

# ARS \* CSREES \* ERS \* NASS *Manual*

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## 9.4.4 Biosafety Level 3 Agriculture (BSL-3Ag)

A. In ARS, special features are required when research involves certain biological agents in large animal species. To support such research, ARS has developed a special facility designed, constructed and operated at a unique containment level called Biosafety Level 3 Agriculture (BSL-3Ag). Using the containment features of the standard BSL-3 facility as a starting point, BSL-3Ag facilities are specifically designed to protect the environment by including almost all of the features ordinarily used for BSL-4 facilities as enhancements. All BSL-3Ag containment spaces must be designed, constructed and certified as primary containment barriers.

The BSL-3Ag facility can be a separate building, but, more often, it is an isolated zone contained within a facility operating at a lower biosafety level, usually a BSL-3. This isolated zone has strictly controlled access, and special physical security measures, and functions on the “box within a box” principle.

B. All BSL-3Ag facilities require the features listed in sections 9.4.2(A) through

9.4.2(F), and sections 9.4.3(C)(1) through 9.4.3(C)(3), and 9.4.3(C)(8).

C. In addition, the mandatory special features for a BSL-3Ag facility include:

1) Personnel change and shower rooms that provide for the separation of street clothing from laboratory clothing and that control access to the containment spaces. The facility is arranged so that personnel ingress and egress are only through a series of rooms (usually one series for men and one for women) consisting of: a ventilated vestibule with compressible gaskets on the two doors, a “clean” change room outside containment, a shower room at the non-containment/containment boundary, and a “dirty” change room within containment. Complete laboratory clothing (including undergarments, pants and shirts or jump suits, and shoes and gloves) is provided in the “dirty” change room, and put on by personnel before entering the research areas. In some facilities, complete laboratory clothing and personal protective equipment (PPE) are provided in the “clean” change room, where they can be stored and stowed for use without entry into containment.

In general, when leaving a BSL-3Ag laboratory, where all open handling of infectious materials is done in BSCs or other physical containment equipment, personnel need not take a shower to go to any other containment space within the facility, and would be required to take only the access control shower to leave the facility.

However, when leaving a BSL-3Ag large animal space (an animal room, necropsy room, carcass disposal area, contaminated corridor, etc.) that acts as the primary barrier and that contains large volumes of aerosols holding highly infectious agents, personnel usually would be required to remove their “dirty” lab clothing, take a shower, and put on “clean” lab clothing immediately after leaving this high risk BSL-3Ag animal space and before going to any other part containment space within facility. When leaving the facility, these personnel would take another shower at the access control shower and put on their street clothing.

It is very important for the A-E to realize that the location, size and number of change rooms and showers within a facility need to be programmed very carefully with the scientists and staff at the location due to the unique circumstances at each research center.

Soiled clothing worn in a BSL-3Ag space is autoclaved before being laundered. Personnel moving from one space within containment to another will follow the practices and procedures described in the biosafety manual specifically developed for the particular facility and adopted by the laboratory director.

2) Access doors to these facilities are self closing and lockable. Emergency exit doors are provided, but are locked on the outside against unauthorized use. The A-E shall consider the practicality of providing vestibules at emergency exits.

3) Supplies, materials and equipment enter the BSL-3Ag space only through an

airlock, fumigation chamber or an interlocked and double-doored autoclave.

4) Double-door autoclaves engineered with bioseals are provided to decontaminate laboratory waste passing out of the containment area. The double doors of the autoclaves must be interlocked so that the outer door can be opened only after the completion of the sterilizing cycle, and to prevent the simultaneous opening of both doors. All double door autoclaves are situated through an exterior wall of the containment area, with the autoclave unit forming an air tight seal with the barrier wall and the bulk of the autoclave situated outside the containment space so that autoclave maintenance can be performed conveniently. A gas sterilizer, a pass-through liquid dunk tank, or a cold gas decontamination chamber must be provided for the safe removal of materials and equipment that are steam or heat sensitive. Disposable materials must be autoclaved before leaving the BSL-3Ag space, and then incinerated.

5) Dedicated, single pass, directional, and pressure gradient ventilation systems must be used. All BSL-3Ag facilities have independent air supply and exhaust systems. The systems are operated to provide directional airflow and a negative air pressure within the containment space. The directional airflow within the containment spaces moves from areas of least hazard potential toward areas of greatest hazard potential. A visible means of displaying pressure differentials is provided. They can be seen inside and outside of the containment space, and sound an alarm when the preset pressure differential is not maintained. The air supply and exhaust systems must be interlocked to prevent reversal of the directional airflow and the containment spaces becoming positively pressurized, in the event of an exhaust system failure.

6) Supply and exhaust air to and from the containment space is HEPA filtered, with special electrical interlocks to prevent positive pressurization during electrical or mechanical breakdowns. The exhaust air is discharged in such a manner that it cannot be drawn into outside air intake systems. The HEPA filters are outside of containment but are located as near as possible to the containment space to minimize the length of potentially contaminated air ducts. The HEPA filter housings are fabricated to permit the scan testing of the filters in place after installation, and to permit filter decontamination before removal. Backup HEPA filter units are strongly recommended to allow filter changes without disrupting research. (The most severe requirements for these modern, high level biocontainment facilities include HEPA filters arranged both in series and in parallel on the exhaust side, and series HEPA filters on the supply side of the HVAC systems serving "high risk" areas where large amounts of aerosols containing BSL-3Ag agents could be expected [e.g., large animal rooms, contaminated corridors, necropsy areas, carcass disposal facilities, etc.]

For these high risk areas, redundant supply fans are recommended, and redundant exhaust fans are required. The supply and exhaust air systems should be filtered with 80-90 percent efficiency filters to prolong the life of the supply and exhaust HEPA filters. Air handling systems must provide 100 percent outside conditioned air to the containment spaces.

7) Liquid effluents from BSL-3Ag areas must be collected and decontaminated in a central liquid waste sterilization system before disposal into the sanitary sewers. Equipment must be provided to process, heat and hold the contaminated liquid effluents to temperatures, pressures and times sufficient to inactivate all biohazardous materials that reasonably can be expected to be studied at the facility in the future. The system may need to operate at a wide range of temperatures and holding times to process the facility's effluents economically and efficiently. Double containment piping systems with leak alarms and annular space decontaminating capability should be considered for these wastes. Effluents from laboratory sinks, cabinets, floors and autoclave chambers are sterilized by heat treatment. Under certain conditions, liquid wastes from shower rooms and toilets may be decontaminated by chemicals. Facilities must be constructed with appropriate basements or piping tunnels to allow for inspection of plumbing systems.

8) Each BSL-3Ag containment space shall have its interior surfaces (walls, floors, and ceilings) and penetrations sealed to create a functional area capable of passing a pressure decay test with a leak rate established by the ARS RPSO. This requirement includes all interior surfaces of all

BSL-3Ag spaces, not just the surfaces making up the external containment boundary. All walls are constructed slab to slab, and all penetrations, of whatever type, are sealed airtight to prevent escape of contained agents and to allow gaseous fumigation biological decontamination. This prevents cross contamination between individual BSL-3Ag spaces and allows gaseous fumigation in one space without affecting other BSL-3Ag spaces. Exterior windows and vision panels, if required, are breakage-resistant and sealed.

Greenhouses constructed to meet the BSL-3Ag containment level will undergo the following tests, or the latest subsequent standards: (a) an air infiltration test conducted according to ASTM E 283-91; (b) a static pressure water resistance test conducted according to ASTM E 331-93; and (c) a dynamic pressure water resistance test conducted according to AAMA 501.1-94.

9) All ductwork serving BSL-3Ag spaces shall be airtight and pressure tested (see Appendix 9B for testing and certification details).

10) The hinges and latch/knob areas of all passage doors shall be sealed to meet pressure decay testing requirements.

11) All airlock doors shall have air inflated or compressible gaskets. The compressed air lines to the air inflated gaskets shall be provided with HEPA filters and check valves.

12) Restraining devices shall be provided in large animal rooms.

13) Necropsy rooms shall be sized and equipped to accommodate large farm animals.

14) Pathological incinerators, or other approved means, must be provided for the safe disposal of the large carcasses of infected animals. Redundancy and the use of multiple technologies need to be considered and evaluated.

15) HEPA filters must be installed on all atmospheric vents serving plumbing traps, as near as possible to the point of use, or to the service cock, of central or local vacuum systems, and on the return lines of compressed air systems. All HEPA filters are installed to allow in-place decontamination and replacement. All traps are filled with liquid disinfectant.

16) Biological Safety Cabinets must be provided and must be installed where their operations are not adversely affected by air circulation and foot traffic. Class II BSCs use HEPA filters to treat their supply and exhaust air. The exhaust from most Class II cabinets must be connected to the building's exhaust system. Supply air to a Class III cabinet is HEPA filtered, and the exhaust air must be double HEPA filtered (through a cabinet HEPA and then through a HEPA in a dedicated building exhaust system), before being discharged to the atmosphere.

A BSL-3Ag facility will be provided only at those locations where the research mission requires this special type of facility; that is, where the facility barriers, usually considered secondary barriers, now act as primary barriers. Examples are sealed interior surfaces (walls, ceilings and floors of each containment space), ventilation systems, pathological incinerators, effluent sterilization systems, HEPA filters, etc. This requirement exists, in most cases, to contain biologically hazardous aerosols.

The BSL-3Ag facility must undergo special testing and certification procedures.

See Appendix B, "Testing and Certification Requirements for Critical Components of the Biological Containment System," at the end of this chapter, and the Design Details Manual.

D. For a summary of the general containment guidelines for a BSL-3Ag facility, see Table 9-1.

#### **9.4.2 Biosafety Levels 1 and 2 (BSL-1 and BSL-2)**

A. In general, a BSL-1 facility represents a basic level of containment that relies on standard microbiological practices with no special or secondary barriers recommended, other than a sink for hand washing, and self closing and lockable doors. The facility must be insect and rodent proof.

B. BSL-2 facilities, in general, support research with agents that, as aerosols, could increase the risk of infection, and must have available primary containment such as BSCs, safety centrifuge cups and/or personal protection equipment. The BSL-2 facility should include the secondary barriers of a foot, elbow or automatically operated hand

washing station located near the exit of each functional area within containment, and an autoclave, or other appropriate type of biohazardous waste treatment, to process infectious wastes. With appropriate procedural controls, non-infectious wastes from a BSL-2 facility could be decontaminated at a remote site within the same building.

C. If laboratory animals are used, a BSL-2 animal facility must have appropriate cage storage areas and appropriate means of cleaning the cages or caging systems. Any mechanical cage washer should be capable of producing a final rinse temperature of at least 180 degrees F, but should also be able to operate at lower temperatures to save energy and to prevent damage to some types of plastic cages.

D. The BSL-1 and BSL-2 facilities should provide an internal environment which is easily cleanable. The walls and floors should be surfaced with or be constructed of materials which can withstand harsh detergents, disinfectants and decontaminating agents. Horizontal surfaces and open storage cabinets which may collect dust should be minimized, and suspended fixtures, such as fluorescent lighting and exposed service piping, should be accessible for cleaning. Bench tops should be impervious to liquids and resistant to acids, alkalis, organic solvents, and moderate heat.

E. The facility furniture should be sturdy and readily cleanable. Voids in furniture groupings should be accessible for cleaning. The use of carpets, rugs, and cloth-covered, porous furniture is inappropriate in a biocontainment facility. Open shelving should be avoided; closed cabinets minimize dust buildup on their shelves and contain splashes of liquids.

F. Although the primary consideration in the arrangement of the furnishings is their suitability for the research program, floor plans should include environmental control and safety considerations. Work spaces should be planned to be out of through traffic areas. If BSCs are provided, they shall be located deep in the laboratory, preferably at "dead ends," where foot traffic that could disturb the laminar flow of air in the BSCs would be minimized. They shall also be located away from supply air outlets. The floor plans should separate clean and contaminated operations. Extraneous traffic should be minimized. Although formal offices should not be included in the laboratory, an area should be provided to allow researchers to record notes, possibly at a computer workstation with a laptop, or to fax materials. Doors should be equipped with self-closing devices to reduce and control the entry of non-facility personnel, and with locks or key card access.

### **9.4.3 Biosafety Level 3 (BSL-3)**

C. The unique features which distinguish the BSL-3 facility from the BSL-1 and BSL-2 facilities are the provisions for: access control, safety equipment, a specialized ventilation system, and sealed finishes and penetrations.

1) For access control, the BSL-3 laboratory or facility should be completely separated from areas that are open to the public, and from corridors used by laboratory

personnel who do not work in the BSL-3 facility. The change room and shower facility arrangement provides the greatest access control of any of the examples and is strongly recommended for laboratories; this arrangement is required for animal facilities at this level of containment. All facility doors must be self-closing.

2) Safety equipment includes biological safety cabinets and autoclaves.

Each BSL-3 laboratory or module in a BSL-3 facility should be equipped with an appropriate Class II or III BSC to contain certain procedures when moderately infectious agents are being studied. Potentially hazardous procedures shall be confined to ventilated safety cabinets. Protective cabinets shall be used whenever biohazardous materials are handled outside fully contained vessels.

An autoclave for the decontamination of facility wastes must be located within the BSL-3 space. A double door (having two doors in series) and interlocked autoclave with access outside the laboratory or facility provides an excellent method for providing clean/contaminated materials flow. With appropriate procedural controls, an autoclave may be located outside of the BSL-3 laboratory, providing it is located within the same building.

3) A specialized ventilation system to control air movement is a requirement for a BSL-3 facility. A ducted exhaust air ventilation system must be provided. The exhaust air may not be recirculated to any other area of the building. In general, exhaust air may not require filtration or other treatments, but special site requirements, or certain activities with, or uses of, hazardous agents may dictate the use of HEPA filtration. Air from the containment space is to be discharged to the outside so that it either clears occupied buildings and air intakes (this is usually done by locating the exhaust stacks on the roof and discharging upward at a velocity greater than 3,000 fpm). The laboratory staff must ensure that the flow of air is always into the containment space. A visual monitoring device should be provided at the space's entry to confirm the inward direction of the airflow. Supply air systems must be designed to prevent the positive air pressurization of the space and the reversal of airflow from the containment areas to the non-containment areas of the building. A device for monitoring airflow, and possibly an alarm, should be provided to alert facility personnel to an air pressure problem.

8) Any windows in a BSL-3 facility must be inoperable and sealed in the shut position. All facility doors must be self-closing.

**Table 9 -1  
General Containment Guidelines**

<b>Biosafety Levels:</b>	<b>BSL-1</b>	<b>BSL-2</b>	<b>BSL-3</b>	<b>BSL-3 Ag</b>	<b>BSL-4</b>
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**Facility Features:**

1. Personnel Entry/Exit through Clothing Change & Shower Rooms	n/a	n/a	recommended	required	required
2. Materials, Supplies, & Equipment enter/leave through Double-Door Autoclave, Fumigation Chamber, or Airlock	n/a	n/a	required	required	required
3. Work Conducted in Primary Containment Equipment.	open bench tops	as required	required	required (If the space is a lab.)	required
4. Hand Washing Station  *(Foot, elbow or automatically operated)	required	recommended*	required*	required*	required* (not where a suit would be worn)
5. Laboratory and Animal Room Wastes from the Containment Area Decontaminated or Sterilized	n/a	recommended	recommended	required	required
6. Lab Clothing Decontaminated Before Being Washed	n/a	n/a; to be disposed of in the lab or washed by the	required	required	required



		facility			
7. Animal Cages Autoclaved or Thoroughly Decontaminated Before Cleaning	cages washed, then rinsed at 180 degrees.	cages washed, then rinsed at 180 degrees.	cages washed, then rinsed at 180 degrees.	required	required
8. Appropriate Cautionary Signs	n/a	required	required	required	required
9. Separate Building or Isolated Zone Within a Building	n/a	n/a	required	required	required
10. BSC or other Appropriate Personal Protective or Physical Containment Devices	n/a	Class I or Class II BSC	Class II or Class III BSC	Class II or Class III BSC	Class III or Class I or II BSC with ventilated suit
11. Suit Room	n/a	n/a	n/a	n/a	AS REQUIRED
12. Steam and/or Ethylene Oxide Sterilizers:	recommended	required	required (integral, double door)	integral, double-door	integral, double-door
13. Liquid Effluent (Bio-Waste) Treatment System	n/a	not required	required	required	required
14. Personnel Change Room	n/a	n/a	recommended for laboratories; required for animal facilities.	required	required
15. Shower Available Within Facility	n/a	n/a	recommended for laboratories;	required	required

			required for animal facilities.		
16. Lab Contiguous with Shower	n/a	n/a	n/a	as required for lab; required for "high risk" areas	required
17. Work Surfaces: Bench Tops Impervious to Water, Resistant to Acids, Alkalis, Organic Solvents and Moderate Heat.	required	required	required	required	seamless required
18. Interior Surfaces of Walls, Floors, and Ceilings: Monolithic, Resistant to Liquids and Chemicals, all Penetrations Sealed. Any Drains in the Floors Contain Traps Filled with Chemical Disinfectant	n/a	walls, floors, and ceilings are monolithic, resistant to liquids and chemicals.	required	required	required
19. Windows	not recommended for animal rooms. For other areas, if provided, fitted with fly screens	not recommended for animal rooms. For other areas, if provided, fitted with fly screens	all windows closed and sealed.	no windows recommended (If with windows: breakage resistant and sealed)	no windows recommended (If with windows: breakage resistant and sealed)
20. Animal Room: Cages	n/a	n/a	as required	as required	required

Solid-Sided, Cages Ventilated or Filtered, Restraining Devices.					
21. Vacuum Outlets (if provided) Protected by HEPA Filters & Liquid Disinfectant in Traps	n/a	n/a	required	required	required if central vacuum systems are used
22. Other Liquid & Gas Services Protected by Backflow Preventers	n/a	n/a	required	required	required
23. Sewer & Other Vent Lines Protected by HEPA Filters	n/a	n/a	required	required	required
24. Ventilation ( <b>Facility</b> ): Individual Supply & Exhaust Air Systems. (For animal facilities, HVAC to be provided as per latest edition of <i>Guide for Care and Use of Laboratory Animals</i> )	ducted exhaust required	ducted exhaust required	ducted exhaust required	required	required
Single Pass (No Recirculation)	required	required.	required	required	required
Directional Air Flow	required	required	required	required	required

Pressure Gradient	recommended for animal rooms; n/a for other areas.	recommended for animal rooms; n/a for other areas.	required	required	required
Supply/Exhaust Coordination (Exhaust Confirmed before Supply Operates )	n/a	n/a	required	required	required
HEPA Filtered Supply and/or Exhaust	n/a	n/a	HEPA exhaust recommended	HEPA supply & exhaust for labs; HEPA supply and 2 in series HEPAs exhaust for high risk areas	HEPA supply & exhaust for Cabinet Lab; HEPA supply and 2 in series HEPAs exhaust for Suit Areas
25. Ventilation (Containment Equipment):  Class III BSC	n/a	n/a	HEPA supply filters & tandem (2 in series) HEPA exhaust filters	HEPA supply filters & tandem (2 in series) exhaust filters.	HEPA supply filters & tandem (2 in series) exhaust filters
Class I and II BSC	n/a	n/a	Class II; HEPA supply and exhaust	Class II: HEPA supply and exhaust	Class II; In Suit Lab, HEPA supply and exhaust
26. DDC and Building Automation Systems	to be considered	to be considered	required unless impractical	required	required
27. Leak Tightness Testing & Certification of Critical Components of the Biological Containment	n/a	n/a	BSC, HEPA filter assemblies (if required), welded ductwork (if required).	BSC, HEPA filter assemblies, containment room, welded ductwork.	BSC, HEPA filter assemblies, containment room, welded ductwork.

System Prior to Final Acceptance of the Completed Work					
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## [Appendix 9B: Testing and Certification Requirements for the Critical Components of Biological Containment Systems](#)

### **9B-1. General**

This section provides the requirements for testing and certification that must be conducted at the factory and/or the field to verify the containment integrity of the critical components of biological containment systems. Copies of all testing and certification results are to be made to the facility. These copies will be retained indefinitely by and at the facility.

### **9B-2. Testing and Certification of Biological Safety Cabinets**

Biological Safety Cabinets shall be tested in accordance with the latest version of NSF Standard 49, Class II (Laminar Flow) Biohazard Cabinetry.

### **9B-3. Testing and Certification of HEPA Filter Assemblies**

A. Factory Testing. The filter housing pressure boundary shall undergo factory testing per ASME N5-1989 to 10" w.g. with a maximum permissible leak rate of 0.2 percent of the housing volume per hour. The filter element sealing surface shall be factory tested by the pressure decay method as specified in ASME N 510-1989.

B. In Place HEPA Filter Testing. Field test and provide written certification of all HEPA filter units with Polyalphaolefin (PAO) after installation to verify that the filters do not contain pinhole leaks in the filter media, the bond between the filter media and the filter frame and the filter frame gasket to filter housing.

Filter testing is intended to be completed in a similar manner to industry standards for certification of HEPA filters in Biological Safety Cabinets. The testing contractor may submit an alternate written testing procedure for approval by the RPSO prior to making filter certifications. If the alternate testing procedure is not approved, the following procedure shall be used.

#### Approved Testing Procedure:

1) Utilize an aerosol photometer with either a linear or a logarithmic scale and a threshold sensitivity of at least  $1 \times 10^{-3}$  micrograms per liter for 0.3 micrometer diameter PAO particles and a capacity for measuring 80-120 micrograms per liter concentration. The air sampling rate shall be at least 1 cfm.

The PAO generator shall be the Laskin nozzle(s) type which generates an aerosol of PAO particles by flowing air through liquid PAO. The compressed air supply to the generator shall be adjusted to 20 psi, measured at the entrance to the nozzle and downstream of all restrictions. The nozzles shall be with liquid PAO to a depth not to exceed 1 inch.

2) Adjust the air flow to approximately 10 percent of the design air flow rate of the filter. Place the PAO generator to uniformly introduce PAO aerosol upstream of the HEPA filter. Measure and record the upstream PAO concentration approximately in the center of the filter face.

For linear readout photometers (graduated 0\_100), adjust the instrument to read 100 percent while using at least one Laskin type nozzle per 500 cfm airflow, or increments thereof. For logarithmic readout photometers, adjust the upstream concentration to  $1 \times 10^{-4}$  above the concentration necessary for one scale division (using the instrument calibration curve).

3) With the nozzle of the photometer probe not more than 1 inch from the surface, scan the downstream side of the HEPA filters by passing the probe over the entire filter surface in slightly overlapping strokes. Scan the entire periphery of the filter, and the junctions between the filter media and the filter frame, and the filter frame and the housing. Scanning shall be done at a transverse rate of not more than 2 inches per second.

4) Identify and repair all points of leakage which exceed 0.01 percent of PAO penetration at any point, measured by a linear or logarithmic photometer for acceptance.

#### **9B-4. Testing and Certification of a Containment Room**

A. General. The purpose of testing the containment room or envelope is to determine if the walls, floors, ceilings, penetrations, and other containment barrier features have adequate integrity to prevent leakage of air from the containment space. Testing is typically completed by subjecting the containment area to negative or positive air pressure in excess of the anticipated operating conditions, and monitoring the containment air pressure over a test period.

Testing and Certification will typically consist of three progressive steps:

- 1) Pretesting for gross leaks by raising/lowering the containment space air pressure to about ½ inch W.C. (125 Pascal), then looking and listening for major leaks.
- 2) Soap bubble pretesting.
- 3) Pressure decay testing for final certification.

An individual containment testing plan shall be developed for each project and the Contractor's role shall be clearly identified in the project specifications. The Contractor's role may include: (a.) full responsibility for testing and documentation through the use of third-party testing subcontractors; (b.) sealing and repairs as needed to comply with Owner completed/subcontracted testing; or (c.) simple visual inspection. If third-party testing is to be coordinated by the Contractor, the project specifications shall include prior testing experience and submittal of qualifications prior to approval of the testing subcontractor.

For new construction, the Contractor will typically have greater responsibility for testing and certification than for renovation work, where access conditions will vary and all existing conditions may not be known. The project approach may also vary depending on the availability and expertise of location or agency safety staff.

#### B. Pretesting

The integrity of the containment space to prevent leakage will largely be the result of the care used by the Contractor and subcontractors to install products in accordance with the plans and specifications. The project quality assurance/quality control measures should include pretesting prior to testing for certification--even if the Contractor is not responsible for final acceptance testing and certification.

Prior to testing, supply and exhaust ventilation openings shall be sealed closed, and all doors and other openings through the containment perimeter shall be placed in their normal closed positions. If the doors in the containment perimeter are not gasket sealed, they will need to be temporarily caulked or otherwise sealed to complete the testing. The testing plan should address how the openings are to be sealed.

A calibrated digital or inclined manometer shall be installed across the containment perimeter in a manner to minimize interference with wind or ventilation turbulence and to accurately represent the interior and exterior

differential air pressure. The manometer shall have a display with capabilities to be easily read to an accuracy of 0.05 inch W.C. (10 Pascal) and capability to accurately read pressures to 3 inches W.C. (750 Pa).

When pretesting for large/gross leaks, the containment space may be pressurized or depressurized by installing a variable speed "blower door" or other approved means to generate a nominal ½ inch W.C. (125 Pa) differential pressure across the containment perimeter. The building surfaces, joints, penetrations, etc., are then inspected for air leakage and sealed in accordance with the plans and specifications. The testing plan should include a warning that generating excessive negative or positive pressures can apply significant stress to the facility, and may cause damage that will be repaired at the Contractor's expense. The testing plan and specifications should also remind the Contractor to complete sealing repairs while the space is not under test pressures, and that adequate time is to be allowed for sealants to properly cure before retesting.

Following completion of sealing of all leaks identified at ½ inch W.C. (125 Pa), pretesting may proceed to soap bubble testing. Depending on the location of the containment barrier and construction, soap bubble testing may be completed under positive or negative differential pressure. Typically testing is completed under negative pressure, when the soap bubbles are readily visible on the inside surface of the containment barrier.

Provide a fan/blower unit with the capacity to create and maintain a 2 inch W.C. (500 Pa) differential pressure for the time required to inspect all surfaces and to mark leaks. As the containment zone is sealed, the fan/blower capacity required to maintain adequate differential pressure becomes significantly smaller. A simple shop vacuum unit may be adequate for a large building. Provide a valve or other means of throttling the fan/blower unit to slowly "load" the building with pressure differential, and to keep from creating too large a pressure differential and causing damage to the structure.

Apply a soap or detector solution (e.g., a liquid detergent with a low surface tension, or a commercial test solution such as "Leak-Tek," "Search," or "Snoop") to all joints, corners, sealed penetrations, or other locations which could be point sources of air leakage. Potentially porous construction surfaces such as wood, masonry units, and mortar joints should be carefully checked. Mark all locations of bubble formations and air leaks. Remove the pressure differential and repair the leaks in accordance with the plans and specifications. Following adequate curing time, repeat the soap bubble testing.

Repeat testing and sealing cycles until it appears that the containment zone will pass pressure decay testing. If a ball valve is located in the fan/blower piping from the containment zone, the valve can be closed to seal the



containment zone. With the valve closed, monitor the time for the containment pressure to drop from 2 inches W.C. (500 Pa) to 1 inch W.C. (250 Pa). If the time approaches 20 minutes or more, the containment zone may be ready for pressure decay testing.

### C. Pressure Decay Testing and Certification

Prepare for testing by closing openings at the perimeter of the containment envelope and setting up testing equipment as described for pretesting. The fan/blower unit shall be capable of creating a 2-inch W.C. (500 Pa) pressure differential in the containment zone, and shall have a ball valve in the piping to the containment zone to allow the room/zone to be sealed once the testing pressure differential has been reached.

Testing shall be completed under generally stable conditions of outside wind, temperature, barometric pressure, and humidity. Testing shall be under negative differential pressure with respect to the surrounding environment. Air pressure testing ports/openings for the digital or inclined manometer instruments shall be located where the readings will not be affected by wind, air disturbances, or traffic.

#### Pressure Decay Testing Procedure:

- 1) Operate fan/blower unit to slowly (5 to 10 minutes) bring the differential pressure to 2 inches W.C. (500 Pascal).
- 2) Close the valve between the fan/blower and the test zone to seal the containment zone at 2 inches W.C. negative pressure with respect to the adjacent areas.
- 3) Record the differential pressure each minute for 20 minutes.
- 4) Slowly open the seal valve to allow the room/containment zone to return to normal pressure.

Decay testing may be repeated after a 20 minute wait period. Visually inspect the containment surfaces between testing and make repairs as necessary. If the acceptance criterion is not met, repeat the soap bubble testing and make repairs before retesting.

#### Acceptance Criterion:

Two consecutive pressure decay tests demonstrating a minimum of 1 inch W.C. (250 Pa) negative differential pressure remaining after 20 minutes, from an

initial negative pressure differential of 2 inches W.C. (500 Pa).

#### Reports:

At a minimum, reports for each decay test shall include start time, start and end room temperature, date, manometer data (brand, model, serial number, date of last calibration, full scale reading, and smallest scale increment), description of fan/blower unit and control means, tabulation of pressure differential readings for each test minute, a graphical plot of test data (time on the horizontal scale and differential pressure on the vertical axis), a floor plan illustrating the containment envelope and location of the fan/blower unit, and a description of the test, including seals and blockouts. Reports shall be signed and dated by the person completing the test.

### **9B-5. Testing and Certification of Gas Tight Ductwork and Isolation Valves**

Testing shall include all portions of the gas tight ductwork and filter systems that may potentially be exposed to contamination: from the rooms to the respective isolation dampers on the upstream side of the supply HEPA filters and on the downstream side of the exhaust HEPA filters.

Perform in-place positive pressure testing and written certification. All welds and /or duct joints shall remain fully exposed and accessible for inspection and repair until testing is completed and certified.

A. Preliminary testing shall be completed using soap bubble leak detection and/or helium gas to detect leaks for repair prior to final testing and certification. Use of "Freon" or other CFC gas is not acceptable.

B. Certification testing shall be completed using helium gas and a leak detector. The detector shall be an industrial type, capable and adjusted for detection of leaks of  $1 \times 10^{-7}$  cc/sec. Pressurize duct or assemblies to 4 inches w.g. (1,000 Pa) with a helium concentration adequate to insure leaks will be detected. Scan the interior surfaces of all ducts, seams, joints, gaskets, and other areas of possible leakage at a distance of 1/4 to 1/2 inch from the surface and at an approximate rate of 1 inch per second. Acceptance shall be no detected leaks in excess of  $1 \times 10^{-5}$  cc/sec.

At a minimum, the testing certification report shall include the date, time, detailed location, description of materials being tested, brand and serial number and calibration date of detector, name and signature of the person completing the testing, and shall be submitted in a format approved by the COR.

C. Alternative pressure testing may be approved on a case-by-case basis if temperature and other environmental conditions will not affect the test. Pressure testing shall be completed by pressurizing the gas tight assembly or ductwork to the specified pressure criteria, closing all valves and monitoring for pressure drop. Acceptance shall be zero pressure drop in one hour.

## **9B-6. Testing and Certification of Biocontainment Greenhouses**

Greenhouses constructed to meet the BSL-3Ag containment level will undergo the following tests: (a) an air infiltration test conducted according to ASTM E 283-91; the test pressure difference will be 6.24 pounds per square foot positive static pressure; the allowable leakage rate is 0.03 cfm per square foot; (b) a static pressure water resistance test conducted according to ASTM E 331-93; the minimum test pressure will be 10 pounds per square foot; the passing standard is no water penetration to the interior surface; and (c) a dynamic pressure water resistance test conducted according to AAMA 501.1-94; the minimum test pressure will be 10 pounds per square foot; the passing standard is no water penetration to the interior surface.