NSF/ANSI 49 – 2007
Class II (laminar flow) biosafety cabinetry

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 3.18, “readily accessible.”]).

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

3.3 biosafety levels:1 The essential elements of the four biosafety levels for activities involving infectious microorganisms and laboratory animals are summarized in the *Biosafety in Microbiological and Biomedical Laboratories*.2 The levels are designated in ascending order, by degree of protection provided to personnel, the environment, and the community. Standard microbiological practices are common to all laboratories. Special microbiological practices enhance worker safety, environmental protection, and address the risk of handling agents requiring increasing levels of containment.

The combination of laboratory practices and techniques, safety equipment, and laboratory facilities appropriate for the operations performed and the hazard posed by the infectious agents and the laboratory function or activity. These biosafety levels are described in *Biosafety in Microbiological and Biomedical Laboratories*.

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

Practices, safety equipment, and facility design and construction are appropriate for undergraduate and secondary educational training and teaching laboratories and for other laboratories in which work is done with defined and characterized strains of viable microorganisms not known to consistently cause disease in healthy adult humans. *Bacillus subtilis*, *Naegleria gruberi*, infectious canine hepatitis virus, and exempt organisms under the NIH

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


Guidelines for Research Involving Recombinant DNA Molecules\textsuperscript{2} are representative of those microorganisms meeting these criteria. Many agents not ordinarily associated with disease processes in humans are, however, opportunistic pathogens and may cause infection in the young, the aged, and immunodeficient or immunosuppressed individuals. Vaccine strains that have undergone multiple in vivo passages should not be considered avirulent simply because they are vaccine strains.

Biosafety Level 1 represents a basic level of containment that relies on standard microbiological practices with no special primary or secondary barriers recommended, other than a sink for hand washing.

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

- laboratory personnel have specific training in handling pathogenic agents and are supervised by scientists competent in handling infectious agents and associated procedures;
- access to the laboratory is restricted when work is being conducted; and
- all procedures in which infectious aerosols or splashes may be created are conducted in BSCs or other physical containment equipment.

Practices, equipment, and facilities are applicable to clinical, diagnostic, teaching, and other facilities in which work is done with the broad spectrum of indigenous moderate-risk agents present in the community and associated with human disease of varying severity. With good microbiological techniques, these agents can be used safely in activities conducted on the open bench, provided that the potential for producing aerosols is low. Hepatitis B virus, human immunodeficiency virus, the \textit{salmonellae}, and \textit{Toxoplasma spp.} are representative of microorganisms assigned to this containment level. Biosafety Level 2 is appropriate when work is done with any human-derived blood, body fluids, tissues, or primary human cell lines where the presence of an infectious agent may be unknown. (Laboratory personnel working with human-derived materials should refer to the OSHA Bloodborne Pathogen Standard for specific required precautions.)

Primary hazards to personnel working with these agents may include accidental percutaneous or mucous membrane exposures or ingestion of infectious materials. Extreme caution should be taken with contaminated needles and sharp instruments. Even though organisms routinely manipulated at Biosafety Level 2 are not known to be transmissible by the aerosol route, procedures with aerosol or high splash potential, which may increase the risk of such personnel exposure, must be conducted in primary containment equipment or in devices such as biosafety cabinets (BSCs) or safety centrifuge cups. Other primary barriers should be used as appropriate, such as splash shields, face protection, gowns, and gloves.

Secondary barriers such as hand washing sinks and waste decontamination facilities must be available to reduce potential environmental contamination.

3.3.3 biosafety Level 3 (BSL-3): Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities where work is performed with indigenous or exotic 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.

Practices, safety equipment, and facility design and construction are applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indigenous or

\textsuperscript{2} Department of Health and Human Services, National Institute of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985
exotic agents with a potential for respiratory transmission and that may cause serious and potentially lethal infection. Mycobacterium tuberculosis, St. Louis encephalitis virus, and Coxiella burnetii are representative of the microorganisms assigned to this level. Primary hazards to personnel working with these agents relate to autoinoculation, ingestion, and exposure to infectious aerosols.

At Biosafety Level 3, more emphasis is placed on primary and secondary barriers to protect personnel in contiguous areas, the community, and the environment from exposure to potentially infectious aerosols. For example, all laboratory manipulations should be performed in a BSC or other enclosed equipment, such as a gas-tight aerosol generation chamber. Secondary barriers for this level include controlled access to the laboratory and ventilation requirements that minimize the release of infectious aerosols from the laboratory.

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

There are two models for BSL-4 laboratories:

— A Cabinet Laboratory where all handling of agents must be performed in a Class III BSC.

— A Suit Laboratory where personnel must wear a positive pressure protective suit.

BSL-4 Cabinet and Suit Laboratories have special engineering and design features to prevent microorganisms from being disseminated into the environment. Practices, safety equipment, and facility design and construction are applicable for work with dangerous and exotic agents that have a high individual risk of life-threatening disease, which may be transmitted via the aerosol route and for which there is no available vaccine or therapy. Agents with a close or identical antigenic relationship to Biosafety Level 4 agents also should be handled at this level. When sufficient data are obtained, work with these agents may continue at this level or at a lower level. Viruses such as Marburg or Congo-Crimean hemorrhagic fever are manipulated at Biosafety Level 4.

The primary hazards to personnel working with Biosafety Level 4 agents are respiratory exposure to infectious aerosols, mucous membrane or broken skin exposure to infectious droplets, and autoinoculation. All manipulations of potentially infectious diagnostic materials, isolates, and naturally or experimentally infected animals pose a high risk of exposure and infection to laboratory personnel, the community, and the environment.

The laboratory worker's complete isolation from aerosolized infectious materials is accomplished primarily by working in a Class III BSC or in a full-body, air-supplied, positive-pressure personnel suit. The Biosafety Level 4 facility itself is generally a separate building or completely isolated zone with complex, specialized ventilation requirements and waste management systems to prevent release of viable agents to the environment.

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

### 3.4.1 Class I

The Class I BSC provides personnel and environmental protection, but no product protection. It is similar in air movement to a chemical fume hood, but has a HEPA filter in the exhaust system to protect the environment. In the Class I BSC, unfiltered room air is drawn across the work surface. Personnel protection is provided by this inward airflow as long as a minimum velocity of 75 linear feet per minute (lfpm) is maintained through the front opening. Because product protection is provided by the Class II BSCs, general usage of the Class I BSC has declined. However, in many cases, Class I BSCs are used specifically to enclose equipment (e.g., centrifuges, harvesting equipment or small fermenters), or procedures with potential to generate aerosols (e.g. cage dumping, culture aeration or tissue homogenation).

The classical Class I BSC is not equipped with an integral exhaust fan and must be directly connected to the building exhaust system, and the building exhaust fan provides the negative pressure necessary to draw room air into the cabinet. Cabinet air is drawn through a HEPA filter as it enters the cabinet exhaust plenum. A second HEPA filter may be installed in the terminal end of the building exhaust prior to the exhaust fan.

**Reason:**

The paragraphs below for Class I have been removed after the task group discussed the various alternative Class I’s. They decided to stay with a classical Class I definition. If anyone would like more information on any other equipment on the market this could be put into an informative annex via an issue paper.

Some Class I BSCs are equipped with an integral exhaust fan and a HEPA filter in the exhaust plenum must be canopy connected. If the BSC is connected to the building’s exhaust system it shall be canopy connected. The cabinet exhaust fan must be interlocked with the building exhaust fan. In the event that the building exhaust fan fails, the cabinet exhaust fan must turn off so that the building exhaust ducts are not pressurized. If the exhaust ducts are pressurized and the HEPA filter develops a leak, then contaminated air could be discharged into other parts of the building or the environment. Note that cabinets with an auxiliary air supply opening should have a filter installed on the cabinet air supply intake.

A panel with openings to allow access for the hands and arms to the work surface can be added to the Class I cabinet. The restricted opening results in increased inward air velocity, increasing worker protection. For added safety, arm-length gloves can be attached to the panel. Makeup air is then drawn through an auxiliary air supply opening (which may contain a filter) and/or around a loose-fitting front panel.

Some Class I models used for animal cage changing are designed to allow recirculation of air into the room after HEPA filtration and may require more frequent filter replacement due to filter loading and odor from organic materials captured on the filter. The recirculating Class I BSC should be annually certified for sufficient airflow and filter integrity.

A ventilated cabinet for personnel and environmental protection, having an un-recirculated inward airflow away from the operator that exhausts all air to the atmosphere after filtration through a HEPA filter. Class I cabinets are suitable for work where no product protection is required.

**NOTE**—Although the traditional Class I BSC is exhausted to the atmosphere without recirculation into the lab, it is recognized that some of the benefits of the Class I BSC can be obtained even when the unit’s HEPA filtered exhaust is vented back into the laboratory.
3.4.2 Class II: Class II BSCs are partial barrier systems that rely on the laminar movement of air to provide containment. If the air curtain is disrupted (e.g., movement of materials in and out of a cabinet, rapid or sweeping movement of the arms) the potential for contaminant release into the laboratory work environment is increased as is the risk of product contamination.

The Class II (Types A1, A2, B1 and B2) BSCs provide personnel, environmental and product protection. Airflow is drawn into the front grille of the cabinet, providing personnel protection. In addition, the downward laminar flow of HEPA-filtered air provides product protection by minimizing the chance of cross-contamination across the work surface of the cabinet. Because cabinet exhaust air is passed through a certified HEPA filter, it is particulate-free (environmental protection), and may be recirculated to the laboratory (Type A1 and A2 BSCs) or discharged from the building via a canopy connection. Exhaust air from Types B1 and B2 BSCs must be discharged to the outdoors via a hard connection.

HEPA filters are effective at trapping particulates and thus infectious agents but do not capture volatile chemicals or gases. Only Type A2-exhausted or Types B1 and B2 BSCs exhausting to the outside should be used when working with volatile, toxic chemicals, but amounts must be limited. All Class II cabinets are designed for work involving microorganisms assigned to biosafety levels 1, 2 and 3. 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. Class II BSCs may be used with organisms requiring BSL-4 containment if used in a BSL-4 suit laboratory by a worker wearing a positive pressure protective suit.

A ventilated cabinet for personnel, product, and environmental protection having an open front with inward airflow for personnel protection, downward HEPA filtered laminar airflow for product protection, and HEPA filtered exhausted air for environmental protection.

NOTE — When toxic chemicals or radionuclides are used as adjuncts to biological studies or pharmaceutical work, Class II cabinets designed and constructed for this purpose should be used.

3.4.2.1 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 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 filtered air back into the laboratory or to the environment through an exhaust canopy; and
- may have positive pressure contaminated ducts and plenums that are not surrounded by negative pressure plenums.

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

3.4.2.2 Class II, Type A2 cabinets (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 filtered downflow air that is a portion of the mixed downflow and inflow air from a common exhaust plenum;
– may exhaust HEPA filtered air back into the laboratory or to the environment through an exhaust canopy; and

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

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

3.4.2.3 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 filtered downflow air composed largely of uncontaminated recirculated inflow air;

– exhaust most of the contaminated downflow air through a dedicated duct exhausted to the atmosphere after passing through a HEPA filter; and

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

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

3.4.2.4 Class II Type B2 cabinets (sometimes referred to as “total exhaust”): cabinets that

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

– have HEPA 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 after filtration through a HEPA 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 toxic chemicals and radionuclides required as adjuncts to microbiological studies.

3.4.3 Class III: The Class III BSC was designed for work with highly infectious microbiological agents and for the conduct of hazardous operations and provides maximum protection for the environment and the worker. It is a gas-tight (no leak greater than $1 \times 10^{-7}$ cc/sec with 1% test gas at 3 inches pressure Water Gauge) enclosure with a non-opening view window. Access for passage of materials into the cabinet is through a dunk tank, that is accessible through the cabinet floor, or double-door pass-through box (e.g., an autoclave) that can be decontaminated between uses. Reversing that process allows materials to be removed from the Class III BSC safely. Both supply and exhaust air are HEPA filtered on a Class III cabinet. Exhaust air must
pass through two HEPA filters, or a HEPA filter and an air incinerator, before discharge to the outdoors. Airflow is maintained by an exhaust system exterior to the cabinet, which keeps the cabinet under negative pressure (minimum of 0.5 inches of water gauge.) The exhaust fan for the Class III cabinet is generally separate from the exhaust fans of the facility ventilation system.

Long, heavy-duty rubber gloves are attached in a gas-tight manner to ports in the cabinet and allow direct manipulation of the materials isolated inside. Although these gloves restrict movement, they prevent the user’s direct contact with the hazardous materials. The trade-off is clearly on the side of maximizing personal safety. Depending on the design of the cabinet, the supply HEPA filter provides particulate-free, albeit somewhat turbulent, airflow within the work environment. Laminar airflow is not a characteristic of a Class III cabinet.

Several Class III BSCs can be joined together in a “line” to provide a larger work area. Such cabinet lines are custom-built; the equipment installed in the cabinet line (e.g., refrigerators, small elevators, shelves to hold small animal cage racks, microscopes, centrifuges, incubators, etc.) is generally custom-built as well.

A totally enclosed, ventilated cabinet of leak-tight construction. Operations in the cabinet are conducted through attached rubber gloves. The cabinet is maintained under negative air pressure of at least 0.50 in w. g. (120 Pa). Downflow air is drawn into the cabinet through HEPA filters. The exhaust air is treated by double HEPA filtration or by HEPA filtration and incineration.  