Learning Event 6: Operating and Maintaining Laboratory Equipment

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Contents

Guidance on Collaborative Review

Dear Subject Matter Experts,

Origin of the Content Outline:

This content outline has been crafted based on our collaborative workshops. The details and structure have been derived from the diverse resources you generously provided, ensuring a foundation that is both comprehensive and reflective of our collective expertise.

Role of the Designer:

As the instructional designer, my responsibility is to organize, structure, and present information in an effective and coherent manner. I take the intricate details of our subject and ensure they are communicated effectively to our intended audience.

Your Expertise:

Your role is crucial. You ensure that the information is accurate, relevant, and up to date. Your deep knowledge helps us maintain the integrity and credibility of our content.

Actionable Feedback Defined:

When reviewing, we kindly ask for "actionable feedback." This means:

1. **Specificity:** Instead of saying "This section isn't clear," it would be helpful to indicate which part is unclear and why.
2. **Suggestions:** If something seems off, please provide an alternative or explain how it can be improved.
3. **References & Resources:** If you believe additional sources or references should be included, please provide the exact details or links.

Your detailed feedback ensures that we create a product that is both authoritative and user-friendly. Thank you for your commitment to excellence.

Modality

2 hours of eLearning: Operating and Maintaining BSL-3 Laboratory Equipment

Asynchronous e-learning modules will provide participants with the foundational knowledge and information they need to understand critical concepts related to Operating and Maintaining BSL-3 Laboratory Equipment. This includes hazards associated with common laboratory equipment, preventative maintenance, and engineering controls. The modules will include interactive activities, videos, scenarios, case studies, self-assessments, and quizzes to reinforce key concepts and assess participants' understanding of the material. This will allow participants to review the material at their own pace and deepen their understanding of the concepts. Existing assets will be utilized to design and develop eLearning modules to provide a comprehensive understanding of equipment operation and maintenance in laboratory settings.

Topics to be Covered

- ✓ Laboratory Equipment:
  - Hazards associated with common laboratory equipment
  - Use of glassware, autoclaves, high-speed centrifuges, incubators, ultra-low freezers, liquid nitrogen freezers, bead beaters, vortex, and microcentrifuges
- ✓ Evaluating Risks:
  - Risks associated with using common laboratory equipment in a BSL-3 laboratory
  - Sharps – as they relate to equipment or other usage
- ✓ Preventative Maintenance:
  - Procedures for planned or preventative maintenance of laboratory equipment
  - Decontamination of equipment
- ✓ Safely Moving Equipment:
  - Concepts related to safely moving equipment or supplies into or out of a BSL-3 environment
  - Equipment awareness, mobile containment enclosure, movement, and decontamination
- ✓ Engineering Controls:
  - Purpose, functionality, and proper use of commonly used BSL-3 laboratory engineering controls
  - Biological safety cabinets (BSC), high-efficiency particulate air (HEPA), air lock, dunk tanks, effluent decontamination system (EDS), negative airflow, sealed rotors, anteroom, shower, lab doors, self-closing doors, sealed floors, and hands-free sinks
- ✓ Ensuring Proper Functioning of Engineering Controls:
  - Familiarity with engineering control functions, alarms, checking for negative airflow, and troubleshooting autoclaves
- ✓ Addressing and Reporting Compromised, Malfunctioning, or Non-Functioning Engineering Controls:
  - Procedures to address and report when engineering controls are compromised, malfunctioning, or non-functioning
  - Communication of issues with all users of the space.

Note: The list of topics included was generated during the content outlining phase of our curriculum design process. This was based on initial discussions and planning sessions aimed at ensuring comprehensive coverage and relevance to our objectives.

Course Learning Objectives

Lesson 1: Laboratory Equipment

- Evaluate risks associated with using common laboratory equipment in a BSL-3 laboratory
- Describe procedures for planned or preventative maintenance of BSL-3 laboratory equipment

Lesson 2: Safely Moving Equipment

- Identify the concepts related to safely moving equipment or supplies into or out of a BSL-3 environment

Lesson 3: Engineering Controls

- Describe the purpose, functionality, and proper use of commonly used BSL-3 laboratory engineering controls
- Determine if BSL-3 laboratory engineering controls are functioning properly
- Describe the procedures to address and report when engineering controls are compromised, malfunctioning, or nonfunctioning
- Identify procedures to follow when non-negative airflow (pressure) values are indicated in the BSL-3 environment

Note: The list of objectives provided here was formulated during the content mapping and content outline phases of our development process. This approach ensures that our objectives are thoroughly planned and aligned with the overall educational goals.

Operating and Maintaining BSL-3 Laboratory Equipment

Lesson 1: Laboratory Equipment

Hazards of Common Laboratory Equipment

While using BSL-3 laboratory equipment, personnel must prevent contamination and maintain a safe working environment. Responsible equipment use protects laboratory staff, prevents the spread of infectious agents to the broader community, and ensures the integrity of scientific research.

In this learning event, we'll look at key pieces of equipment in BSL-3 laboratories, such as sharp instruments, autoclaves, centrifuges, stacked incubators, biosafety cabinets, freezers, and vortexes, and their associated hazards.

Sharps

Description
Sharp instruments are items such as needles, scalpels, and glassware. They can sometimes easily penetrate personal protective equipment (PPE). A single mishap can result in the introduction of pathogens into the bloodstream, posing a risk of severe infection. Therefore, handling sharp instruments requires meticulous attention to detail and adherence to strict safety protocols. Potentially contaminated materials must be disposed of safely.
Hazards or Issues
• Cuts and punctures • Aerosol generation • Needlestick injuries • Breakage of glassware • Improper disposal
Example BSL-3 Pathogen Risk
• Mycobacterium tuberculosis (M. tuberculosis) can cause serious respiratory infections if introduced into the bloodstream through a cut or puncture • Francisella tularensis, the pathogen that causes tularemia, has a very low infectious dose, making even minor cuts a significant risk.
Precautions
• Minimize the use of glassware. Use substitutes when possible. • Dispose of needles- do not reuse. Avoid recapping needles to prevent needlestick injuries • Use engineered sharps safety devices (safety syringes, retractable needles, or scalpel blades) • Wear puncture-resistant gloves to provide an additional barrier against punctures or cuts • Dispose of sharps immediately, directly into designated puncture-resistant sharps containers • Perform procedures involving sharps within a biosafety cabinet (BSC) to minimize aerosols

Autoclaves

Description
An autoclave is a sterilizing chamber used to decontaminate biohazardous waste and other contaminated materials or equipment. The decontamination is achieved through the introduction of steam into the chamber to maintain a temperature of 121^{0}C or greater with a pressure of 15 PSI or greater for predetermined period of time. These parameters are programmed in your designated autoclave.
Hazards or Issues
• Exposure to Pathogens (due to autoclave failure) • Burns and Scalds • Pressure Explosions • Chemical Hazards (dangerous reactions or release of toxic fumes) • Mechanical Failure (door seal failures, chamber cracks/leaks, exposure to high-pressure steam) • Accidental Spills
Example BSL-3 Pathogen Risk
Some BSL3 pathogens are capable of airborne transmission, making them particularly dangerous if not properly sterilized. The risk arises if the autoclave fails to achieve the necessary temperature, pressure, and duration to inactivate these pathogens fully. Any failure in the autoclave's operation, such as inadequate steam penetration or temperature control, can result in incomplete decontamination, potentially leading to accidental exposure to infectious agents.
Precautions
The autoclave's **interlocking mechanism** prevents the door from being opened while the chamber is pressurized. The feature ensures users are not exposed to high-pressure steam or hazardous materials. It also keeps the autoclave from beginning its sterilization cycle unless the door is securely closed and sealed. Wearing **heat-resistant gloves** while operating an autoclave protects against burns and scalds when handling hot materials, touching the autoclave's heated surfaces, or unloading the autoclave. **Effective Response to Autoclave Failure:** • Cease all autoclave operations • Make sure autoclave doors are sealed. Affected materials should remain inside. • Communicate the failure to all personnel within the laboratory. • Notify laboratory management. • Implement emergency response plan (e.g. evacuation of non-essential personnel; decontamination procedures) • Potentially exposed personnel should undergo medical evaluation. • Inspect and repair the autoclave before any further use

Centrifuges

Description
A centrifuge separates biological samples at very high rotational speeds, often exceeding 20,000 RPM. This centrifuge must be operated under strict biosafety protocols, as it handles potentially infectious materials that could generate aerosols if mishandled. To ensure safety, the centrifuge is typically equipped with sealed rotor buckets and is often placed within a biosafety cabinet or an enclosed room to contain any accidental releases of hazardous agents. If the centrifuge is too large to be placed in a biosafety cabinet (BSC), rotors are loaded and closed and then unloaded in the BSC. A microcentrifuge is a small, high-speed laboratory centrifuge designed for spinning down small volumes of liquid, typically in microcentrifuge tubes (0.5 to 2.0 mL). It operates at speeds ranging from a few thousand to tens of thousands of revolutions per minute (RPM), generating significant centrifugal forces. This device is commonly used to separate biological molecules, such as nucleic acids and proteins, from a liquid sample through sedimentation. Microcentrifuges are essential for tasks like DNA purification, RNA extraction, and sample concentration.
Hazards or Issues
• Aerosol Generation • Mechanical Failure (rotor imbalance, improper loading, or structural fatigue) • Improper Sealing (Sample Leakage) • Tube Breakage • Overheating (results in sample damage or equipment failure) • Electrical Hazards (electric shocks, short circuits) • Vibration and Noise (hearing damage, physical damage to centrifuge) • Imbalanced Loads (centrifuge failure, mechanical failure, sample loss) • Inadequate Decontamination
BSL-3 Pathogens
When using BSL-3 pathogens in a centrifuge, samples must be carefully sealed in leak-proof containers, an the centrifuge should be equipped with aerosol-tight rotor lids to contain any potential leaks. Rotors shoul be well-greased, balanced, and sealed. • If a small centrifuge is used with a pathogen and centrifuge safety cups are not available, operate the centrifuge in a biosafety cabinet. • Fill and decant all centrifuge tubes and bottles within a biosafety cabinet. • Wipe the outside of tubes with a disinfectant compatible with the rotor before placing them in safety cups or rotors. • Open safety buckets or rotors in a biosafety cabinet. • Decontaminate safety carriers or rotors and the centrifuge interior after each use. • Some centrifuge models have the capability to connect to a vacuum pump. Connect when needed and use an in-line HEPA filter on the exhaust.

After use, the centrifuge and all associated equipment should be thoroughly decontaminated to eliminate any residual contamination. BSL3 pathogens often require the use of a microcentrifuge for processing small volumes of highly infectiou samples. A microcentrifuge enables the precise separation of components such as bacterial cells, viral particles, or nucleic acids from biological fluids. Separation facilitates accurate analyses, using PCR, sequencing, or other molecular biology techniques, which are necessary for diagnosing, studying, or developing treatments for these pathogens. The small size and high speed of microcentrifuges make them ideal for quick and efficient processing of samples, minimizing the time the pathogens are not contained.
Precautions
• Load and unload centrifuge rotors in a biosafety cabinet • Use centrifuge safety buckets or sealed centrifuge tubes in sealed rotors. • For ultracentrifuges, keep a log book that contains operation records for each centrifuge and their rotors to assist in determining service requirements. • Check tubes and bottles for cracks or stress marks before using them. • Never fill centrifuge tubes more than recommended by the manufacturer. • Make sure all tubes that are on the opposite side of a rotor are equal in weight. • Sealed Tubes to Prevent Leakage • Always cap tubes, if required, before spinning. • Inspect the “O” ring seal of the safety bucket and the inside of the safety buckets or rotors. • Never exceed safe rotor speed. • Stop the centrifuge immediately if an unusual noise or vibration begins. Get away from any centrifuge which starts to “walk.” • Wait five minutes to allow aerosols to settle after the run and before opening the centrifuge. • Pre- and Post-Operation Decontamination • Spill Response Preparation

Knowledge Check 1

1. Which of the following precautions should be taken when working with sharps? Select all that apply.

A) Minimize the use of glassware. Use substitutes when possible.
B) Dispose of needles; do not re-use. Avoid recapping needles to prevent needlestick injuries.
C) Wear puncture-resistant gloves.
D) Keep sharps in the work area even when they are not in use and you are finished with them.
E) Use as many sharp instruments as possible so that personnel have practice using them.

Correct answers: A, B, and C. “Correct. The following are precautions that should be taken with sharps: Minimize use of glassware, substitute glassware when possible, dispose of needles, do not re-use needles, avoid recapping needles, and wear puncture-resistant gloves. Note: these guidelines are not exhaustive.”

Incorrect answers: Try again. The following are precautions that should be taken with sharps: Minimize use of glassware, substitute glassware when possible, dispose of needles, do not re-use needles, avoid recapping needles, and wear puncture-resistant gloves. Note: these guidelines are not exhaustive."

Knowledge Check 2

True or False: If an autoclave failure occurs, you can continue autoclave operations while you investigate what went wrong.

Correct Answer (False): Correct. The statement is false. You should cease autoclave operations if an autoclave error occurs. Autoclave errors indicate a malfunction in sterilization processes, which can compromise the safety and effectiveness of the equipment. Continuing operations during an error could lead to incomplete sterilization, risking contamination or infection. Ceasing operations allows for proper troubleshooting and ensures that corrective actions are taken before resuming safe use.

Incorrect Answer (True): Try again. The correct answer is False. You should cease autoclave operations if an autoclave error occurs. Autoclave errors indicate a malfunction in sterilization processes, which can compromise the safety and effectiveness of the equipment. Continuing operations during an error could lead to incomplete sterilization, risking contamination or infection. Ceasing operations allows for proper troubleshooting and ensures that corrective actions are taken before resuming safe use.

Stacked Incubator

Description
A stacked incubator is designed for the controlled growth of biological cultures. These incubators are typically configured with multiple shelves or compartments. This allows for the simultaneous cultivation of different samples under precisely regulated conditions, such as temperature, humidity, and CO2 levels. The stacked design maximizes space efficiency. The incubators are constructed with airtight seals and HEPA filters to prevent the escape of potentially hazardous pathogens. Additionally, incubators are equipped with robust monitoring and alarm systems to promptly detect and address any deviations from the set conditions.
Hazards or Issues
• Contamination Risk (improper sealing or handling of pathogen culture) • Mechanical Failure (malfunction of temperature, humidity, or CO2 controls) • Space Constraints (increases the risk of accidental spills) • Airflow Obstruction (leads to uneven temperature and humidity distribution) • HEPA Filter Failure • Electrical Hazards (fires; equipment damage) • Human Error

• Maintenance Challenges (difficult access to incubator parts; cleaning and service challenges)
BSL-3 Pathogens
Various BSL3 pathogens require the use of a stacked incubator due to their need for controlled environmental conditions for growth and study. Stacked incubators maintain specific CO2 concentrations, temperature, and humidity.
Precautions
• Ensure Proper Sealing (doors, gaskets, access ports, HEPA filters, etc.) • Regular Calibration (temperature, CO2, and humidity) • Routine Maintenance (HEPA filters, alarm systems) • Monitor and Test Alarm Systems • Proper Loading (maintain proper airflow and prevent temperature or humidity imbalances) • Safe CO2 Handling (secure connections, no leaks, accurate incubation conditions) • Follow Decontamination Protocols

Biosafety Cabinets

Description
Biosafety cabinets (BSCs) are designed to serve as a primary barrier to contain potential aerosols (e.g. viruses, bacteria, fungi, etc.) that might be generated from laboratory activities and equipment. BSCs are equipped with high efficiency particulate air (HEPA) filters that have an optimum efficiency capture rating of 99.97% for particles 0.3 µm in size. HEPA filters have a higher filtration efficiency for particles that are smaller or larger than 0.3 µm.
Hazards or Issues
• Aerosol Generation from Improper Use • Cross-Contamination from Inadequate Cleaning • HEPA Filter Failure • Improper Airflow • Electrical Hazards: (sparks, short circuits in the presence of flammable substances) • UV Light Exposure (skin burns, eye damage) • Ergonomic Strain (repetitive stress injury, awkward postures, limited mobility) • Human Error (inadequate training or lapses in protocol adherence)
BSL-3 Pathogens
All BSL3 pathogens require the use of a BSC.
Precautions
• Proper Training • Pre-operation Checklist (check airflow gauges, ensure the sash is at the correct height, and verify that the UV light [if available] is off) • Minimize Disruptions (Avoid quick movements or placing objects near the air vents of the BSC) • Decontaminate Before and After Use • Work Within the Safe Zone (at least four inches from the front grill) • Proper Waste Disposal • Routine Maintenance and Certification

Ultra-Low-Temperature (ULT) Freezers

Description
Ultra-Low-Temperature (ULT) freezers are used to store biological samples, chemicals, and other sensitive materials at specific temperature ranges, often well below freezing, to preserve the integrity of the materials they store. Some laboratory freezers are designed to achieve regular freezer temperatures (-16°C to -24°C), while ULT freezers can achieve extremely low temperatures between -40°C and -86°C. ULT freezers are critical in research involving vaccines, enzymes, and cell cultures. They may also include advanced features such as temperature alarms and data logging.
Hazards or Issues
• **Frostbite Risk** • **Melted Ice Slip** • **Electrical Hazards** (electrical shock or fire if there are issues with wiring or grounding) • **Mechanical Hazards** (moving parts such as compressors and fans) • **Physical Strain** (size and weight of ULT freezer doors) • **Temperature Fluctuations (from f**requent opening of the ULT freezer; can compromise integrity of stored samples) • **Freezer Thaws (**leads to materials expansion or seals failure; leaks) • **Power Failure Risk** • **Compressed Gas Hazards** (gas leaks or explosions if the system is not properly maintained)
BSL-3 Pathogens
Most BSL3 pathogens require storage in ultra-low temperature (ULT) freezers, typically at temperatures ranging from -70°C to -80°C, to maintain their viability and stability over extended periods. The ULT storage conditions help preserve the genetic material and proteins of these pathogens, ensuring that they remain suitable for research, diagnostics, or vaccine development. Proper storage helps maintain the integrity of the samples and ensures biosafety, as low temperatures help to reduce the risk of accidental release or proliferation of these dangerous pathogens.
Precautions
• Wear Cryogenic Gloves • Avoid Prolonged Door Openings • Regular Maintenance • Emergency Backup Systems • Proper Sample Labeling and Inventory • Monitor Temperature Continuously • Avoid Overloading • Safe Handling of Liquid Nitrogen

Vortex

Description

A vortex is a compact, high-speed mixing device used to rapidly blend small volumes of liquid samples by creating a vortex motion in the container. It is commonly used to mix reagents, resuspend pellets, or homogenize solutions containing BSL-3 pathogens. The device is equipped with variable speed controls, allowing for precise adjustments based on the sensitivity and requirements of the samples being mixed.
Hazards or Issues
• Aerosol Generation • Spill Risk (Improper seals or loosely capped containers) • Cross-Contamination (from inadequate cleaning of the vortex or mixing containers between uses) • Equipment Malfunction • Vibration Hazards (can cause improperly balanced loads, leading to falls and breakages) • Electric Shock (shocks or short circuits, particularly in a high-humidity environment) • Glass Breakage (sharps) • Plastic Container Cracks (spills/leaks) • Injury from Improper Handling (cuts, bruises)
BSL-3 Pathogens
Vortexing homogenizes BSL3 pathogen solutions, resuspends pellets, and ensures that reagents are evenly distributed.
Precautions
• Use a BSC • Use sealed or capped tubes during vortexing to reduce the risk of aerosol generation

Knowledge Check 3

Match the equipment type with its description, function, or characteristic.

A. **Stacked Incubator -** Designed for the controlled growth of biological cultures. typically configured with multiple shelves or compartments.
B. **Biosafety Cabinet -** Designed to serve as a primary barrier to contain potential aerosols (e.g. viruses, bacteria, fungi, etc.) that might be generated from laboratory activities and equipment. Equipped with HEPA filters.
C. Ultra-Low-Temperature (ULT) - Used to store biological samples, chemicals, and other sensitive materials at specific temperature ranges, often well below freezing, to preserve the integrity of the materials they store.
D. Vortex – A compact, high-speed mixing device used to rapidly blend small volumes of liquid samples. Commonly used to mix reagents, resuspend pellets, or homogenize solutions.

Correct: You matched everything correctly.

Incorrect. Try again. The correct matches are as follows: A **stacked Incubator is** designed for the controlled growth of biological cultures. typically configured with multiple shelves or compartments. A **biosafety cabinet is d**esigned to serve as a primary barrier to contain potential aerosols (e.g. viruses, bacteria, fungi, etc.) that might be generated from laboratory activities and equipment. Equipped with

HEPA filters. An **Ultra-Low-Temperature (ULT) Freezer** is used to store biological samples, chemicals, and other sensitive materials at specific temperature ranges, often well below freezing, to preserve the integrity of the materials they store. A **vortex** is a compact, high-speed mixing device used to rapidly blend small volumes of liquid samples. Commonly used to mix reagents, resuspend pellets, or homogenize solutions.

Knowledge Check 4

Biosafety Cabinets (BSCs) are equipped with high efficiency particulate air (HEPA) filters that have an optimum efficiency capture rating of ______% for particles 0.3 µm in size.

A) 80%
B) 99.97%
C) 50%
D) 90%

Correct: Biosafety Cabinets (BSCs) are equipped with high efficiency particulate air (HEPA) filters that have an optimum efficiency capture rating of 99.97% for particles 0.3 µm in size.

Incorrect: Try again. Biosafety Cabinets (BSCs) are equipped with high efficiency particulate air (HEPA) filters that have an optimum efficiency capture rating of 99.97% for particles 0.3 µm in size.

Knowledge Check 5

True or False: Tubes do not need to be sealed or capped during vortexing since there is no risk of aerosol generation.

Correct (False): Tubes do need to be sealed or capped during vortexing to reduce the risk of aerosol generation.

Incorrect (True): Try Again. The correct answer is as follows: Tubes do need to be sealed or capped during vortexing to reduce the risk of aerosol generation.

Equipment Maintenance

Annual Shutdown

BSL-3 laboratories at CDC shut down once a year for planned maintenance to ensure the continued safety and integrity of the facility and its equipment. The shutdown is recommended but not necessarily required of all facilities.

Planned Maintenance

During this period, the laboratory is thoroughly decontaminated, and all essential equipment, including biosafety cabinets, autoclaves, and HVAC systems, undergoes rigorous inspection, maintenance, and certification.

Floors, walls, and doors are checked for signs of wear, damage, or breaches in containment, which are promptly repaired to maintain the laboratory's airtight integrity. This shutdown allows for the replacement or repair of any faulty or aging components, ensuring that the laboratory meets the stringent safety standards required for handling high-risk pathogens.

Additionally, the process provides an opportunity to update protocols and train staff on any new safety measures, ensuring that the laboratory operates at peak safety and efficiency when it reopens.

External inspectors are typically present during planned maintenance of BSL-3 laboratories to ensure that the facility meets all regulatory standards and safety protocols required for the containment of high-risk pathogens. They certify that equipment, such as biosafety cabinets and autoclaves, is functioning correctly and that the laboratory's structural integrity is intact.

Emergency Facility Maintenance

Emergency facility maintenance requires a shutdown of the laboratory. It could be required in cases of

- **Critical Equipment Failure:** If essential systems like the HVAC, biosafety cabinets, or autoclaves fail, a shutdown may be needed to repair or replace the equipment and prevent exposure to hazardous materials.
- **Structural Integrity Compromise:** Any damage to the lab's structure, such as breaches in walls, doors, or containment seals, may require a shutdown to repair and restore the lab's containment capabilities.
- **Incident Response:** In the event of a significant spill, contamination, or accidental exposure, a shutdown may be necessary to thoroughly decontaminate the laboratory and address any safety concerns before resuming operations.

During emergency maintenance, external inspectors may not always be immediately present, as the priority is to quickly address and resolve the issue to prevent potential exposure or contamination.

After emergency repairs are completed, however, an inspection or review by external authorities is often required to verify that the laboratory is safe to resume operations, ensuring that all corrective actions have restored the lab to its full containment and safety standards.

Knowledge Check 6

True or False: All BSL-3 facilities have an annual shutdown for planned maintenance.

Correct: False – Yes. The statement is false. Not all BSL-3 facilities have an annual shutdown for planned maintenance. BSL-3 laboratories may not have an annual planned shutdown for maintenance because these labs often handle ongoing critical research and diagnostic activities that cannot be easily paused. Continuous operations are necessary to maintain vital work on high-priority pathogens, such as those causing infectious diseases, which may require real-time testing and monitoring. Instead of annual shutdowns, BSL-3 laboratories typically perform staggered or phased maintenance, ensuring both safety and uninterrupted research.

Incorrect: True – Try again. The statement is False. BSL-3 laboratories may not have an annual planned shutdown for maintenance because these labs often handle ongoing critical research and diagnostic activities that cannot be easily paused. Continuous operations are necessary to maintain vital work on high-priority pathogens, such as those causing infectious diseases, which may require real-time testing and monitoring. Instead of annual shutdowns, BSL-3 laboratories typically perform staggered or phased maintenance, ensuring both safety and uninterrupted research.

Knowledge Check 7

What is the primary role of external inspectors during planned maintenance of BSL-3 laboratories?

A) To assist laboratory personnel in operating autoclaves and biosafety cabinets.
B) To ensure that the laboratory meets regulatory standards and safety protocols for containment
C) To conduct research alongside laboratory personnel
D) To perform routine cleaning and maintenance tasks

Correct answer: B) External inspectors' primary role is to ensure that the laboratory meets regulatory standards and safety protocols for containment. They also certify that equipment, such as biosafety cabinets and autoclaves, is functioning correctly and that the laboratory's structural integrity is intact.

Incorrect Answers: Try Again. The correct answer is B. External inspectors' primary role is to ensure that the laboratory meets regulatory standards and safety protocols for containment. They also certify that equipment, such as biosafety cabinets and autoclaves, is functioning correctly and that the laboratory's structural integrity is intact.

Preparation for Maintenance (Video)

Decontamination of Maintenance Equipment

Tools used during equipment preventative maintenance and the decontamination equipment itself must be decontaminated beforehand to ensure the safety of personnel and prevent the release of hazardous pathogens. All maintenance equipment and tools must be thoroughly decontaminated using appropriate methods such as chemical disinfectants (e.g., 70% ethanol, sodium hypochlorite), autoclaving, or vaporized hydrogen peroxide, depending on the material and type of contamination.

Any removable parts should be sterilized separately, and non-removable parts should undergo rigorous surface decontamination. The decontamination process must be documented, and maintenance personnel should be equipped with appropriate personal protective equipment (PPE) to handle the decontaminated items safely. Decontamination equipment is maintained by OLSS (OLSR).

Full Room Decontamination

Vaporized Hydrogen Peroxide

For full room decontamination, equipment should be able to withstand contamination with Vaporized Hydrogen Peroxide (VHP**)**. VHP is a commonly used decontamination method that involves the generation of hydrogen peroxide vapor to sterilize equipment, surfaces, and spaces within the laboratory. VHP can penetrate hard-to-reach areas. This method is effective in killing a wide range of microorganisms, including bacteria, viruses, and spores, making it an essential tool for maintaining biosafety in high-containment laboratories like BSL-3 facilities.

Peracetic Acid

Prior to use, ensure the room can withstand decontamination with peracetic acid (PA), a chemical sterilant which when generated into a fine mist or vapor that can reach all surfaces, including hard-to-access areas. The room is sealed, and the peracetic acid vapor is introduced and maintained for a specific duration to ensure that all microorganisms are effectively neutralized. After the exposure time, the room is aerated to remove any residual peracetic acid, ensuring the area is safe for re-entry.

Sealing Equipment

Before full room decontamination takes place, incubators, freezers, and other equipment can be carefully sealed to prevent the infiltration of decontamination agents and to protect sensitive contents from exposure. Equipment doors are secured with tape or other sealing materials, and any vents or openings are covered to ensure a tight seal. In some cases, plastic sheeting or specialized covers are used to fully encapsulate larger equipment.

If equipment is to be left on during the shutdown, most equipment, such as incubators, centrifuges, etc.), are left open to allow for VHP decontamination. This completely decontaminates the equipment and prepares it for preventative maintenance.

Equipment being opened and unsealed could be a potential breach in biocontainment.

Decontamination Tags

Decontamination tags track and verify that all equipment and surfaces in the laboratory have been properly decontaminated. These tags typically include the date of decontamination, the method used, and the initials of the personnel responsible for the procedure.

Knowledge Check 8

Select the statement below that is not true about decontamination of maintenance equipment.

A. Tools used during equipment preventative maintenance must be decontaminated beforehand
B. Chemical disinfectants are used to decontaminate maintenance equipment.
C. Removable parts of equipment can be sterilized while attached to the main part and other parts.
D. The decontamination process must be documented.

Correct: B. Removable parts of BSL-3 equipment need to be sterilized separately to ensure that all surfaces, including internal or hidden components, are properly decontaminated. Sterilizing them while attached to the main equipment may leave areas unexposed to the sterilization agent, increasing the risk of contamination by dangerous pathogens.

Incorrect: Try Again. The correct answer is B. Removable parts of BSL-3 equipment need to be sterilized separately to ensure that all surfaces, including internal or hidden components, are properly decontaminated. Sterilizing them while attached to the main equipment may leave areas unexposed to the sterilization agent, increasing the risk of contamination by dangerous pathogens.

Knowledge Check 9

True or False: If equipment is to be left on during the shutdown, most equipment, such as incubators, centrifuges, etc.), are left open to allow for VHP decontamination.

Correct/True: Yes. If equipment is to be left on during the shutdown, most equipment, such as incubators, centrifuges, etc.), are left open to allow for VHP decontamination. This ensure that it can undergo vaporized hydrogen peroxide (VHP) decontamination, which requires active circulation. By keeping equipment like incubators and centrifuges open and running, all surfaces are exposed to the sterilizing agent, ensuring complete decontamination.

Incorrect/False. Try Again. The statement is true. If equipment is to be left on during the shutdown, most equipment, such as incubators, centrifuges, etc.), are left open to allow for VHP decontamination. This ensure that it can undergo vaporized hydrogen peroxide (VHP) decontamination, which requires active circulation. By keeping equipment like incubators and centrifuges open and running, all surfaces are exposed to the sterilizing agent, ensuring complete decontamination.

Lesson 2: Safety Moving Equipment

Equipment Awareness

Personnel should be aware of the potential hazards associated with each piece of equipment in a BSL-3 laboratory. The equipment should be properly used, maintained, and decontaminated.

High Occupancy Time (HOT)

Equipment is HOT during high-occupancy times when active work with infectious agents is taking place. During these periods, all equipment, including that which is mobile or on wheels, is potentially contaminated and must be treated with the highest level of caution. Equipment should not be removed from the laboratory without undergoing thorough decontamination procedures to prevent the spread of pathogens outside the containment area. This includes surface decontamination with appropriate disinfectants and, if necessary, using autoclaving or gas sterilization methods to ensure all microbial life is neutralized.

Robotics and Automated Equipment

In BSL-3 laboratories, robotics and automated equipment help enhance safety and efficiency. Robotic systems minimize human interaction by automating repetitive tasks, such as sample handling, reducing the risk of contamination and exposure. Automated equipment also ensures precision and consistency in experiments, which is essential for reliable results. Moreover, robotics can operate continuously without fatigue, improving productivity while adhering to stringent safety protocols. This technology not only safeguards researchers but also accelerates the pace of scientific discovery in high-risk environments.

Moving Equipment and Materials

Robotics and automated equipment enable precise and controlled movement of instruments and materials, minimizing human contact and reducing the chance of accidental spills, contamination, and exposure to infectious agents. Automated systems can be programmed to handle specific tasks, such as transporting samples between workstations or loading and unloading equipment, all while maintaining the stringent containment protocols required in these labs.

Robotics and automated equipment may help move the following:

- **Biological Samples**: These may include bacteria, viruses, and other microorganisms that are classified as BSL-3 pathogens.

- **Laboratory Containers**: Automated systems can handle Petri dishes, culture flasks, test tubes, and other containers used to grow or store biological samples.
- **Reagents and Chemicals**: Robotics can be used to move and dispense potentially hazardous chemicals, reducing the risk of exposure.
- **Pipetting Systems**: Automated liquid-handling systems are commonly used for precise pipetting of biological samples and reagents to avoid human error and contamination.
- **Plates and Assay Systems**: Robotics can handle multi-well plates used in assays, moving them between incubators, readers, and other devices.
- **Centrifuges**: Automated centrifuge loading and unloading can be done to handle samples that need to be separated without manual intervention.
- **Incubators**: Robotics can move samples in and out of incubators, ensuring consistent environmental conditions while reducing human exposure.
- **Sterilization Equipment**: Automated systems may handle items that need to be sterilized before and after use, such as by moving them through autoclaves or UV chambers.
- **Specimen Transport Systems**: Robotics can be used for the internal transport of specimens between different areas of the lab, ensuring secure and contained movement.
- **Data Collection Devices**: Automated systems might also be used to operate and collect data from laboratory instruments like microscopes, flow cytometers, and PCR machines.

Knowledge Check 10

What are benefits of robotics and automated equipment in BSL-3 laboratories? Select all that apply.

- They minimize human interaction by automating repetitive tasks, such as sample handling, to reduce the risk of contamination and exposure.
- Can operate continuously without fatigue, improving productivity while adherent to stringent safety controls.
- Require specialized expertise for maintenance, and any malfunction can delay critical operations.
- The acquisition, installation, and maintenance of robotic systems are costly.
- Improperly calibrated or maintained equipment could introduce contamination risks, particularly in handling high-risk pathogens.

Correct. A & B. Yes. Two benefits of robotics and automated equipment in BSL-3 laboratories are that they minimize human interaction by automating repetitive tasks, such as sample handling, to reduce the risk of contamination and exposure, and that they can operate continuously without fatigue, improving productivity while adherent to stringent safety controls.

Incorrect: Try Again. The correct answers are A & B. Two benefits of robotics and automated equipment in BSL-3 laboratories are that they minimize human interaction by automating repetitive tasks, such as sample handling, to reduce the risk of contamination and exposure, and that they can operate continuously without fatigue, improving productivity while adherent to stringent safety controls.

Recalibration

Laboratory equipment often needs to be recalibrated after being moved to ensure accurate and reliable results. The need for recalibration depends on several factors, including the type of equipment, the distance and conditions under which it was moved, and the sensitivity of the measurements it performs.

Circumstances

Situations that could necessitate recalibration include

- **Movement Over Long Distances**: When equipment is moved across long distances, especially if transported by vehicle, vibrations and shocks can disrupt calibrations.
- **Changes in Environmental Conditions**: Moving equipment to a location with different temperature, humidity, altitude, or air pressure can affect performance and accuracy, necessitating recalibration.
- **Relocation Within the Lab**: Even small movements within the laboratory, such as moving equipment from one bench to another, can affect sensitive instruments like balances and spectrophotometers.
- **Mechanical Disturbances**: If equipment is bumped, jarred, or subjected to mechanical stress during a move, recalibration is usually necessary.
- **Change in Power Supply**: Moving equipment to a different electrical outlet or power supply can sometimes affect its calibration, especially for highly sensitive electronic equipment.
- **Installation in a New Environment**: When equipment is moved to a new laboratory or facility, recalibration ensures it functions correctly under the new conditions.
- **Regulatory Requirements**: Some laboratory protocols or quality control standards may require recalibration whenever equipment is moved, regardless of the perceived impact.

Equipment Type

The equipment types that could need recalibration include

- **Balances and Scales**: These are sensitive to changes in position, leveling, and environmental factors like vibrations and temperature. Even small changes can affect their accuracy.
- **Pipettes and Liquid Handling Systems**: Precision in volume measurement can be affected by changes in altitude, air pressure, and handling conditions.
- **Spectrophotometers**: Movement can affect the alignment of optical components, which may require recalibration to ensure accurate absorbance or transmittance readings.
- **Microscopes**: The alignment of optical paths and mechanical components may shift, requiring recalibration for accurate imaging.
- **Centrifuges**: Rotor balance and speed settings may be affected by movement, necessitating recalibration to maintain proper operation.

- **Chromatography Systems (e.g., HPLC, GC)**: These systems are sensitive to alignment, pressure, and flow rate settings, which may be disturbed during transport.
- **Temperature-Controlled Equipment (e.g., Incubators, Freezers, Ovens)**: Changes in position or environment can affect temperature uniformity and control, requiring recalibration.
- **pH Meters**: Sensor calibration may be affected by movement, particularly if the probes or sensors are disturbed.
- **Autoclaves**: Recalibration might be needed to ensure proper temperature and pressure settings for sterilization after relocation.
- **Lab Automation and Robotics**: Mechanical and alignment settings might need recalibration to ensure precise operation after being moved.

Knowledge Check 11

True or False: The need for equipment calibration after movement takes into consideration equipment type, distance and conditions of movement, and sensitivity of measurements performed.

Correct/True: You answered correctly. The need for equipment calibration after movement takes into consideration equipment type, distance and conditions of movement, and sensitivity of measurements performed.

Incorrect/False: Try again. The statement is true. The need for equipment calibration after movement takes into consideration equipment type, distance and conditions of movement, and sensitivity of measurements performed.

Knowledge Check 12

Why might balances and scales in a BSL-3 laboratory need recalibration after being moved?

A) They are sensitive to changes in position, leveling, and environmental factors like vibrations and temperature.

B) The internal software of the balance resets automatically when the equipment is moved, which requires recalibration.

C) Moving the balance causes the weighing platform to shrink slightly, affecting weight measurements.

D) Recalibration is needed because the balance loses power when disconnected and cannot remember previous settings.

Correct: Yes. Balances and scales are sensitive to changes in position, leveling, and environmental factors like vibrations and temperature. Even small changes can affect their accuracy.

Incorrect: Try again. The correct answer is A. Balances and scales are sensitive to changes in position, leveling, and environmental factors like vibrations and temperature. Even small changes can affect their accuracy.

Movement

When moving equipment in a BSL-3 laboratory, ensure that all items are properly decontaminated, using approved disinfectants known to be effective for specific pathogens. The equipment should be sealed to prevent the release of any hazardous agents.

Always wear appropriate personal protective equipment (PPE) and follow strict protocols to avoid contamination or exposure.

Decontamination Before Movement

When moving equipment, also make sure the space through which you are moving it is properly decontaminated beforehand. An exception to this guideline is when you are moving equipment from a clean area to a dirty area.

Decontamination

All equipment, including pipettors, pipette aids, and sample transport carriers, require surface decontamination before removal from a laboratory to prevent the accidental transfer of infectious agents. Sample transport carriers often come into direct contact with biological samples, increasing the risk of contamination from spills, leaks, or aerosol generation. Pipettors and pipette aids may not directly contact hazardous materials, but aerosols or splashes can still deposit pathogens on their surfaces.

Lesson 3: Engineering Controls

Engineering controls in a BSL-3 laboratory are designed to minimize the risk of exposure to infectious agents. These controls include features such as negative air pressure, which ensures that air flows into the laboratory rather than out, preventing the escape of airborne pathogens. Additionally, HEPA-filtered ventilation systems capture and remove any harmful particles from the air before it is recirculated or exhausted. Other key engineering controls, such as biosafety cabinets and sealed windows, provide physical barriers that protect laboratory personnel and maintain containment of hazardous biological materials.

Types of Engineering Controls

Biosafety Cabinets

Biosafety cabinets (BSCs) protect laboratory personnel, the environment, and the research material by providing a controlled, sterile environment for handling infectious agents and hazardous materials.

BSCs use HEPA-filtered airflow to create a barrier between the user and the work area, effectively capturing and filtering out airborne contaminants to prevent exposure and contamination. The cabinet's airflow patterns ensure that harmful particles are contained within the cabinet and do not escape into the laboratory environment.

To ensure safety and effectiveness, laboratory personnel must work within the designated area inside the BSC, avoid obstructing airflow vents, regularly decontaminate surfaces before and after use, and ensure that the cabinet is properly maintained and certified annually.

High-Efficiency Particulate Air (HEPA)

High-Efficiency Particulate Air (HEPA) filters remove airborne contaminants, including infectious agents and hazardous particles, to maintain a sterile and safe environment for laboratory personnel and research.

HEPA filters can trap 99.97% of particles that are 0.3 microns in size or larger, using a dense, fibrous material that captures particles through mechanisms like interception, impaction, and diffusion. They are installed in ventilation systems and biosafety cabinets to filter incoming and outgoing air, ensuring that any potentially harmful particles are contained.

To maintain their effectiveness, HEPA filters must be regularly inspected, tested, and replaced as needed, following the manufacturer's guidelines and laboratory safety protocols. Proper sealing and installation are essential to prevent leaks, and airflow should be monitored to ensure that the filters are functioning correctly and providing the necessary level of protection.

Note that some BSL-3 laboratories do not have HEPA filtration. They have them instead on laboratory exhaust lines or supply lines.

Knowledge Check 13

HEPA filters are installed in _________ and __________ to filter incoming and outgoing air, ensuring that any potential harmful particles are contained.

A. ventilation systems; biosafety cabinets
B. centrifuges; autoclaves
C. incubators; freezers
D. refrigerators; fume hoods

Correct. Correct. HEPA filters are installed in **ventilation systems** and **biosafety cabinets** to filter incoming and outgoing air, ensuring that any potential harmful particles are contained.

Incorrect. Try again. The correct answer is A. HEPA filters are installed in **ventilation systems** and **biosafety cabinets** to filter incoming and outgoing air, ensuring that any potential harmful particles are contained.

Airlocks

Some BSL-3 laboratories have airlocks. An airlock is a controlled entry and exit space designed to prevent the escape of airborne pathogens and maintain a safe containment environment. It typically consists of two doors, one leading into the laboratory and the other to the outside, which are never open at the same time, ensuring that air from the laboratory does not flow out. The airlock often includes features like HEPA filtration, negative air pressure, and decontamination protocols to further prevent the release of infectious agents. This setup helps protect both laboratory personnel and the surrounding environment by minimizing the risk of contamination and maintaining strict containment barriers.

Airlocks and other designated areas allow equipment to be safely passed from the outside into the laboratory. However, it is not necessarily the case that equipment can be passed from the laboratory to an outside area.

Autoclave

Autoclaves sterilize equipment, waste, and biological materials, effectively killing or inactivating pathogens to prevent contamination and the spread of infectious agents.

Autoclaves use high-pressure steam at temperatures typically ranging from 121°C to 134°C to sterilize materials. The combination of heat and pressure over a set period (usually 15-30 minutes, depending on the load) ensures that all microorganisms, including spores, are destroyed. Autoclaves are equipped with safety interlocks, temperature and pressure controls, and monitoring systems to verify that sterilization parameters are met consistently.

Proper Use of Autoclave

Proper use of autoclaves involves following standardized procedures for loading, operating, and unloading the unit. Materials must be prepared correctly (e.g., using autoclave bags and containers), and the autoclave's cycle settings should be selected according to the type and volume of the load. Regular maintenance, including calibration, validation of sterilization efficacy, and adherence to safety protocols, is essential to ensure reliable performance and laboratory safety.

When autoclave errors occur, laboratory personnel should call their Biosafety Officer (BSO).

Knowledge Check 14

Which statement is **true** about autoclaves and the operation of autoclaves?

A. Laboratory personnel should troubleshoot autoclave problems when an autoclave error occurs.
B. Autoclaves use high-pressure steam at temperatures typically ranging from 40°C to 60°C to sterilize materials.
C. Autoclaves are equipped with safety interlocks, temperature and pressure controls, and monitoring systems to verify that sterilization parameters are met consistently.

Correct. Yes, you answered correctly. Autoclaves are equipped with safety interlocks, temperature and pressure controls, and monitoring systems to verify that sterilization parameters are met consistently.

Incorrect. Try again! Choice C is the true statement. Autoclaves are equipped with safety interlocks, temperature and pressure controls, and monitoring systems to verify that sterilization parameters are met consistently.

Dunk Tanks

A dunk tank is a specialized container used for the decontamination of equipment and tools by fully immersing them in a disinfectant solution. This process ensures that all surfaces, including those with intricate designs or hard-to-reach areas, come in contact with the disinfectant for an adequate amount of time, effectively inactivating any hazardous pathogens. Dunk tanks are commonly sealed to contain splashes and spills, enhancing safety and minimizing exposure risks for laboratory personnel during the decontamination process.

Regular monitoring and replacement of the disinfectant solution are essential to maintain the dunk tank's effectiveness.

Effluent Decontamination System (EDS)

Effluent Decontamination Systems (EDS) are designed to treat and neutralize liquid waste that may contain harmful biological agents before they are released into the environment. These systems ensure that all waste materials are thoroughly decontaminated to prevent the spread of infectious agents.

EDS typically use methods such as chemical disinfection, heat treatment (e.g., autoclaving), or UV irradiation to effectively kill or inactivate pathogens in the wastewater. The system monitors and controls the decontamination process to ensure that it meets safety standards and regulatory requirements.

To ensure the efficacy of the EDS, it must be regularly maintained and monitored by trained personnel. Proper use includes adhering to standard operating procedures, conducting routine inspections, calibrating sensors, and performing maintenance to prevent malfunctions or system failures. Documentation and validation of the decontamination process are essential for compliance and safety.

Negative Airflow

Negative airflow prevents the escape of airborne pathogens. By maintaining a lower air pressure inside the laboratory compared to surrounding areas, negative airflow ensures that any potentially hazardous microorganisms are contained within the laboratory environment, minimizing the risk of exposure and contamination outside the lab.

Negative airflow is achieved using specialized HVAC (Heating, Ventilation, and Air Conditioning) systems that control and monitor air pressure differentials. Air is drawn into the laboratory from adjacent areas, and exhaust air is filtered through high-efficiency particulate air (HEPA) filters before being released. This process ensures that contaminated air is not recirculated and is safely removed from the laboratory space.

To maintain effective negative airflow, the laboratory should have sealed doors and windows, and entry should be limited to authorized personnel only.

Non-Negative Airflow

Regular testing and calibration of the airflow systems are required to ensure proper pressure differentials. Laboratory personnel must follow strict protocols for entering and exiting the laboratory to prevent any disruption of airflow patterns, which could compromise containment.

Regularly check pressure indicators. If airflow is not pulling negative, personnel should not enter.

If there are people already in the space, immediately notify them of the situation so they can exit appropriately. Cease all ongoing work involving infectious agents to minimize potential exposure. Close and secure all samples, tubes, and containers. Then, notify laboratory supervisors, safety officers, and facility maintenance personnel immediately. The number to call maintenance is 404-639-3216.

Do not open the laboratory door until proper airflow has been restored. Maintenance staff should inspect the ventilation system to identify and correct the problem, such as fixing leaks, blockages, or malfunctions in the air handling unit. Regular monitoring and validation of the airflow systems should be conducted to prevent recurrence, ensuring a safe working environment for all personnel.

Additionally, maintaining proper HVAC system maintenance and ensuring all doors and seals are secure is essential for preserving the negative airflow required for safe operation.

Knowledge Check 15

True/False: Negative airflow ensures that potentially hazardous microorganisms are contained within the laboratory by maintaining a higher air pressure inside the laboratory compared to surrounding areas.

Correct/False. The statement is False. Negative airflow ensures that potentially hazardous microorganisms are contained within the laboratory by maintaining a **lower** air pressure inside the laboratory compared to surrounding areas.

Incorrect/True. Try again. The statement is False. Negative airflow ensures that potentially hazardous microorganisms are contained within the laboratory by maintaining a **lower** air pressure inside the laboratory compared to surrounding areas.

Knowledge Check 16

Which of these is a specialized container used for the decontamination of equipment and tools by fully immersing them in a disinfectant solution?

A. Effluent Decontamination System (EDS)
B. Autoclave
C. Airlock
D. Dunk Tank

Correct. A **dunk tank** is a specialized container used for the decontamination of equipment and tools by fully immersing them in a disinfectant solution.

Incorrect. Try again. The correct answer is D. A **dunk tank** is a specialized container used for the decontamination of equipment and tools by fully immersing them in a disinfectant solution.

Sealed Rotors and Safety Caps

Sealed rotors or safety caps function to contain potentially infectious aerosols that may be generated during high-speed centrifugation of biological samples. They provide a physical barrier that encloses samples within a sealed chamber. This containment system ensures that any aerosols or droplets produced by the samples are trapped inside the sealed rotor or cap, which can then be safely opened in a biosafety cabinet to minimize the risk of aerosol exposure.

To ensure effectiveness, laboratory personnel must inspect components of rotors and safety caps for integrity and secure fit before each use. It's important to follow manufacturer guidelines for proper assembly, sealing, and operation. After centrifugation, sealed rotors or caps should be carefully transferred to a biosafety cabinet before opening to safely handle and process the contents. Regular maintenance and decontamination of these devices are essential to maintain their protective function.

Anterooms

Anterooms provide a controlled transition space between the containment laboratory and non-containment areas, ensuring that pathogens do not escape. Double-door entry systems, often designed as airlocks, help maintain proper pressure differentials and directional airflow, preventing contaminated air from flowing into cleaner areas. This setup requires that only one door is open at a time, reducing the risk of cross-contamination and enhancing the overall safety of the laboratory environment.

Anterooms are equipped with features such as hands-free sinks, airlocks, and changing stations for donning and doffing personal protective equipment (PPE).

Proper use of anterooms requires strict adherence to entry and exit protocols. Personnel must follow a designated sequence for putting on and removing PPE, washing hands, and decontaminating materials. Only authorized personnel should access the anteroom, and doors to the laboratory and anteroom should not be opened simultaneously to maintain airflow integrity. Regular monitoring and maintenance of the anteroom’s airflow systems and decontamination procedures are important for effective operation.

Personal Showers

Showers are installed to decontaminate personnel before they exit the containment area, reducing the risk of spreading infectious agents.

The showers, which use high-pressure water, are typically located at exits leading out of high-containment areas, often within or adjacent to anterooms. The drainage system is designed to handle contaminated water safely, ensuring it is treated and decontaminated before disposal.

Personnel should follow established protocols for using showers, including removing PPE in a specified order before showering to prevent contamination spread. The showering process should be thorough, covering all exposed skin and hair to ensure complete decontamination. Regular maintenance and inspection of shower systems are essential to ensure proper functioning and availability when needed.

Doors

Doors at BSL-3 are designed to maintain containment and control access, ensuring that potentially hazardous biological agents are confined within the designated high-containment areas.

BSL-3 laboratory doors are typically constructed to be airtight and are often equipped with automatic closing mechanisms, interlocking systems, and access control features such as keycards or biometric scanners. The self-closing element helps prevent doors from being left open inadvertently.

These doors are integrated into the laboratory's ventilation and pressure control systems to maintain negative air pressure, ensuring that air flows into the laboratory rather than out, thereby containing pathogens.

Sealed Floors and Seams

Sealed floors and seams in a BSL-3 laboratory are designed to prevent the accumulation and spread of infectious agents, ensuring that biological contaminants cannot penetrate or be absorbed into the surfaces.

Floors and seams are sealed using specialized, chemical-resistant materials that are durable and easy to clean, forming a continuous, impermeable surface. The seamless design eliminates cracks and crevices where contaminants could harbor, facilitating thorough decontamination and disinfection procedures.

To maintain the integrity of sealed floors and seams, regular inspections should be conducted to identify any damage or wear that could compromise the containment barrier. Laboratory personnel should follow established cleaning and maintenance protocols, using approved disinfectants to decontaminate surfaces effectively. Prompt repair of any damage to the sealed surfaces is essential to ensure continued containment and biosafety.

Hands-Free Sinks

Hands-free sinks are designed to minimize the risk of contamination and the spread of infectious agents by eliminating the need to touch sink handles, which can harbor pathogens. These sinks are typically equipped with motion sensors, foot pedals, or knee levers to operate the water flow. They may also feature automatic soap dispensers and touchless drying mechanisms.

Regular maintenance and inspection of the hands-free mechanisms are essential to ensure they function correctly. Any malfunctions should be addressed immediately.

Knowledge Check 17

Which statement is not true of doors at BSL-3?

A. The self-closing element of doors is intended to prevent them from being left open inadvertently.
B. Keycards and biometric scanners are never used with BSL-3 doors.
C. BSL-3 doors are designed to maintain containment and control access.
D. BSL-3 doors are integrated into the laboratory's ventilation and pressure control systems to maintain negative air pressure.

Correct. Correct. The statement that is not true is B. Keycards and biometric scanners may be used with BSL-3 doors to control access.

Incorrect. Try again. The statement that is not true is B. Keycards and biometric scanners may be used with BSL-3 doors to control access.

Alarm Sounds

Alarm sounds alert personnel to various safety concerns, such as airflow disruptions, equipment malfunctions, or emergency situations. Different alarms, such as those for negative airflow, fire, or chemical spills, have distinct tones to ensure that laboratory workers can quickly identify and respond to specific threats. Regular testing and maintenance of these alarms ensure they function properly.

Negative Airflow Alarm

A negative airflow alarm indicates a loss of negative pressure within the laboratory. This means air is not flowing as intended to keep potentially hazardous agents contained. The alarm usually emits a continuous or intermittent beeping sound.

Emergency Exit Alarm

An emergency exit alarm is triggered when an emergency exit is used or when an unauthorized exit is attempted. The alarm is often a loud, high-pitched siren to ensure immediate attention.

Biosafety Cabinet (BSC) Alarm

A BSC alarm activates when there is an airflow disruption or blockage in the biosafety cabinet, such as a filter problem or improper sash position. The sound is typically a consistent, moderate-pitched beep.

Autoclave Failure Alarm

If an autoclave fails to reach the required temperature or pressure for decontamination, an autoclave failure alarm will sound. It is usually a steady beep to alert personnel to address the issue.

Fire Alarm

A standard fire alarm sound, usually a loud, continuous ringing or siren, indicates the presence of smoke or fire in the laboratory, prompting immediate evacuation.

Personal Safety Alarm

Personnel working in BSL-3 laboratories may carry personal alarms to signal distress or an emergency. These alarms typically emit a loud, piercing sound to quickly alert others to assist.

Knowledge Check 18

When is an emergency exit alarm triggered?

A. When the fire alarm is deactivated.
B. When laboratory doors are left open for too long.
C. When any door in the facility is opened after working hours.
D. **When an emergency exit is used or when an unauthorized exit is attempted.**

Correct. Yes. An emergency exit alarm is triggered when an emergency exit is used or when an unauthorized exit is attempted.

Incorrect. Try again. The correct answer is D. An emergency exit alarm is triggered when an emergency exit is used or when an unauthorized exit is attempted.

Incidents and Reporting

Engineering Controls Functionality

If any functionality issues arise with autoclaves, showers, or airflow systems, contact BSL-3 facility dispatch immediately.

When reporting broken engineering controls, immediately inform your supervisor and the laboratory manager and Biosafety Officer (BSO) to ensure the safety of all personnel and the integrity of experiments. Document the issue by reporting it in MyCority, providing details such as the equipment type, nature of the malfunction, and time of discovery. Ensure that the broken equipment or system is clearly labeled and removed from service until it can be repaired or replaced. Follow all biosafety protocols to prevent contamination.

Note that documentation of issues related to engineering controls may vary between programs. Ensure there is a process to communicate these issues with all users of the space.

Non-Conforming Events

Likewise, if a non-conforming event (NCE) occurs, you must file a report.

Examples of NCEs include improper use of personal protective equipment (PPE), breaches in containment practices, or incorrect handling of infectious agents.

Documentation of NCEs may also vary between programs but always involve contacting the lab supervisor and BSO.

Final Assessment Questions

1. What is a potential outcome of an autoclave failure, such as inadequate steam penetration or temperature control?

A. Accidental exposure to infectious agents
B. Incomplete denaturation of proteins in biohazardous waste
C. Increased microbial resistance to sterilization
D. Formation of non-sterile condensate within the chamber

2. Which of the following are appropriate responses to an autoclave failure? (Select all that apply.)

A. Immediately open the autoclave and remove all materials.
B. Cease all autoclave operations.
C. Make sure autoclave doors are sealed. Affected materials should remain inside.
D. Increase the autoclave temperature and attempt another cycle without checking the failure.
E. Continue using the autoclave for non-hazardous materials while awaiting repairs.
F. Communicate the failure to all personnel within the laboratory.

3. True/False: High efficiency particulate air (HEPA) filters have an optimum efficiency capture rating of 99.97% for particles 0.3 µm in size.

4. Which of these is NOT a reason a BSL-3 laboratory would have an emergency facility maintenance shutdown?

A. Critical equipment failure (HVAC, biosafety cabinets, autoclave)
B. Structural integrity compromise (breaches in walls, doors, or containment seals)
C. Incident response (significant spill, contamination, or accidental exposure)
D. Routine calibration of laboratory equipment (balances/scales, pipettes, thermometers)

5. Which of these are roles of robotics and automated equipment in a BSL-3 laboratory? (Select all that apply.)

A. Replaces the need for all human oversight and monitoring in the laboratory.
B. Lowers the required biosafety level of the laboratory by reducing risks associated with pathogens.
C. Minimizes human interaction by automating repetitive tasks, such as sample handling, reducing the risk of contamination and exposure.
D. Ensures precision and consistency in experiments, which is essential for reliable results.
E. Operates continuously without fatigue, improving productivity while adhering to stringent safety protocols.

6. True/False: After equipment movement, the decision to recalibrate the equipment or not considers the distance over which the equipment was moved and whether vibrations and shocks occurred.

7. True/False: Some laboratory protocols or quality control standards require recalibration whenever equipment is moved, regardless of the perceived impact.

8. Which statements are true about airlocks? Select all that apply.

A. Airlocks and other equipment can always be safely passed from the laboratory to an outside area.
B. All BSL-3 laboratories have airlocks.
C. Airlocks typically consist of two doors, one leading into the laboratory and the other to the outside.
D. Airlocks often include features like HEPA filtration and negative air pressure.
E. An airlock is a controlled entry space but not a controlled exit space.

9. Which methods do Effluent Decontamination Systems (EDS) typically use to effectively kill or inactivate pathogens in wastewater?

A. Mechanical filtration and sedimentation
B. Electrostatic precipitation and cryogenic freezing
C. Ozone injection and magnetic field manipulation
D. Chemical disinfection, autoclaving, or UV irradiation

10. If functionality issues arise with autoclaves, showers, or airflow systems, who should laboratory personnel immediately contact?

A. Building maintenance staff
B. BSL-3 facility dispatch
C. Local health department
D. Security personnel