

BSL-3/ABSL-3 Verification Process and Requirements

May 18, 2023



Agenda

- **Verification Components**
 - BSL-3/ABSL-3 HVAC verification
 - Major changes
 - Major problems
 - Failure conditions
 - BSL-3/ABSL-3 facility verification
 - Minimum requirements
 - Decontamination systems
 - Filter verification
 - Primary barriers



Rationale

- Federal regulations require that for entities that possess select agents and toxins, “biosafety and containment procedures must be sufficient to contain the select agent or toxin (e.g., physical structure and features of the entity, and operational and procedural safeguards).” See 42 CFR 73.12(b), 9 CFR 121.12(b), 7 CFR 331.12 (b)
- The BSL-3 facility design, operational parameters, and procedures are verified and documented prior to operation. Facilities are tested annually or after significant modification to ensure operational parameters are met. Verification criteria are modified as necessary by operational experience. (BMBL, BSL-3, D.15)
- The laboratory shall be designed such that under failure conditions the airflow will not be reversed. (BMBL, BSL-3, D.9)
- The ABSL-3 animal facility shall be designed such that under failure conditions the airflow will not be reversed. (BMBL, ABSL-3, D6)



Two Distinct Verification Components

- BSL-3/ABSL-3 HVAC verification
 - To be completed initially and repeated only if:
 - Major changes to the system are performed
 - Major problems are identified
- BSL-3/ABSL-3 Facility verification
 - All listed items to be completed *annually*



BSL-3/ABSL-3 HVAC Verification

- Initial HVAC design verification must be performed and documented by someone with experience and expertise with the HVAC system prior to operation.
 - This initial HVAC design verification ensures that secondary containment is maintained under failure conditions to prevent possible exposure of personnel outside the containment boundary.
- After HVAC verification is initially documented, the testing need not be repeated, providing no major changes have been made to, or major problems noted with, the HVAC system.



Examples of Major Changes

- Replacement of exhaust or supply fans that serve the BSL-3/ABSL-3 containment areas
- Replacement of ductwork valves or dampers that serve these areas
- Replacement or repair of HVAC system control wiring
- Building automation system (BAS) logic programming changes
- Structural changes to the BSL-3/ABSL-3 rooms
- Addition or removal of hard-ducted Biological Safety Cabinets (BSCs) or fume hoods



Examples of Major Problems

- Frequent failures of the HVAC system
- Supply-exhaust interlocking system failure
- Observation that directional airflow is reversed under normal conditions or incidents (e.g., power failure)
- Observation that HVAC alarms are not working
- Observation that any BSCs with an HVAC connection are not working properly



Failure Conditions

- Mechanical failure of exhaust fan or fan component(s)
- Simultaneous power failure supporting supply and exhaust fan components
- Return from power failure to “normal” operating conditions



HVAC Verification

Note: A facility may be considered to pass the HVAC verification tests as long as laboratory air does not exit the containment barrier of the facility.

- The BSL-3 anteroom is considered to be within the containment envelope.
- A positive pressure excursion is not necessarily an airflow reversal; if a brief, weak positive pressure excursion is noted, a repeat test may be performed with airflow observation using an airflow indicator such as a smoke stick, or dry ice in a container of water, at the base of the closed laboratory door to confirm whether airflow reversal is occurring.



Minimum Facility Verification Requirements (1 of 2)

1. The means of detecting airflow has been confirmed to accurately reflect observed airflow. It is recommended, but not required, that digital or magnehelic gauges be calibrated annually.
2. Inward directional airflow has been confirmed by observation for the laboratory.
3. Decontamination systems (autoclave, room decontamination systems, digesters, liquid effluent systems, etc.) have been confirmed to be operating correctly.
4. If a Building Automation System (BAS) has the capacity to monitor and record performance measurements (e.g., differential pressures), the entity is encouraged to capture and store data from potential failure events, drills, etc. This information may provide verification of system performance. In addition, any programmed BAS alarms should be verified for proper functioning.



Minimum Facility Verification Requirements (2 of 2)

5. All alarms (fire, airflow, security, etc.) have been checked and are functioning according to established specifications.
6. Laboratory HVAC HEPA filters, if present, have been certified annually.
7. Exhaust fan motors have been checked and routine maintenance conducted.
8. The laboratory has been checked for unsealed penetrations, cracks, breaks, etc., and these have been repaired if present.
9. All biological safety cabinets have been certified annually.
10. Seals on centrifuges, Class III cabinets, gloves on Class III cabinets, etc., have been checked and replaced if required.
11. Drench showers, eye wash stations, and hands-free sinks have been confirmed to be operating properly.



BSL-3/ABSL-3 Facility Verification (1 of 9)

1. Means of detecting airflow has been confirmed

- Visual observation of any gauges at laboratory entries both prior to and after opening the door is sufficient. .
- Calibration is not required; however, it is considered best practice to routinely calibrate to ensure accuracy.
- Other methods such as BAS readings may also be used.

2. Inward air flow has been confirmed by observation

- May be performed at the same time as the above.
- smoke visualization may be employed to observe flow in a direction consistent with design parameters (usually inward).



BSL-3/ABSL-3 Facility Verification (2 of 9)

3. Decontamination systems have been confirmed to be operating correctly

- Effluent Decontamination System (EDS)
- Tissue Digestors
- Autoclaves
 - Annual Preventative Maintenance (PM)
 - Vent filter replacement or testing
 - Documentation demonstrating validated time and temperature has been reached prior to discharge for all cycles
 - Biological Indicators (BIs) and/or Bowie-Dick testing (BMBL, BSL-3, B.11)



Autoclaves

- Cycle data

- Ensures validation parameters are met.
- Inclusion of Biological Indicators (BIs) ensures uniform temperature throughout the load, especially because material is placed in bags.

- Preventative maintenance

- Bioseal integrity
- Door seals
- Valves and piping is operational
- Interlocks are operational
- Vent filter verification: Many autoclaves include filter systems that may be decontaminated at the end of the cycle.



Effluent Decontamination System (EDS)

- Batch cycle data
 - Temperature/time
 - Pressure
 - Fill level
- Preventative maintenance
 - Load cell/radar calibration
 - Temperature/pressure indicator testing and calibration
 - Valves and piping are operational
 - Operational testing
 - Vent filter verification



Tissue Digester

- Cycle data
 - Typically, a chemical process. Ensure proper chemical concentrations are reached.
 - Ensure cycle load weight is not greater than weight used during validation.
 - Are BIs used?
- Preventative maintenance
 - Load cell calibration
 - Lid seals should be checked
 - Temperature/pressure indicator testing and calibration
 - Vent filter verification



Batch Cycle Validation

- BIs are the most commonly used validation method.
- Chemical indicators ensure previously defined validation parameters have been reached.
- Indicators may be included in every decontamination cycle, but this is not required.
 - Frequency of BI validation is dependent on risk-based institutional policy.



Vent Filters

- Operational **AND** emergency vent filters must be verified annually:
 - Water intrusion test is the most common method.
 - Poly Alpha Olefin (PAO) challenge may be used; however, this method is not recommended by most manufacturers, and may result in a general concern.
 - A risk assessment should be performed prior to the use of this method.



BSL-3/ABSL-3 Facility Verification (3 of 9)

4. If a BAS has the capacity to monitor and record performance measurements, the entity is encouraged to capture and store data from potential failure events, drills, etc.
5. All alarms (fire, air flow, security, etc.) have been checked and are functioning according to established specifications.



BSL-3/ABSL-3 Facility Verification (4 of 9)

6. Laboratory HVAC HEPA filters, if present, have been certified annually.

- Filter penetration test
 - Gross probe
 - This test measures total filter efficiency (HEPA minimum = 99.97%).
- Filter in-place leak test
 - Scan test
 - This test identifies pinpoint leaks in the filter media, gasket area, and may identify HEPAs that are not seated properly.



HEPA Test Methodology

- Regardless of test performed, challenge media (PAO) is introduced upstream of the HEPA filter(s).
- The upstream and downstream concentrations are compared; any value that lies outside of the stated acceptance criteria is considered a leak.
- HEPA filters must either be patched (if applicable) or replaced if a leak is detected.



HEPA Filter Standards

- NEBB Procedural Standards for Certified Testing of Cleanrooms (National Environmental Balancing Bureau)
- IEST-RP-CC0034: HEPA and ULPA Filter Leak Tests (Institute of Environmental Sciences and Technology)
- IEST-RP-CC001: HEPA and ULPA Filters (Institute of Environmental Sciences and Technology)
- NSF/ANSI 49 Biosafety Cabinetry: Design, Construction, Performance, and Field Certification



BSL-3/ABSL-3 Facility Verification (5 of 9)

7. Exhaust fan motors have been checked and routine maintenance conducted.

- Facilities preventative maintenance records – typical checks:
 - Bearings
 - Belts
 - Proper lubrication levels
 - Variable Frequency Drive(s) reading properly
 - Air Handler Units: Temp., Relative Humidity, flow rate



BSL-3/ABSL-3 Facility Verification (6 of 9)

8. The laboratory has been checked for unsealed penetrations, cracks, breaks, etc., and these have been repaired if present.

- Typically smoke visualization around all penetrations into laboratory including:
 - All plumbing lines and conduit.
 - Fire suppression piping.
 - Fixtures such as lighting and light switches, etc.
 - Paint on walls is free from cracks.
 - Cracks in floors and ceilings are sealed.
 - Demonstrate that air transfer ducts can be easily sealed.



BSL-3/ABSL-3 Facility Verification (7 of 9)

9. All BSCs have been certified annually.

- Test standards employed for BSCs:

- NSF/ANSI 49 Biosafety Cabinetry: Design, Construction, Performance, and Field Certification



Primary Barriers

- BSC
- Flexible film isolators (bioBubble™)
- Downdraft/necropsy table



Biosafety Cabinets (BSCs)

- Certified annually
 - Must be certified on or before due date listed on previous year's certification document.
 - May be certified later than due date listed provided *no select agent or toxin work has taken place in the cabinet. Unit should include signage precluding its use with select agents and toxins.*



Class II

- Inward airflow
- Downward airflow
- Smoke testing at sash opening
- Site installation assessment tests (NSF 49, section F.7)
- HEPA filter verification (supply and exhaust)



Site Installation Assessment Tests –Required (1 of 2)

- Class II, Type A1/A2
 - Alarm Functions
 - Sash Alarms
 - Exhaust airflow alarms, if present
 - Canopy connected cabinets only
 - Inflow alarm system, if present



Site Installation Assessment Tests - Required (2 of 2)

- Class II, Type B1/B2
 - Alarm Functions
 - Sash Alarms
 - Exhaust airflow alarms and interlocks, if present
 - Exhaust alarm test
 - Interlock shuts down internal blower within 15 seconds of loss of exhaust volume.
 - Inflow alarm system, if present



Site Installation Assessment Tests (Optional)

- Class II
 - Electrical leakage and ground circuit resistance and polarity tests
 - Lighting intensity test
 - Vibration test
 - Noise level tests



Record of Field Certification (1 of 2)

- Field certification label:
 - Date of field certification
 - Date cabinet should be field recertified
 - Certifiers report number
 - Name, address, and telephone number of certifying company
 - Signature of the person who performed the field certification tests



Record of Field Certification (2 of 2)

- Field certification report:

- 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
- Specific field test data as detailed in Annex F
- Acceptance criteria for each field test
- Printed name of field certification technician
- Field test date
- Field retest date



Class III

- HEPA filters
 - 1x supply
 - 2x exhaust
- Ventilation rate/negative pressure
- Alarms/interlocks
- Integrity testing if moved or panels removed



Flexible Film Isolators

- Smoke visualization at openings and other areas to ensure airflow is proper.
- Proximity of discharge to supply or exhaust vents
- Check decontamination methods:
 - Are they appropriate for the given agents/activities?
 - Are they appropriate for the materials of construction?
- HEPA verification
- Alarms, if present



Necropsy Tables

- HEPA filters
- Decontamination methods
- Airflow tests performed



BSL-3/ABSL-3 Facility Verification (8 of 9)

10. Seals on centrifuges, Class III cabinets, gloves on Class III cabinets, etc., have been checked and replaced if required:

- Seals may be checked by visual inspection.



BSL-3/ABSL-3 Facility Verification (9 of 9)

11. Drench showers, eye wash stations, and hands-free sinks have been confirmed to be operating properly:

- These may be checked by simply activating them and confirming operation.
 - Hands-free sinks and eyewash stations should release water for a sufficient amount of time, but should also stop the supply of water in a timely manner to prevent any overflowing of effluent decontamination systems, if present.
 - The sinks, eyewash station, and the water supply and drainage piping should be free from rust or any other signs of corrosion.



Summary

- Total of 11 facility-related items to be checked *annually*.
 - Some inspection items may simply include visual observation.
 - Others require detailed documentation to confirm compliance.
 - Risk assessments may be helpful in defining test methodology and frequency.
 - Consider employing best practice, even if it is not required.



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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention or the Animal and Plant Health Inspection Service.

