# BSL-3/ABSL-3 and BSL-4/ABSL-4 Verification

# Agenda

FSAP Policy – Heating, Ventilation, Air Conditioning (HVAC) and Facility Verification Components

- BSL-3/ABSL-3 Verification Policy Statement
  - [BMBL 5th Ed.: (BSL-3) D9; (ABSL-3) D6; (BSL-3 D15); and (ABSL-3) D14]
- Biosafety Level 4 (BSL-4)/Animal BSL-4 (ABSL-4) Laboratory Facility Verification Policy Statement
  - [BMBL 6th Ed.: (BSL-4) IV D16a Cabinet laboratory; (ABSL-4) V D16a Cabinet facility; (BSL-4) IV Cabinet laboratory D17 and Suit laboratory D20); (ABSL-4) V Cabinet facility D18, Suit facility D21
- Basic components (similarities)
- Additional components for BSL-4 facilities

# Links to Policy Statements at www.selectagents.gov

[Policy Statement: BSL-3/ABSL-3 Verification | Select Agents Regulations | Federal Select Agent Program]

## Policy Statement: BSL-3/ABSL-3 Verification

**Date: November 20, 2014**

**Subject:** BSL-3/ABSL-3 Verification

The Federal Select Agent Program (FSAP) is a collaboration between the Center for Disease Control and Prevention (CDC), Division of Select Agents and Toxins (DSAT) and the Animal and Plant Health Inspection Service (APHIS) Division of Agricultural Select Agents and Toxins (DASAT) to regulate the possession, use, and transfer of biological agents listed in 7 C.F.R. Part 331, 9 C.F.R. Part 121, and 42 C.F.R. Part 73 (select agents and toxins). The FSAP administers the select agents and toxins regulations in close coordination with the Federal Bureau of Investigation's Criminal Justice Information Services (CJIS).

**Authority:**
For biological agents and toxins determined by HHS to have the potential to pose a severe threat to public health and safety (select agents and toxins), the Public Health Security and Bioterrorism Preparedness and Response Act of 2002 (42 U.S.C. 262a) directs the promulgation of regulations to establish and enforce safety procedures for the possession and use of select agents and toxins, including measures to ensure proper training and appropriate skills to handle such agents and toxins. 42 U.S.C. § 262a(c)

For biological agents and toxins determined by USDA to have the potential to pose a severe threat to animal health or animal products (select agents and toxins), the Agricultural Bioterrorism Act of 2002 (7 U.S.C. 8401) directs the promulgation of regulations to establish and enforce safety procedures for the possession and use of such select agents and toxins, including measures to ensure proper training and appropriate skills to handle such select agents and toxins. (7 U.S.C. 8401(c))

Zoonotic select agents and toxins are regulated by both HHS and USDA. See 42 CFR 73.4 and 9 CFR 121.4.
Federal regulations require that for entities that possess select agents and toxins their "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).

[Policy Statement: BSL-4/ABSL-4 Laboratory Facility Verification Requirements | Select Agents Regulations | Federal Select Agent Program]

CDC USDA
FEDERAL SELECT AGENT PROGRAM

## Policy Statement: Biosafety Level 4 (BSL-4)/Animal BSL-4 (ABSL-4) Laboratory Facility Verification Requirements

**Date**: February 9, 2023

**Subject:** Biosafety Level 4 (BSL-4)/Animal BSL-4 (ABSL-4) Laboratory Facility Verification

Biosafety Level 4 (BSL-4) and Animal BSL-4 (ABSL-4) facility design parameters and operational procedures are used when working with dangerous and exotic biological agents that are easily transmitted by the aerosol route, cause severe to fatal disease in humans, and typically do not have available vaccines or treatments. These laboratories must establish and exercise the highest level of biosafety precautions to ensure facility containment is maintained.

Entities that possess, use, or transfer select agents and toxins must comply with the federal select agent and toxin regulations found in 42 CFR Part 73, 7 CFR Part 331, and 9 CFR Part 121. To meet the biosafety sufficiency requirement in Section 12(b) of the federal select agent and toxin regulations, it is the policy of the Federal Select Agent Program (FSAP) that entities that store or use select agents and toxins in BSL-4 and/or ABSL-4 laboratories verify that the facility parameters and operational procedures, including heating, ventilation, and air conditioning (HVAC) systems, are functioning as intended. To verify these systems are functioning properly in laboratories, entities need to ensure:

- **HVAC operational verification is conducted initially, annually [1], and after any major changes or after resolving major problems.**
  HVAC operational verification is performed and documented prior to initial operation of the BSL-4/ABSL-4 containment areas (suit and cabinet), annually, and after major changes to ensure operational parameters are maintained. The HVAC design verification process provides evidence that secondary containment will be maintained during both normal operating conditions and during failure conditions to prevent air-flow reversals into non-containment areas or positive pressurization events (e.g., outside the containment boundary, hallways).
  - HVAC operational verification includes "under failure conditions" (e.g., failure of primary supply air fan, failure of primary exhaust

# HVAC Verification Completion

- BSL-3/ABSL-3 HVAC verification
  - To be completed initially and repeated when:
    - Major changes to the system are performed
    - Major problems are identified

- BSL-4/ABSL-4 HVAC verification
  - To be completed initially, **annually,** and repeated if:
    - Major changes to the system are performed
    - Major problems are identified

# 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

# Examples of HVAC Major Changes for BSL-3/ABSL-3s

- Replacement of HVAC equipment that serves these areas
- Replacement of **exhaust or supply fans**
- Replacement of ductwork valves or dampers
- Repair or replacement HVAC system control wiring
- Building automation system (BAS) logic programming changes
- Structural changes to the laboratory rooms
- Addition or removal of hard-ducted Biological Safety Cabinets (BSCs) or fume hoods

# Examples of HVAC Major Changes for BSL-4/ABSL-4s

- Replacement, repairs, or modifications of:
  - Exhaust and supply fans
  - Ductwork valves or dampers
  - HVAC control system components
- Building automation system (BAS) logic programming changes
- Structural changes
- Addition or removal of hard-ducted BSCs, Class III cabinets, or decontamination systems
- Events that compromise the containment envelope

# Examples of HVAC 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

# Tested HVAC Failure Conditions

Failure scenarios for HVAC performance verification include:

- 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 BSL-3 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

# HVAC BSL-4/ABSL-4 Failure Test Results

Results of failure tests must demonstrate that there is no airflow reversal that originates within the BSL-4/ABSL-4 containment areas that escape the containment boundary

# Facility Verification

# Facility Verification

BSL-3/ABSL-3 and BSL-4/ABSL-4 Facility verification

- All listed items to be completed *annually*

# 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

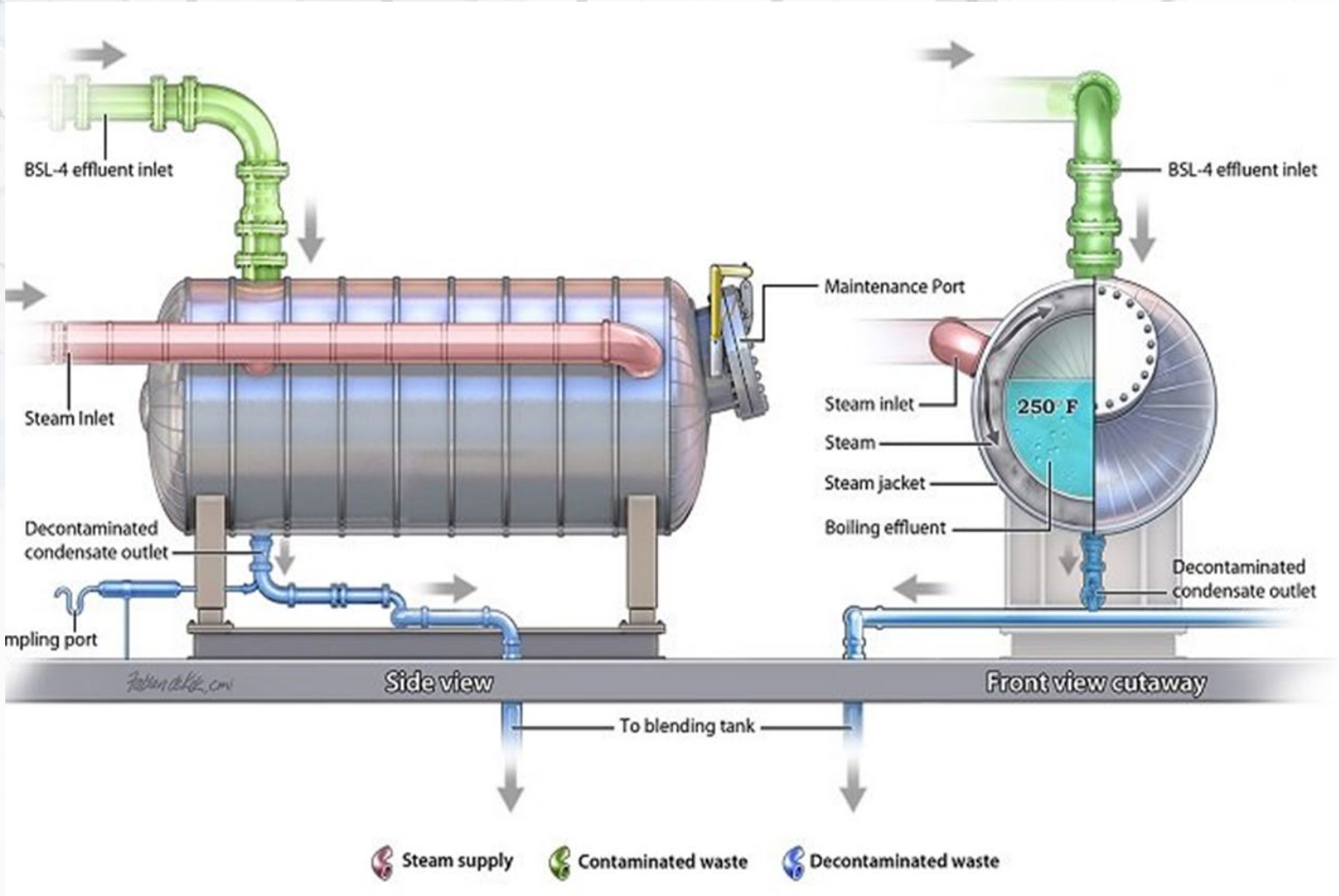
2. Inward air flow has been confirmed by observation
   - May be performed at the same time as the above
   - Smoke visualization may be employed to observe flow in a direction consistent with design parameters (usually inward)

# 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)

# Effluent Decontamination System (EDS)

- Batch cycle data
  - Temperature/time
  - Pressure
  - Fill level



# Effluent Decontamination System (EDS) II

- Preventative maintenance
  - Load cell/radar calibration
  - Temperature/pressure indicator testing and calibration
  - Verifying valves and piping are operational
  - Operational testing
  - Vent filter verification

- **Vent filter – Decontaminate prior to opening filter housing and changing filter**
  - **Opening filter housing without decontamination may result in a Form 3**

# Vent Filters

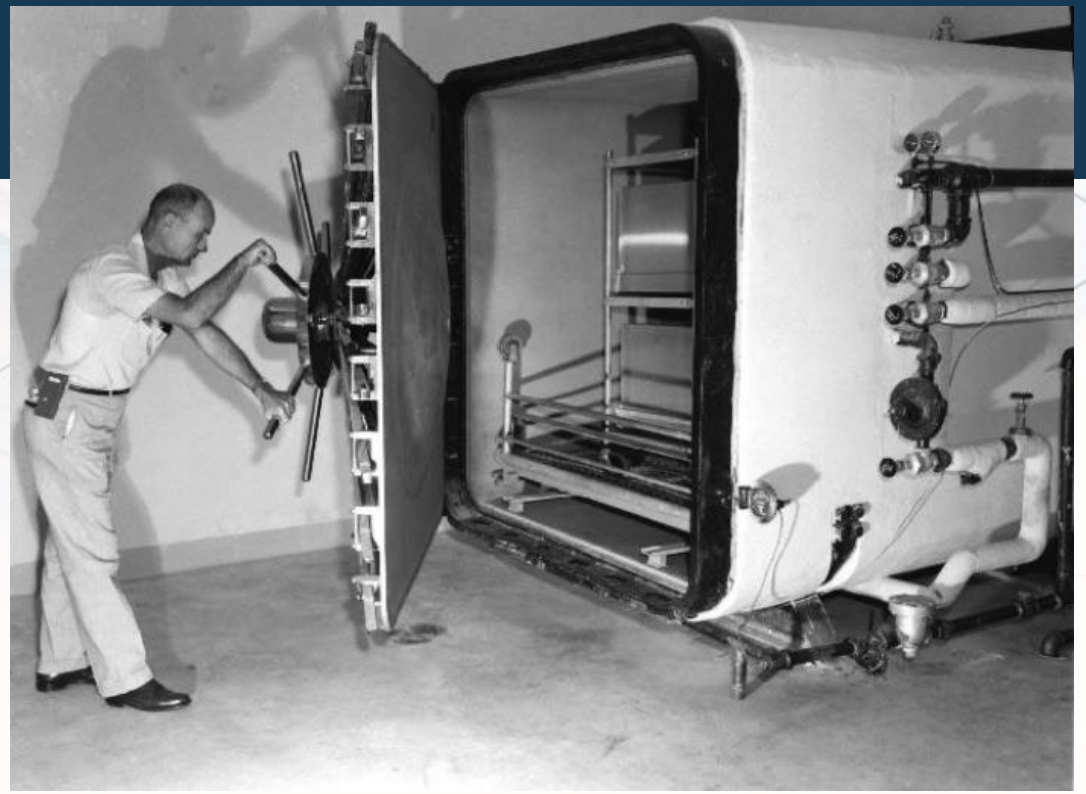
Operational **AND** emergency vent filters verified annually:

- Water intrusion test (WIT) is the most common method
- Poly Alpha Olefin (PAO) challenge is sometimes used; however, this method is not recommended by most manufacturers
  - A risk assessment should be performed prior to the use of this method

# 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 (Decontamination of filter housing prior to opening)

# Autoclaves



- Cycle data
  - Ensures validation parameters are met
- 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

# 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

# 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

# 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: temperature, relative humidity, flow rate

**Annual Fan Failure Testing is NOT required for BSL-3/ABSL-3**

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

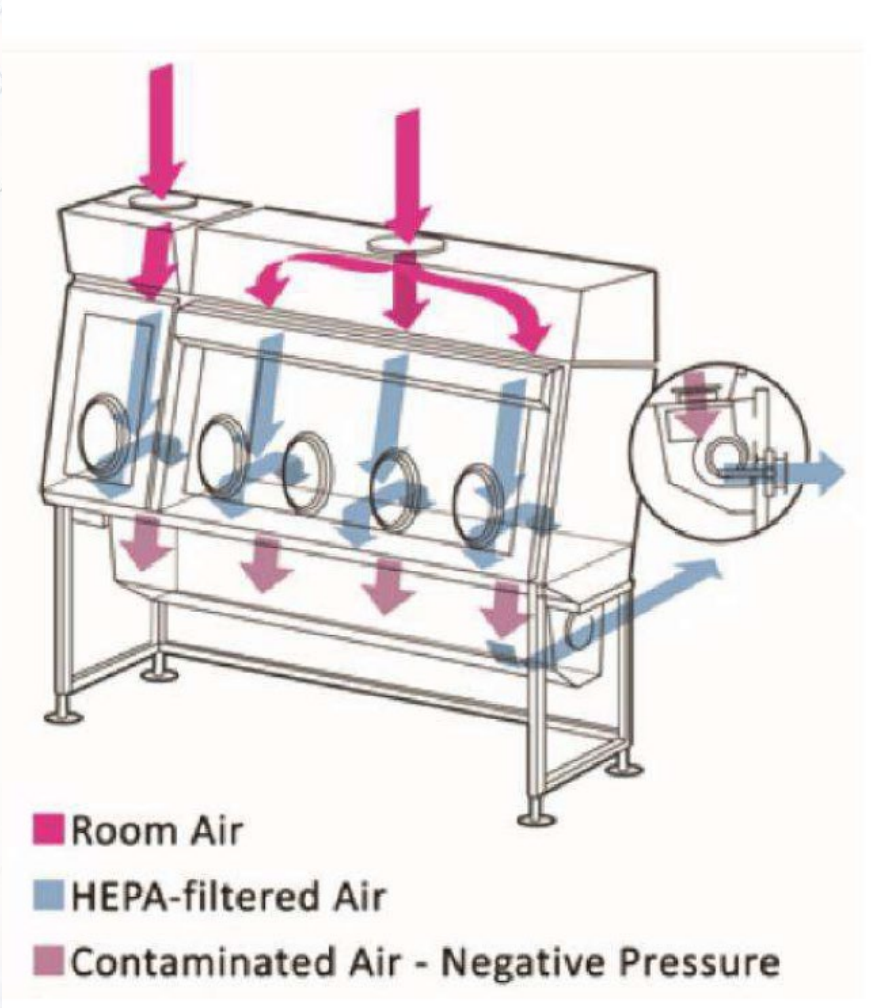
8. The laboratory has been checked for unsealed penetrations, cracks, breaks, etc., and these have been repaired if present.
   - Typically, smoke visualization around all penetrations into laboratory including:
     - All plumbing lines and conduit
     - Fire suppression piping
     - Fixtures such 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.
   - Must be certified on or before due date listed on previous year's certification document
   - Test standards employed for BSCs:
     - NSF/ANSI 49 Biosafety Cabinetry: Design, Construction, Performance, and Field Certification

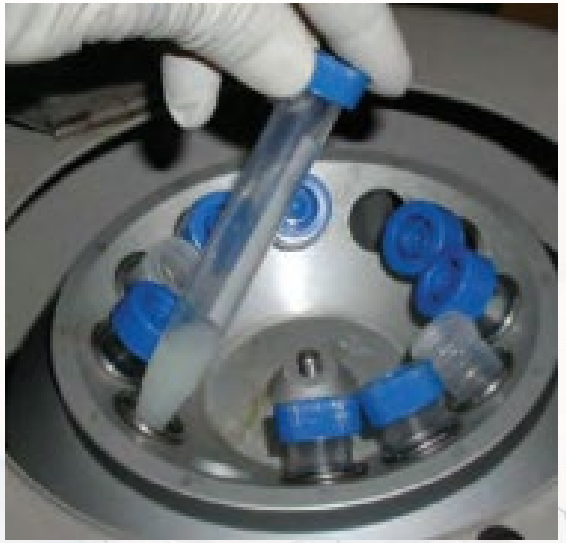
# Class III BSC

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



# 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
        - The sinks, eyewash station, and the water supply and drainage piping should be free from rust or any other signs of corrosion

# Conducting BSL-4/ABSL-4 Facility Verification

**BSL-4/ABSL-4 facility verification is conducted initially, annually, and after any major changes or after resolving major problems**

- Minimum verification components performed and documented
- Eight items

**For the purposes of the BSL-4/ABSL-4 Facility Verification Policy, the term "annual" is defined as every 365 days**

# Minimum Verification Components (1-2)

1. Recalibration of components that monitor containment parameters (e.g., differential pressure gauges, pressure transducers, BAS)

2. Verification of BAS-programmed alarm communication

# Minimum Verification Components (3A)

3. Confirmation that decontamination systems are operating as designed (e.g., autoclave, room decontamination systems, tissue digesters, liquid effluent systems)
    A. Effluent, tissue, and autoclave decontamination systems:
        i. Annual verification of system operational components to ensure biologically validated set point parameters are maintained (e.g., volumetric, pressure, temperature components);
        ii. Biological validation is performed at least annually or more often, if required by institutional policy and/or risk assessment;
        iii. Annual certification testing of associated high-efficiency particulate air (HEPA) filters, if applicable (e.g., operating vent, pressure relief vent, chamber effluent/vent);

# Minimum Verification Requirements (3A) Continued

A. Effluent, tissue, and autoclave decontamination systems:

iv. Annual verification that system failure communication systems are operating as designed (e.g., alarms, leak detection);

v. Verify appropriate filter media is selected and maintained annually (e.g., HEPA, polytetrafluoroethylene [PTFE]); and

vi. Implementation of a risk-based preventative maintenance for other equipment that is critical to containment components, but is not specifically included above (e.g., cook tanks, etc.);

# Minimum Verification Requirements Continued (3B,C)

B. Annual verification that chemical shower delivery systems are operating as designed (e.g., system delivery components and conductivity and alarm monitoring);

C. Annual verification that room decontamination systems are operating as designed and tested using biological indicators (e.g., biological indicator strips);

# Minimum Verification Requirements (4-8)

4. Certification of laboratory HVAC, plumbing vent line, and decontamination system filters by appropriate means and appropriate acceptance criteria to ensure integrity (e.g., HEPA, PTFE).
5. Assessment of breathing air quality for both main and backup air breathing air supply.
6. Certification of BSCs.
7. Verification of primary containment integrity (e.g., centrifuges, Class III cabinets, animal caging) with component replacement if defective.
8. Confirmation that all alarms (e.g., air supply, exhaust, life support, BAS alarms, fire, airflow, security, access systems, water supply backflow prevention devices) have been checked and are functioning according to approved design specifications.

# BSL-4/ABSL-4 Ongoing Facility Verification

Four items that the entity must perform and document on an ongoing, routine basis

# BSL-4/ABSL-4 Ongoing Facility Verification (1, 2)

1. Surveillance and implementation of routine maintenance programs for HVAC supply and exhaust fans, main and backup breathing air systems, and decontamination systems.
2. Surveillance of containment envelope for penetrations, cracks, breaks, and performance of related repairs. Identifying and confirming proper operation of various BSL-4/ABSL-4 containment boundary points of failure (such as penetrations, cracks, breaks, etc.) may be successfully tested through proper pressure decay testing.

# BSL-4/ABSL-4 Ongoing Facility Verification (3, 4)

3. Surveillance and verification of secondary containment envelop integrity (e.g., Air Pressure Resistant (APR) door gaskets, HVAC dampers).
4. Confirmation and testing of critical interlocks and manual overrides (e.g., between exhaust fans and air handling units, between laboratory exhaust fan airflows and supply airflows, and between mechanical and electronic door interlocks).

**Note:** Entities may need to perform additional facility verification in addition to these and other systems due to the entity's facility design, operation, work objectives, etc., to ensure containment of select agents and toxins.

# HVAC Verification Summary

HVAC verification must be completed and documented:

- BSL-3/ABSL-3: Initially and following major changes or problems
- BSL-4/ABSL-4: Initially, **annually** and following major changes or problems

**For BSL-4/ABSL-4, "annually" refers to 365 days**

# Facility Verification Summary

For both BSL-3/ABSL-3 and BSL-4/ABSL-4 facility verification:

Items to be checked *annually.*

- Some inspection items may simply include visual observation.
- Others include detailed documentation to confirm compliance.
- Risk assessments may be helpful in defining test methodology and frequency.

For BSL-4/ABSL-4, ongoing facility verification should be conducted