mm); and openings over 20 inches (500 mm), to be ± 0.13 inch (3.2 mm). The squareness of filter mountings should have diagonals within 0.063 inch (1.6 mm) total allowance. Flatness at the filter gasket seal surface should be ± 0.015 inch (0.4 mm) within any 10 inches (250 mm) run.\(^{59}\)

I-3.6 Sealants

I-3.6.1 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,\(^{60}\) or equivalent, is acceptable when used in accordance with the manufacturer's recommendations.

I-3.6.2 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/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.

I-3.6.3 The medium within HEPA/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.

I-3.7 Fans

Fan(s) should be direct connected and conform to Air Movement and Control Association (AMCA)\(^{61}\) standards.

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\(^{60}\) The Dow Chemical Company. 2030 Dow Center, Midland, MI 48642. <www.dow.com>

\(^{61}\) Air Movement and Control Association (AMCA). 30 West University Dr., Arlington Heights, IL 60004. <www.amca.org>
I-3.8 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), or Underwriters Laboratories (UL), whichever is applicable and provides the highest standard.

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Informative Annex 4
(formerly Annex I)

Reference standards and specifications pertinent to Class II biosafety cabinetry

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 this Standard.

I-4.1 Miscellaneous publications

I-4.1.1 Air Movement and Control Association (AMCA)

— ANSI/AMCA 99, Standards Handbook
— AMCA 210-67, Test Code for Air Moving Devices
— AMCA 99-2406, Designation for Rotation and Discharge of Centrifugal Fans
— AMCA 211, Certified Ratings Program Product Rating Manual for Fan Air Performance

I-4.1.2 American National Standards Institute, Inc. (ANSI)

— ANSI S1.4-1984, Specification for Sound Level Meters
— ANSI S2.2-1959 (R1982), Methods for the Calibration of Shock and Vibration Pickups

I-4.1.3 Illuminating Engineering Society (IES)

— The Lighting Handbook: Reference and Application

I-4.1.4 National Electrical Code

I-4.1.5 National Electrical Manufacturers’ Association (NEMA)

I-4.1.6 Underwriters Laboratories, Inc.

— UL 62-1965, Flexible Cord and Fixture Wire
— UL 94-1985, Tests for Flammability of Plastic Materials for Parts in Devices and Appliances
— UL 181, Factory-Made Air Ducts and Connectors
— UL 586-1985, High Efficiency Particulate Air Filter Units
— UL 817-1987, Cord Sets and Power-Supply Cords
— UL 1262-1984, Safety Laboratory Equipment

I-4.1.7 US Department of Energy

— ERDA 76-11, Nuclear Air Cleaning Handbook (March 1976)

63 Latest edition in effect at the time of manufacture.
I-4.1.8 US Department of Labor

— 29 CFR § 1910.134, Occupational Safety and Health Administration (OSHA) – Safety and Health Standards for Respiratory Protection

I-4.1.9 US Department of Health and Human Services

— 30 CFR § 2, Centers for Disease Control (CDC), National Institute of Occupational Safety and Health (NIOSH) – Requirements for Respirator

I-4.1.10 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-4.1.11 US Naval Research Laboratory

— Report 5959 (July 1963)

I-4.1.12 American Society for Testing and Materials (ASTM)

— ASTM C1048, Standard Specification for Heat-Strengthened and Fully Tempered Flat Glass

I-4.2 Federal specifications

— CC-M-636, Motor, Alternating-Current (Fractional Horsepower)
— J-C-145, Cable, Power, Electrical and Wire, Electrical; (Weather Resistant)
— 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
— 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
— W-C-00596, Connector, Plug, Electrical; Connector Receptacle, Electrical
— W-S-00896, Switch, Toggle
— W-S-893, Switch, Toggle, and Mounting Strap (Interchangeable)

I-4.3 Federal standards

— Federal Standard No. 102, Preservation, Packaging and Packing Levels
— Federal Standard No. 123, Marking for Domestic Shipment

I-4.4 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
Informative Annex 5
(formerly Annex J)

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 this Standard.

I-5.1 Helium leak test

I-5.1.1 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.

I-5.1.2 Apparatus

The helium leak detector shall be calibrated in accordance with the manufacturer's instructions using a calibrated leak standard.

I-5.1.3 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 Section I-5.1.1).

c) Pressurize the cabinet with air to 2 inches w.g. (500 Pa). If the cabinet holds this pressure without more than ± 10% loss for 30 minutes, then release pressure. If the cabinet does not hold this pressure, examine for gross leaks with liquid leak detector (see Section I-5.1.1), 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 inches 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 inches w.g. (500 Pa).

e) Turn on the cabinet blower for 30 seconds to circulate gas.

f) Adjust the helium leak detector to a sensitivity setting of 1 ± 10⁻⁵ mL/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 (25 mm/s), keeping the probe 0.25 to 0.50 inch (6.3 to 13 mm) away from the surface (see Figure 43).

I-5.1.4 Acceptance

Measured leakage from any point in the cabinet shall not exceed a leak rate of 1 × 10⁻⁵ mL/s when pressurized to 2 inches w.g. (500 Pa) with at least 15% concentration of helium.
I-5.2 Sulfur hexafluoride (SF₆) leak test

I-5.2.1 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.

I-5.2.2 Apparatus

— an industrial-type SF₆ leak detector (Ion Track Inc. [ITI] Leakmeter, or equivalent capable of detecting a halide leak of 1 × 10⁻⁷ mL/s); and

— the SF₆ leak detector (shall be calibrated in accordance with the manufacturer's instructions using a calibrated leak standard).

I-5.2.3 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 Section I-5.1.1).

c) Pressurize the cabinet with air to 2 inches w.g. (500 Pa). If the cabinet holds this pressure without more than ± 10% loss for 30 minutes, release pressure. If the cabinet does not hold this pressure, examine for gross leaks with liquid leak detector (see Section I-5.1.1), repair, and retest.

d) Pressurize the air filled cabinet at atmospheric pressure to 2 inches w.g. (500 Pa) with SF₆ gas.

e) Turn on the cabinet blower for 30 seconds to circulate gas.

f) Adjust the SF₆ leak detector to a sensitivity setting of 5 × 10⁻⁷ mL/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 (25 mm/s), keeping the probe 0.25 to 0.50 inch (6.4 to 13 mm) away from the surface (Figure 43).

I-5.2.4 Acceptance

Measured leakage from any point in the cabinet shall not exceed a leak rate of 5 × 10⁻⁷ mL/s to compensate for the dilution of halide gas.
0.25 to 0.5 inches (6.4 to 13 mm) maximum distance from seam being tested

test probe

Figure 43
Scanning for tracer gas leaks
Informative Annex 6  
(formerly Annex K)

Protocol for the validation of alternate biosafety cabinet decontaminating methods and agents

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I-6.1 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 (BSCs). 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 (BI) 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.

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 BI, 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.

I-6.2 Protocol

I-6.2.1 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 BSCs. 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 inch w.g. (75 Pa) (50%) of their starting clean value.

c) Typically BIs 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 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 BIs within the BSC. Locations include, where possible:

— 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 inches from the nearest outside corner of the exhaust HEPA/ULPA filter;

— one pair of BIs is placed within a potentially contaminated positive pressure plenum;

— one pair of BIs is placed beneath the work surface in the plenum below the cabinet work area; and

— 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.

I-6.2.2 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.

— if possible, the procedure should not designate the use of equipment provided by unique manufacturers;

— 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, or time, or both) than what is intended for ultimate field usage;

— 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;

— the decontamination method may have requirements of permitted humidity, or temperature, or both, 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; and

— 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 TLV level, stop generation of the decontaminant 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.

I-6.2.3 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.

I-6.2.4 Analysis

a) Collect BIs.

b) Have go / no-go analysis (3 to 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 one or two 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 six site tests are successful by the criteria given above. It would be considered unsuccessful (a failure) if the site tests failed at more than one 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.

I-6.2.5 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.
Interpretation Annex

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 this Standard.

Section(s) of the Standard for interpretation

Normative Annex 1 – Performance tests

N-1.9 Inflow velocity (face velocity) test

Normative Annex 5 – Field Tests

N-5.3 Inflow velocity (face velocity) test

Requestor’s question

It is understood that the Direct Inflow Measurement method is the primary method and even with the alternate methods with individual readings across the access opening (thermal anemometer) the overall average is always the value considered. If there is an individual reading below 75 ft/min (A1) or 100 ft/min (A2), however the average value is above these minimal values, do we consider the inflow velocity correct? At a personnel protection stand-point, shouldn’t the entire face velocity be above the minimum value?

Interpretation decision

The requirement is for the average. If taking measurements with a DIM device, individual readings could calculate out to an inflow velocity less than 75 or 100 fpm, provided the average meets the requirement. Personnel protection is evaluated with the inflow velocity reduced by 10 fpm from the nominal set point to help provide added assurance that a deviation like this has been addressed. When thinking about constricted opening readings, I do not believe you can correlate the velocity at one point across the opening to the velocity at that same point when the access opening has been returned to the normal height. Remember also that no biosafety cabinet has air moving at the nominal set point everywhere across the opening. Near the bottom of the opening, the velocity is much higher. Near the top of the opening, the air velocity is much lower.

Requestor’s question

N-1.9.3.4.2 states “Method for Type A1, A2, B1, B2 and C1 …… to measure velocity through a constricted access opening…” while N-5.3.3.3.2 states only “Method for Type A1, A2 and B2….” Is this difference in CSB Types between Annex N-1 and Annex N-5 intentional?

Interpretation decision

It is not intentional. The language has been updated in Annex N-1 recently. Not updating this in Annex N-5 was an oversight.

Requestor’s question

Some manufactures (e.g., Labconco) have an alternative method were they constrict 3 to 5 inches for their measurements. NSF/ANSI 49 does not mention what values of constrictions are acceptable and in what cases do we constrict more or less. Is it acceptable that we can consider this restriction (3 to 5 in) for any CSB with the capability of closing the sash when a manual or certified installation / performance test is not available?
**Interpretation decision**

There is no option to measure the inflow velocity using a method that has not been established by the BSC manufacturer. You cannot use a 5-in constricted opening with a Baker or Kewaunee cabinet just because Labconco specifies a 5-in constricted opening for one of their cabinets. If Baker or Kewaunee specify a 5-in constricted opening, that is what you use for their cabinet. If they specify a 3-in opening, you use a 3-in opening. If you are testing a Labconco cabinet where the manufacturer has specified a 5-in opening for measuring inflow velocity by the secondary method, you cannot use a 3-in opening just because most other manufacturers do that. Furthermore, you must use the manufacturer provided and approved probe holder and gauge blocks to set the access opening height. If no secondary method is available, you use the DIM method, which is what you should be using anyway, virtually every time you measure the inflow velocity. If the cabinet was built before introduction of the DIM instrument, you should still be able to find the method for measuring inflow velocity and use it. I cannot imagine circumstances where there is no manufacturer approved primary method available. If such circumstances exist, I would like to hear about them.

**Requestor’s question**

For all individual readings concerning airflow, being it downflow, exhaust and N-1.9.3.4.3 / N-5.3.3.3.3 - B1 access opening inflow velocity, there is always a mention of measuring 4 to 6 inches away from borders. In N-1.9.3.4.2 / N-5.3.3.3.2, there is no such mention. Is it acceptable that the first reading start 4 inches away from the lateral border?

**Interpretation decision**

Annex N-1 establishes requirements for the method that the manufacturer must comply with. Once they have established their grid according to Annex N-1, you are obligated to use that same grid when following this method under Annex N-5. If the manufacturer has not established this as a secondary method for the cabinet you are testing, you may not use it to set the inflow velocity.

**Requestor’s question**

Do these methods, N-1.9.3.4.2 / N-5.3.3.3.2, have to be constricted? Is it acceptable that we do this measurement at the recommended access opening height as in N-1.9.3.4.3 / N-5.3.3.3.3 for the B1?

**Interpretation decision**

It is never acceptable to perform the test this way.

**Requestor’s question**

Concerning equipment / apparatus: Throughout the years we have done measurements with all devices mentioned in the standard – DIM, thermal anemometer and pitot tube – always complying with the apparatus criteria (accuracy). We have also measured with a vane anemometer that complies with the standard accuracy (with values better than our thermal one). It is our experience, that the vane is much more consistent and stable during the readings compared to the thermal. Especially with the constricted access opening of 4 inches for the inflow velocity. In your opinion, is a vane anemometer that complies with the standard accuracy an acceptable apparatus?

**Interpretation decision**

The standard specifies a thermal anemometer. Therefore, a rotary vane anemometer may not be used. If you want to champion inclusion of a rotary vane anemometer in the standard, you can submit an issue paper and follow through with in person or phone in attendance at the Joint Committee meeting when the issue is being discussed.
Requestor’s question

Class I – Testing: NSF/ANSI 49 clearly states that the standard scope is for Class II – CSB, even though it does mention inflow velocity criteria for Class I. Some manufacturers state an NSF/ANSI 49 Class I compliance, yet there is no test method in this standard (for what I’ve seen), only the criteria. Is there any other standard for performance testing or field testing Class I so that we can also be compliant?

Interpretation decision

I suggest testing to manufacturer specifications. Contact the equipment manufacturer for guidance. They may also be able to call out general requirements from other standards. CETA probably has guidelines for testing Class I cabinets.

Robert W. Powitz, PhD, MPH, RS, DLAAS
Chair, Joint Committee on Biosafety Cabinetry
The following Standards established and adopted by NSF as minimum voluntary consensus Standards are used internationally:

<table>
<thead>
<tr>
<th>Std. #</th>
<th>Standard title</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Food Equipment</td>
</tr>
<tr>
<td>3</td>
<td>Commercial Warewashing Equipment</td>
</tr>
<tr>
<td>4</td>
<td>Commercial Cooking, Rethermalization, and Powered Hot Food Holding and Transport Equipment</td>
</tr>
<tr>
<td>5</td>
<td>Water Heaters, Hot Water Supply Boilers, and Heat Recovery Equipment</td>
</tr>
<tr>
<td>6</td>
<td>Dispensing Freezers</td>
</tr>
<tr>
<td>7</td>
<td>Commercial Refrigerators and Freezers</td>
</tr>
<tr>
<td>8</td>
<td>Commercial Powered Food Preparation Equipment</td>
</tr>
<tr>
<td>12</td>
<td>Automatic Ice Making Equipment</td>
</tr>
<tr>
<td>13</td>
<td>Refuse Processors and Processing Systems</td>
</tr>
<tr>
<td>14</td>
<td>Plastics Piping System Components and Related Materials</td>
</tr>
<tr>
<td>18</td>
<td>Manual Food and Beverage Dispensing Equipment</td>
</tr>
<tr>
<td>20</td>
<td>Commercial Bulk Milk Dispensing Equipment</td>
</tr>
<tr>
<td>21</td>
<td>Thermoplastic Refuse Containers</td>
</tr>
<tr>
<td>24</td>
<td>Plumbing System Components for Recreational Vehicles</td>
</tr>
<tr>
<td>25</td>
<td>Vending Machines for Food And Beverages</td>
</tr>
<tr>
<td>29</td>
<td>Detergent and Chemical Feeders for Commercial Spray-Type Dishwashing Machines</td>
</tr>
<tr>
<td>35</td>
<td>High Pressure Decorative Laminates (HPDL) for Surfacing Food Service Equipment</td>
</tr>
<tr>
<td>37</td>
<td>Air Curtains for Entranceways in Food and Food Service Establishments</td>
</tr>
<tr>
<td>40</td>
<td>Residential Wastewater Treatment Systems</td>
</tr>
<tr>
<td>41</td>
<td>Non-liquid Saturated Treatment Systems</td>
</tr>
<tr>
<td>42</td>
<td>Drinking Water Treatment Units – Aesthetic Effects</td>
</tr>
<tr>
<td>44</td>
<td>Residential Cation Exchange Water Softeners</td>
</tr>
<tr>
<td>46</td>
<td>Evaluation of Components and Devices Used in Wastewater Treatment Systems</td>
</tr>
<tr>
<td>49</td>
<td>Biosafety Cabinety – Design, Construction, Performance, and Field Certification</td>
</tr>
<tr>
<td>50</td>
<td>Equipment and Chemicals for Swimming Pools, Spas, Hot Tubs, and Other Recreational Water Facilities</td>
</tr>
<tr>
<td>51</td>
<td>Food Equipment Materials</td>
</tr>
<tr>
<td>52</td>
<td>Supplemental Flooring</td>
</tr>
<tr>
<td>53</td>
<td>Drinking Water Treatment Units – Health Effects</td>
</tr>
<tr>
<td>55</td>
<td>Ultraviolet Microbiological Water Treatment Systems</td>
</tr>
<tr>
<td>58</td>
<td>Reverse Osmosis Drinking Water Treatment Systems</td>
</tr>
<tr>
<td>59</td>
<td>Mobile Food Carts</td>
</tr>
<tr>
<td>60</td>
<td>Drinking Water Treatment Chemicals – Health Effects</td>
</tr>
<tr>
<td>61</td>
<td>Drinking Water System Components – Health Effects</td>
</tr>
<tr>
<td>62</td>
<td>Drinking Water Distillation Systems</td>
</tr>
<tr>
<td>140</td>
<td>Sustainable Carpet Assessment</td>
</tr>
<tr>
<td>169</td>
<td>Special Purpose Food Equipment and Devices</td>
</tr>
<tr>
<td>170</td>
<td>Glossary of Food Equipment Terminology</td>
</tr>
<tr>
<td>173</td>
<td>Dietary Supplements</td>
</tr>
<tr>
<td>Std. #</td>
<td>Standard title</td>
</tr>
<tr>
<td>-------</td>
<td>----------------</td>
</tr>
<tr>
<td>177</td>
<td>Shower Filtration Systems – Aesthetic Effects</td>
</tr>
<tr>
<td>184</td>
<td>Residential Dishwashers</td>
</tr>
<tr>
<td>223</td>
<td>Conformity Assessment Requirements for Certification Bodies that Certify Products Pursuant to NSF/ANSI 60 Drinking Water Treatment Chemicals – Health Effects</td>
</tr>
<tr>
<td>240</td>
<td>Drainfield Trench Product Sizing for Gravity Dispersal Onsite Wastewater Treatment and Dispersal Systems</td>
</tr>
<tr>
<td>244</td>
<td>Drinking Water Treatment Units Supplemental Microbiological Water Treatment Systems – Filtration</td>
</tr>
<tr>
<td>245</td>
<td>Wastewater Treatment Systems – Nitrogen Reduction</td>
</tr>
<tr>
<td>305</td>
<td>Personal Care Products Containing Organic Ingredients</td>
</tr>
<tr>
<td>321</td>
<td>Goldenseal Root (<em>Hydrastis canadensis</em>)</td>
</tr>
<tr>
<td>330</td>
<td>Glossary of Drinking Water Treatment Unit Terminology</td>
</tr>
<tr>
<td>332</td>
<td>Sustainability Assessment for Resilient Floor Coverings</td>
</tr>
<tr>
<td>336</td>
<td>Sustainability Assessment for Commercial Furnishings Fabric</td>
</tr>
<tr>
<td>342</td>
<td>Sustainability Assessment for Wallcovering Products</td>
</tr>
<tr>
<td>347</td>
<td>Sustainability Assessment for Single-Ply Roofing Membranes</td>
</tr>
<tr>
<td>350</td>
<td>Onsite Residential and Commercial Water Reuse Treatment Systems</td>
</tr>
<tr>
<td>350-1</td>
<td>Onsite Residential and Commercial Greywater Treatment Systems for Subsurface Discharge</td>
</tr>
<tr>
<td>358-1</td>
<td>Polyethylene Pipe and Fittings for Water-Based Ground-Source “Geothermal” Heat Pump Systems</td>
</tr>
<tr>
<td>358-2</td>
<td>Polypropylene Pipe and Fittings for Water-Based Ground-Source “Geothermal” Heat Pump Systems</td>
</tr>
<tr>
<td>358-3</td>
<td>Cross-linked Polyethylene (PEX) Pipe and Fittings for Water-based Ground-Source (Geothermal) Heat Pump Systems</td>
</tr>
<tr>
<td>358-4</td>
<td>Polyethylene of Raised Temperature (PE-RT) Tubing and Fittings for Water-based Ground-Source (Geothermal) Heat Pump Systems</td>
</tr>
<tr>
<td>359</td>
<td>Valves for Cross-linked Polyethylene (PEX) Water Distribution Tubing Systems</td>
</tr>
<tr>
<td>360</td>
<td>Wastewater Treatment Systems – Field Performance Verification</td>
</tr>
<tr>
<td>363</td>
<td>Good Manufacturing Practices (GMP) for Pharmaceutical Excipients</td>
</tr>
<tr>
<td>372</td>
<td>Drinking Water Treatment System Components – Lead Content</td>
</tr>
<tr>
<td>375</td>
<td>Sustainability Assessment for Water Contact Products</td>
</tr>
<tr>
<td>385</td>
<td>Disinfection Mechanics</td>
</tr>
<tr>
<td>401</td>
<td>Drinking Water Treatment Units – Emerging Compounds / Incidental Contaminants</td>
</tr>
<tr>
<td>416</td>
<td>Sustainability Assessment for Water Treatment Chemical Products</td>
</tr>
<tr>
<td>418</td>
<td>Effluent Filters – Field Longevity Testing</td>
</tr>
<tr>
<td>419</td>
<td>Public Drinking Water Equipment Performance – Filtration</td>
</tr>
<tr>
<td>426</td>
<td>Environmental Leadership and Corporate Social Responsibility Assessment of Servers</td>
</tr>
<tr>
<td>455-1</td>
<td>Terminology for the NSF 455 Portfolio of Standards</td>
</tr>
<tr>
<td>455-2</td>
<td>Good Manufacturing Practices for Dietary Supplements</td>
</tr>
<tr>
<td>455-3</td>
<td>Good Manufacturing Practices for Cosmetics</td>
</tr>
<tr>
<td>455-4</td>
<td>Good Manufacturing Practices for Over-the-Counter Drugs</td>
</tr>
<tr>
<td>457</td>
<td>Sustainability Leadership Standard for Photovoltaic Modules and Photovoltaic Inverters</td>
</tr>
<tr>
<td>600</td>
<td>Health Effects Evaluation and Criteria for Chemicals in Drinking Water</td>
</tr>
<tr>
<td>14159-1</td>
<td>Hygiene Requirements for the Design of Meat and Poultry Processing Equipment</td>
</tr>
<tr>
<td>14159-2</td>
<td>Hygiene Requirements for the Design of Hand-held Tools Used in Meat and Poultry Processing Equipment</td>
</tr>
<tr>
<td>14159-3</td>
<td>Hygiene Requirements for the Design of Mechanical Belt Conveyors Used in Meat and Poultry Processing Equipment</td>
</tr>
</tbody>
</table>
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.