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## Biosafety level 4 suit laboratory suite entry and exit procedures

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| **Abstract:** | Biosafety level 4 (BSL-4) suit laboratories are specifically designed to study high-consequence pathogens for which neither infection prophylaxes nor treatment options exist. The hallmarks of these laboratories are: custom-designed airtight doors, dedicated supply and exhaust airflow systems, a negative-pressure environment, and mandatory use of positive-pressure ("space") suits. The risk for laboratory specialists working with highly pathogenic agents is minimized through rigorous training and adherence to stringent safety protocols and standard operating procedures. Researchers perform the majority of their work in BSL-2 laboratories and switch to BSL-4 suit laboratories when work with a high-consequence pathogen is required. Collaborators and scientists considering BSL-4 projects should be aware of the challenges associated with BSL-4 research both in terms of experimental technical |

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| | limitations in BSL-4 laboratory space and the increased duration of such experiments. Tasks such as entering and exiting the BSL-4 suit laboratories are considerably more complex and time-consuming compared to BSL-2 and BSL-3 laboratories. The focus of this particular article is to address basic biosafety concerns and describe the entrance and exit procedures for the BSL-4 laboratory at the NIH/NIAID Integrated Research Facility at Fort Detrick. Such procedures include checking external systems that support the BSL-4 laboratory, and inspecting and donning positive-pressure suits, entering the laboratory, moving through air pressure-resistant doors, and connecting to air-supply hoses. We will also discuss moving within and exiting the BSL-4 suit laboratories, including using the chemical shower and removing and storing positive-pressure suits. |
| **Author Comments:** | As discussed with Nandita, filming would be dependent on the BSL-4 laboratories being "cold", which means filming would need to take place during scheduled shut-down times. This can be scheduled once the proposal is accepted and needs the input of NIH Public Relations and NIH Security, which will be coordinated by Linda Coe and Michael Holbrook with/through me. |
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**Editor(s)**
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Dear Editor(s),

We would like you to consider our revised manuscript entitled "**Biosafety level 4 suit laboratory suite entry and exit procedures**" by Janosko *et al.* for publication in the *Journal of Visualized Experiments*.

Maximum-containment research or the study of highly virulent viral pathogens (i.e., Risk Group 4 agents that can only be evaluated in biosafety level 4 (BSL-4) facilities), is an integral part of all medical countermeasure programs. Countermeasure development for Risk Group 4 agents is a high priority because neither infection prophylaxes nor treatment options exist for infections with these pathogens. Most of these agents are endemic in a number of areas of the world and could be imported from endemic areas. In addition, many Risk Group 4 agents are considered possible starting materials for the construction of biological weapons.

Because only few BSL-4 facilities exist worldwide due to their high construction and maintenance costs, Risk Group 4 pathogen countermeasure research is usually performed in tight collaboration between a BSL-4 facility (for "live" pathogen experiments) and outside facilities belonging to the government, industry, or Academia. Although collaborative researchers are generally knowledgeable about the procedures and safety precautions required for BSL-1/2 and sometimes BSL-3 experiments, they most likely are not be familiar with experimental procedures in BSL-4 suit laboratories.

Our article provides a detailed visual demonstration of BSL-4 suit laboratory systems check, laboratory entry, movement, and exit procedures. We aim to further joint projects between collaborators and scientists considering BSL-4 projects by increasing awareness of the challenges associated with BSL-4 research, such as experimental technical limitations in BSL-4 laboratory space and the increased duration of such projects.

In this article, we describe the practices and procedures at the NIH/NIAID Integrated Research Facility at Fort Detrick in Maryland, USA, for checking external systems, and inspecting and donning positive-pressure suits, entering the laboratory, moving through air pressure-resistant doors, and connecting to air-supply hoses. We also discuss moving within and exiting the BSL-4 suit laboratories, including using the chemical shower and removing and storing positive-pressure suits.

Importantly, our article is not meant as an instruction on how individual work steps have to be performed, as standard operating procedures differ slightly among facilities. Instead, our article gives non-BSL-4 experts a glimpse into the BSL-4 environment and describes facility-specific procedures that can be generalized to other situations.

The reviewers' comments have been extremely helpful to streamline our article and to correct minor oversights. We are confident that we have addressed all of the reviewers' concerns and that our revised manuscript is now suitable for publication.

Thank you for your consideration.

Best Regards,
Jens H. Kuhn

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**KEYWORDS:**
Biosafety; biosafety level 4 suit laboratory; biosecurity; BSL4; BSL-4; positive pressure suit; maximum containment; high containment; personal protective equipment; PPE

**SHORT ABSTRACT:**
Although researchers are generally knowledgeable about procedures and safety precautions required for biosafety level 1 or 2 (BSL-1/2) experiments, they may not be familiar with experimental procedures in BSL-4 suit laboratories. This article provides a detailed visual demonstration of BSL-4 suit laboratory systems check, laboratory entry, movement, and exit procedures.

**LONG ABSTRACT:**
Biosafety level 4 (BSL-4) suit laboratories are specifically designed to study high-consequence pathogens for which neither infection prophylaxes nor treatment options exist. The hallmarks of these laboratories are: custom-designed airtight doors, dedicated supply and exhaust airflow systems, a negative-pressure environment, and mandatory use of positive-pressure ("space") suits. The risk for laboratory specialists working with highly pathogenic agents is minimized through rigorous training and adherence to stringent safety protocols and standard operating procedures. Researchers perform the majority of their work in BSL-2 laboratories and switch to BSL-4 suit laboratories when work with a high-consequence pathogen is required. Collaborators and scientists considering BSL-4 projects should be aware of the challenges associated with BSL-4 research both in terms of experimental technical limitations in BSL-4 laboratory space and the increased duration of such experiments. Tasks such as entering and exiting the BSL-4 suit laboratories are considerably more complex and time-consuming compared to BSL-2 and BSL-3 laboratories. The focus of this particular article is to address basic biosafety concerns and describe the entrance and exit procedures for the BSL-4 laboratory at the NIH/NIAID Integrated Research Facility at Fort Detrick. Such procedures include checking external systems that support the BSL-4 laboratory, and inspecting and donning positive-pressure suits, entering the laboratory, moving through air pressure-resistant doors, and connecting to air-supply hoses. We will also discuss moving within and exiting the BSL-4 suit laboratories, including using the chemical shower and removing and storing positive-pressure suits.

**INTRODUCTION:**
Scientific interest in exotic, high-consequence pathogens has steadily increased in recent years.

Of particular interest are pathogens considered emerging or re-emerging agents that are potential bioweapons. In terms of research priority and risk, these pathogens are classified by the Centers for Disease Control and Prevention (CDC) as Bioterrorism Category A-C Agents [1]. In addition, high-consequence pathogens are classified as Select Agents [and Toxins] in regard to import, export, and access regulations. In the US, the biosafety rules and procedures that must be followed for work with special pathogens that require BSL-2, BSL-3, or BSL-4 containment are outlined in *Biosafety in Microbiological and Biomedical Laboratories* (*BMBL*) [2]. Pathogens that are perceived to present the most significant health risk to humans and/or animals are considered BSL-4 pathogens. Consequently, BSL-4 research requires particular caution, highly specialized training, and a robust and redundant facility infrastructure [3]. To improve general awareness of the challenges associated with BSL-4 research, understanding the requirements for high containment building operations, systems management, and routine validation testing of engineering controls is necessary. We aim to advance this understanding by visually presenting the increased standards of operation and biosafety, and the resulting increased difficulty of hands-on research in BSL-4 containment.

Facilities housing BSL-4 suit laboratories must meet stringent requirements including, but not limited to, dedicated nonrecirculating ventilation systems, rigorous waste handling system and processes [4,5], and building automation systems (BAS) [2,6,7]. Laboratory supply air is filtered once and exhaust air is double-filtered through high efficiency particle air (HEPA) filters, which are recertified annually. In addition, the BMBL imposes strict requirements for decontamination of solid waste and collection and decontamination of all effluent materials before release into the general waste system. Multiple redundancies are built into the system to prevent release of any type of BSL-4 pathogen. The BAS monitor facility operations remotely and can pinpoint the problem area. Facility support systems are checked daily by staff for optimal operation and notification of problems in real time. All systems are tested on a recurring basis to comply with CDC/ Division of Select Agents and Toxins requirements for facility operation.

In addition to these standards for the physical facility, laboratory staff working with Select Agents and Toxins must submit to a Security Risk Assessment (SRA) by the Department of Justice prior to working with or around Select Agents. In addition, staff working with Tier 1 Select Agents (e.g., Ebola virus, *Bacillus anthracis*) must be enrolled in a Personnel Reliability Program (PRP) that continually evaluates the physical and mental health of individual researchers [8]. The health screenings assess whether individuals are physically capable of performing the work in BSL-4 containment in a safe manner. Mental health screenings assess general well-being, psychological welfare and resiliency, and safety awareness of the staff. At the NIAID, scientists working with Select Agents undergo additional scrutiny wherein each individual completes an Access National Agency Check and Inquiries (ANACI) background check that examines educational and professional credentials, criminal history, financial history, and risk of foreign influence.

Compared to BSL-2 laboratory entry, maximum containment entry requires a considerably greater investment of resources, time, and training. After SRA approval and registration with the Division of Select Agents and Toxins, staff must undergo stringent hands-on training before access to the BSL-4 suit laboratories. Laboratory staff receives training on operation of the facility, including daily checks of critical functions and entry / exit procedures [9]. Staff is also trained on laboratory biosafety and care and use of positive-pressure suits. White suits made of polyester fabric with polyvinyl chloride coating are used at the NIH/NIAID Integrated Research Facility at Fort Detrick. Facilities that use other types/commercial brands of positive-pressure suits may require different operational procedures for entering and exiting the BSL-4 laboratory than those outlined here. Researchers in facilities using this article should account for these differences prior to training. Suit training at the NIH/NIAID Integrated Research Facility at Fort Detrick includes proper procedures for donning the suit, ensuring the suit is functioning properly, repairing and maintaining suits (within and outside the laboratory), and moving within the laboratory. Once this training is complete, the laboratory staff member can begin working within the BSL-4 suit laboratories. Initially, another experienced staff member mentors one-on-one the newly trained staff member during the first five visits to the BSL-4 suit laboratories. To work independently at the NIH/NIAID Integrated Research Facility at Fort Detrick, the newly trained staff member completes a minimum of 40 supervised visits into BSL-4 suit laboratories with at least 100 hours of practical working time inside the laboratories.

**PROTOCOL:**

**1. Daily External Checklist**

1.1. Verify safety systems. Check breathing air systems, back up breaking air systems, effluent decontamination system (EDS) systems, chemical shower system, directional airflow and autoclave functionality (Figure 1).

**2. Laboratory Entry Procedures**:

2.1. Buffer Corridor Entrance Procedures by Laboratory Staff

2.1.1. Enter staff person’s name, time of entrance, laboratory location, and pathogens under study in the Personnel Entry/Exit Logbook. Indicate the laboratory location on a magnetic board.

2.1.2. To ensure the BSL-4 suit laboratories’ systems are functioning properly, verify that external systems have been checked and the laboratories have been cleared for entry on the Daily External Systems Safety Checklist (Figure 1).

2.1.3. To assure that laboratory room pressure is within negative pressure set points that maintains proper directional air flow, check the building automation system (BAS) monitor outside of the outer change room entrance. If room pressure is not in the normal range, notify biosafety or facilities staff and delay laboratory entry until such pressure returns to the normal range.

2.1.4. Ensure the availability of an autoclave for sterilization of biological waste and/or laundry following experimental work.

2.1.5. Obtain scrubs, socks, and towels from the storage room containing the facility’s supplies located near the Personnel Entry/Exit Logbook.

2.2. Outer Change Room Entrance Procedures by Laboratory Staff:

2.2.1. Complete security requirements that regulate and document access to the biocontainment laboratory. Enter the outer change room.

2.2.2. Remove all street clothing, undergarments, jewelry and watches, don scrubs, and proceed to the suit room.

2.3. Positive-pressure Protective Suit Inspection and Integrity Testing by Laboratory Staff in the Suit Room:

2.3.1. Upon obtaining a positive-pressure protective suit of an appropriate size, perform a visual inspection of the suit looking for any holes, tears, puncture points, seam rips, or weak spots. Inspect the outer suit gloves thoroughly, since gloves are the most likely place to be damaged.

2.3.2. Change gloves at least every 7-calendar days or if damage to the gloves is identified.

2.3.2.1. To replace the outer suit gloves, remove the duct tape from the existing suit glove, roll the O-ring onto the sleeve, and remove the old suit glove.

2.3.2.2. Put a new glove onto the cuff of the suit and ensure that the glove covers the entire cuff and the thumb is in the proper position. Place the O-ring over the glove, seat the O-ring into the groove on the cuff, and use duct tape to secure outer suit glove.

2.3.3.3. Fold the glove cuff over the duct tape and O-ring, seal the glove with one final piece of duct tape, and re-inspect the newly attached glove.

2.3.3. Apply a thin layer of zipper lubricant to the zipper at least once a week.

2.3.4. Cover each of the exhaust valves, located inside the suit behind the head cover and on the left side of the back with either duct tape or an aluminum pressure test cap, and zip the suit closed.

2.3.5. Connect the quick disconnect/connect attachment at the end of each breathing line to the hose connection on the suit. Inflate the suit until the arms and legs are firm and place the suit into an upright position. Disconnect the air supply.

2.3.6. The suit should not be over-pressurized as excessive pressure can cause serious damage to the suit. Thoroughly inspect the suit for any indication of an air leak for approximately 5 minutes and visually inspect the suit for signs of deflation. Ensure that the suit is not over-pressurized as excess pressure can cause serious damage to the suit.

2.3.7. If the suit loses firmness, check the suit for leaks by re-inflating the suit. Listen and feel for leaks in the suit. If needed, spray all surfaces of the protection suit with a soap solution. Some common areas for leaks are the seams of the suit, the zipper area, and the junction of the visor material with the suit material. Monitor for the formation of bubbles that indicate a leak, paying particular attention to seams and the visor. Check to ensure that the exhaust valves are fully sealed.

2.3.8. Repair small fabric leaks temporarily with duct tape. Complete permanent repairs using material from a suit repair kit. If the suit cannot be repaired easily, decontaminate the suit with an alcohol-based disinfectant and remove from the suit-room. If the suit is beyond repair, retire suit from use and incinerate.

2.3.9. Once the integrity test is completed, unzip the suit and remove the covers from the exhaust valves. Failure to remove the exhaust covers will clog filtration and severely damage the suit. Enter testing and repair information in the Positive-pressure Suit Integrity Test Log.

2.4 Donning Positive-pressure Suit in the Suit Room and Entering into BSL-4 Suit Laboratories:

2.4.1. Tape socks to legs of scrubs. Don inner nitrile gloves and duct tape them to the cuffs of the scrubs. Don a second pair of nitrile gloves (not taped) over the inner gloves. If necessary, clean the inner face shield with glass cleaner for increased visibility.

2.4.2. Don the positive-pressure suit, making sure the suit is zipped completely closed, and connect the suit to a breathing-air line. Enter into the BSL-4 suit laboratories when the BAS monitors are free of alarms.

2.4.3. To enter the BSL-4 suit laboratories, disconnect from the breathing air line in the suit room and push the request to access button for the chemical shower room.

2.4.4. Once the air pressure-resistant (APR) door is activated, the seal around the door deflates and the door magnet disengages. The APR doors within the chemical shower room are interlocking, meaning one APR door must be closed when the other is open to maintain containment at all times. To pass through the chemical shower room, close the APR door to the suit room, wait for the bladder to re-inflate and fully seal the door frame, and activate the second APR door leading to the laboratory.

2.4.5. Upon entry to the BSL-4 suit laboratories, connect the suit to an available breathing air line.

2.4.6. Before proceeding further, close the APR door leading to the laboratory and ensure that chemical shower is automatically activated to disinfect the chemical shower area and the suit. Once disinfected, the APR door in chemical shower room can be opened again from the suit room. If a cycle does not start, contact facility management personnel.

2.4.7. If the suit does not have integral boots, don a pair of overshoes which are located on the shelves adjacent to exit door, the chemical shower APR door. To avoid additional risk/hazards, check that the overshoes are form fitting and snug.

## 3. Movement within the BSL-4 Suit Laboratories

3.1. Move freely around the laboratories by disconnecting and reconnecting to air lines throughout the laboratories. Stay connected to an air line to maintain positive pressure when attempting to bend over or retrieve items close to the ground.

**NOTE:** Such movements while disconnected from the air line will exhaust the breathing air from within the suit and create negative pressure that can allow laboratory air to enter any breaches in the suit.

3.2. Observe the monitors throughout the laboratories for various alarms and current suite status.

**4. Laboratory Exit Procedures from BSL-4 Suit Laboratories**

4.1. Decontaminate gloved hands in detergent disinfectant cleaner in plastic dunk tanks by the sink or in interior hallways. If no dunk tank is present, spray gloved hands thoroughly with disinfectant.

4.2. Inspect the bottom of overshoes or boots for visible material. If overshoes are clean, remove overshoes and place them on the shelves adjacent to the chemical shower APR door. Close all interior doors of the laboratory, disconnect from the breathing air line, and proceed to the chemical shower.

4.3. If the overshoes are dirty, wash them thoroughly in the sink with detergent disinfectant cleaner.

**5. BSL-4 Chemical Shower Procedures**

5.1. Enter the chemical shower by pushing the request to access button to open the APR door, and close the door. Reconnect suit to a breathing air line inside the shower.

5.2. Once the APR door is closed, the chemical shower cycle will automatically activate to release an adequate mixture of detergent disinfectant cleaner to properly disinfect the suit and the chemical shower area. If the cycle does not start automatically, pull on the deluge handle. Scrub the suit for approximately 1 minute, push the deluge handle back into place to prevent fully draining the chemical disinfectant tanks. A manual shower cycle is not available.

5.3. Check for glove and foot leaks by placing gloved hands and feet in the plastic tub containing detergent disinfectant cleaner solution kept inside the chemical shower. Look for liquid or wetness underneath the outer glove and check for tears, rips or weak portions on the outer glove. If a leak is found in one of the outer gloves, wait until the shower cycle is complete, remove outer suit glove and the outermost inner glove, and leave the outer glove in the plastic tub.

5.4. Scrub the suit and integrated suit boots with a long-handled scrub brush or hands to distribute the chemical disinfectant to all surfaces of the suit. Thoroughly rinse suit of detergent disinfectant cleaner during the water cycle of the shower.

5.5. Disconnect the breathing air line, previously exposed to laboratory air, both during the chemical and the water cycle to disinfect the connection on the suit. Rinse the air line connection of any detergent disinfectant cleaner. Without disconnection, the detergent disinfectant cleaner cannot otherwise clean the air line connection.

5. 6. Open the door leading to the suit room and close it after exiting.

5.7. In the event of an emergency, such as a nonlife-threatening cut or fire, follow the same procedure outlined in 5.2.

**6. Suit Room Procedures after Exiting the Chemical Shower**

6.1. Dry the exterior of the suit with a large towel. Unzip the positive-pressure suit, remove one hand from the outer suit glove, and examine the outside of the outer nitrile glove carefully for moisture.

6.2. If moisture has leaked through the outer suit glove, remove the outer suit glove and outer nitrile glove and dispose in biohazard waste trashcan. Remove the other hand and repeat the check.

6.3. Remove suit and continue to dry exterior of suit to prevent dry rot damage. Spray the inside of face shield with glass cleaner and wipe dry. Hang the suit up to dry. Remove duct tape and inner nitrile gloves from scrubs and dispose of these items into designated biohazard waste trash bin.

6.4. Document any leaks or repairs to the suit in the suit log and report them to the facility management.

6.5. Proceed to inner change room, remove dirty scrubs and socks, and place them in the designated laundry bin. Proceed and enter into the Personal Shower.

**7. Personal Shower and Outer Change Room Exit Procedures**

7.1. Activate shower and fully wash hair and body for a minimum of 3 minutes with soap and water. Exit the Personal Shower and proceed to the Outer Change Room.

7.2. Dry off and don street clothing, exit the Outer Change Room, place used towels in the designated laundry bin, and proceed to the Buffer Corridor.

7.3. Indicate time of exit in the Personnel Entry/Exit Logbook and indicate laboratory exit on the locator board.

**REPRESENTATIVE RESULTS:**

Staff has been carefully and thoroughly trained in these techniques to ensure safe and consistent practices inside a BSL-4 facility. By checking that the facility is functioning properly, as indicated on the daily checklist (Figure 1), we are able to ensure that all of the necessary administrative and engineering controls are in place and functioning to maintain a safe and properly functioning environment. The positive-pressure suit provides an additional layer of protection for the staff member. Proper maintenance and use of these suits is integral to personal protection for the staff member. Through strict adherence to these procedures, no laboratory-acquired infections have been recorded at the NIH/NIAID Integrated Research Facility at Fort Detrick.

**FIGURE LEGENDS:**

**Figure 1.** Daily visual checklist for the support and backup systems for the BSL-4.

**DISCUSSION:**

We outlined the BSL-4 entrance and exit procedures used at the NIH/NIAID Integrated Research Facility at Fort Detrick for working with highly hazardous (Risk Group 4) pathogens. One purpose of visualizing the BSL-4 entrance and exit procedures is to emphasize the importance of safety of laboratory staff during work with such pathogens to avoid laboratory-acquired infections. Negative-pressure, BSL-4 laboratories maintain an inward directional airflow to ensure that pathogens will be contained within the laboratory. Positive-pressure suits with dedicated breathing air systems worn by laboratory staff mitigate contact of airborne pathogens with the laboratory staff. After laboratory staff leave the BSL-4 laboratory, a chemical shower disinfects the surface of the potentially contaminated suit and therefore prevents potential contamination of the rest of the building and the person changing out of the suit.

As the integrity of the positive-pressure suit is one of several important primary barriers for preventing potential pathogen exposure, staff is required to check for suit leaks before entrance and after exiting from BSL-4 laboratories. If a leak occurs, staff identifies the location of the leaks and alerts facility management. These practices ensure a swift response to any exposure to high consequence pathogens. Although every effort is taken to eliminate risk to laboratory staff, breaches of the suit may occur through the use of glassware, sharps, animal aggression, or continuous use.

While the procedures presented here generally follow the BMBL specifications outlined by CDC[2], these procedures are specific to the IRF-Frederick. Each BSL-4 facility has different building design specifications that impact the exact methods of laboratory operation. Alternative procedures for entering and exiting BSL-4 laboratories depend in part on the design and operation of these laboratories. In addition, government regulations in different countries may also have an effect on BSL-4 laboratory procedures in each country. Nevertheless, a general understanding of BSL-4 procedures and the building monitoring systems that support the safety of laboratory staff will help health administrators who are contemplating the design of similar buildings and outside collaborators involved in studies of high risk pathogens.

Productivity will increase as more laboratory staff is trained in BSL-4 laboratory entrance and exit procedures and in conducting experiments under BSL-4 conditions. However, when designing protocols with outside collaborators, sufficient time should be allotted to perform even basic laboratory operations, and expectations of time frames for delivering results have to be adjusted by accepting the difficulties inherent with work in BSL-4 laboratories. A generalized assumption is that any experiment performed at BSL-2 (e.g., 2 h) will require twice the amount of time to perform in BSL-4 (e.g., 4 h).

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The authors have nothing to disclose.

**REFERENCES**
1 National Institute of Allergy and Infectious Diseases. *NIAID Category A, B, and C Priority Pathogens*. (National Institute of Allergy and Infectious Diseases. Bethesda, MD, http://www.niaid.nih.gov/topics/biodefenserelated/biodefense/pages/cata.aspx 2013).
2 Chosewood, L. C. & Wilson, D. E. *Biosafety in Microbiological and Biomedical Laboratories*. 5th edn, (U.S. Dept. of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, National Institutes of Health. Washington, D.C. http://www.cdc.gov/biosafety/publications/bmbl5/ 2009).
3 Shurtleff, A. C. *et al.* The impact of regulations, safety considerations and physical limitations on research progress at maximum biocontainment. *Viruses* **4**, 3932-3951 (2012).
4 Walker, J. & Stern, L. *A Guide to the Biosolids Risk Assessments for the EPA Part 503 rule*. (US Environmental Protection Agency, Office of Wastewater Management, Washington, D.C. http://water.epa.gov/scitech/wastetech/biosolids/503rule_index.cfm 1995).
5 Salkin, I. F., Krisiunas, E. & Turnberg, W. L. Medical and infectious waste management. *Appl. Biosaf.* **5 (2)**, 54-69. (2000).
6 de Kok-Mercado, F., Kutlak, F. & Jahrling, P. B. The NIAID integrated research facility at Fort Detrick. *Appl. Biosaf.* **16 (2)**, 58-66. (2011).
7 Jahrling, P. B. *et al.* The NIAID Integrated Research Facility at Frederick, Maryland: a unique international resource to facilitate medical countermeasure development for BSL-4 pathogens. *Pathog. Dis.*, doi:10.1111/2049-632x.12171 (2014 March 29).
8 Skvorc, C. & Wilson, D. E. Developing a behavioral health screening program for BSL-4 laboratory workers at the National Institutes of Health. *Biosecur. Bioterror.* **9**, 23-29, doi:10.1089/bsp.2010.0048 (2011).
9 Le Duc, J. W. *et al.* Framework for leadership and training of Biosafety Level 4 laboratory workers. *Emerg. Infect. Dis.* **14 (11)**, 1685-1688, doi:10.3201/eid1411.080741 (2008).

Figure
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# IRF-Frederick
# BSL-4 Daily External Systems Safety Checklist*

| 1st Floor | | | | |
|---|---|---|---|---|
| **Effluent Decontamination System** | **Alarm Status:** | Clear | Warning (Yellow) | Alarm (Red) |
| **Visual Inspection** (Indicate Conditions and Comments): | | | | |

**2nd Floor (Buffer Corridor)**

| **Containment Status** *Building Automation System (BAS)* Monitor Display | BAS Functioning *(circle one)* | | Alarm Status *(circle one)* | | *Comments:* |
|---|---|---|---|---|---|
| | *Yes* | *No* | *Clear* | *Alarm* | |

| | **Alarm Status** *(circle one)* | | **Control Power Status** *(circle one)* | | **Steam Pressure in Range** (circle one) | | **Comments** |
|---|---|---|---|---|---|---|---|
| **Large Autoclave #1** | *Clear* | *Alarm* | *On* | *Off* | *Yes* | *No* | |
| **Large Autoclave #2** Carcass disposal backup →Rad EDS | *Clear* | *Alarm* | *On* | *Off* | *Yes* | *No* | |
| **Small Autoclave #3** | *Clear* | *Alarm* | *On* | *Off* | *Yes* | *No* | |
| **Med Autoclave #4** | *Clear* | *Alarm* | *On* | *Off* | *Yes* | *No* | |
| **Tissue Digester** | *Clear* | *Alarm* | *On* | *Off* | | | |
| **Large Autoclave #5** | *Clear* | *Alarm* | *On* | *Off* | *Yes* | *No* | |
| **Med Autoclave #6** | *Clear* | *Alarm* | *On* | *Off* | *Yes* | *No* | |
| **Dunk Tank #1** | Level Within Limits (circle one) → | *Yes* / *No* | Conductivity ≥3,500 µS | | *Yes* | *No* | |
| **Small Autoclave #7** | *Clear* | *Alarm* | *On* | *Off* | *Yes* | *No* | |
| **Small Autoclave #8** | *Clear* | *Alarm* | *On* | *Off* | *Yes* | *No* | |
| **Dunk Tank #2** | Level Within Limits (circle one) → | *Yes* / *No* | Conductivity ≥3,500 µS | | *Yes* | *No* | |
| **Dunk Tank #3** | Level Within Limits (circle one) → | *Yes* / *No* | Conductivity ≥3,500 µS | | *Yes* | *No* | |
| **Small Autoclave #9** | *Clear* | *Alarm* | *On* | *Off* | *Yes* | *No* | |

**Laboratory Visual Inspection:** (As viewed through buffer corridor windows. Indicate Conditions and Comments):

**Section I:**
**Systems Clear: Yes / No** *(Circle yes or no based on visual observation of systems at time of inspection)*
**Name:** **Signature:** **Date:** **Time:**

**4th Floor (Mezzanine)**

| **Breathing Air & Compressed Air Systems:** Compressors, Storage Tanks, Backup Systems and Quality Control | | | | | Comments |
|---|---|---|---|---|---|
| **1 – Breathing Air Compressor (BAC-1)** | **Alarm Status:** | | Clear | Alarm | |
| **2 – Breathing Air Compressor (BAC-2)** | **Alarm Status:** | | Clear | Alarm | |
| **3 – Breathing Air Purification Unit (BAPU-1)** Zander System | **Dryer Alarm Status:** Clear / Alarm | **CO Alarm Status:** | Clear | Alarm | |
| **4 – Breathing Air Purification Unit (BAPU-2)** Zander System | **Dryer Alarm Status:** Clear / Alarm | **CO Alarm Status:** | Clear | Alarm | |
| **5 – Breathing Air Storage Tank (BAST-1)** | 80-120 PSI | | Yes | No | |
| **6 – Breathing Air Storage Tank (BAST-2)** | 80-120 PSI | | Yes | No | |
| **7 – Breathing Air Main Header Pressure** | 50-70 PSI | | Yes | No | |
| **8 - Backup Breathing Air Header Pressure** | 75-150 PSI | | Yes | No | |
| **9 – Backup Breathing Air Storage Tanks (BBAST-1)** | ≥ 2300 PSI | | Yes | No | |
| **10 – Backup Breathing Air Storage Tanks (BBAST-2)** | ≥ 2300 PSI | | Yes | No | |
| **11 - Backup Breathing Air Storage Tanks (BBAST-3)** | ≥ 2300 PSI | | Yes | No | |
| **12 - Chemical Shower Air Compressor (CSAC-1)** | **Alarm Status:** | | Clear | Alarm | |
| **13 - Chemical Shower Air Compressor (CSAC-2)** | **Alarm Status:** | | Clear | Alarm | |
| **14 - Chemical Shower Air Compressor Storage Tank (CSACST-1)** | 75-150 psi | | Yes | No | |

**Chemical Disinfectant Storage System**

| **Conductivity (≥3,500 µS):** | Tank 1 (S1) | Yes | No | Tank 2 (S2) | Yes | No | |
|---|---|---|---|---|---|---|---|
| **Chemical Storage Tank Level:** | Tank # 1 | ¼ ½ ¾ Full ○ ○ ○ ○ ○ ○ ○ | | Tank #2 | ¼ ½ ¾ Full ○ ○ ○ ○ ○ ○ ○ | | **Comments:** |
| **Chemical Mixing Tank Level:** | Tank #1 | ¼ ½ ¾ Full ○ ○ ○ ○ ○ ○ ○ | | Tank #2 | ¼ ½ ¾ Full ○ ○ ○ ○ ○ ○ ○ | | |

**Number of Full Micro-Chem Plus Barrels:**____________________ **Number of Empty Micro-Chem Plus Barrels:**_____________________

**Section II:**
**Clear to Enter: Yes / No** (circle yes or no based on visual observation of systems on mezzanine level at time of inspection)
**Name:** **Signature:** **Date:** **Time:**

*Some biocontainment parameters are outside of the normal range; however, after consulting with the HCS and/or DOHS, I am signing below to authorizing entrance.

| **Name and Title:** | **Signature:** |
|---|---|
| **Restrictions:** | |

** This checklist must be completed each day. Prior to entry, BSL-4 personnel must check to ensure that both Sections I and II of this checklist have been completed and entry cleared. After this form is complete, post it on cart in the Buffer Corridor*

Excel Spreadsheet- Table of Materials/Equipment
Click here to download Excel Spreadsheet- Table of Materials/Equipment: Janosko et al. JoVE52317R2 Excel table.xlsx

| Name | Company | Catalog Number |
|---|---|---|
| Micro-Chem Plus | National Chemical Laboratories | 255 |
| Windex | Grainger | 3U560 |
| Sperian aluminum pressure test cap | Honeywell Sperian | CC0199649 |
| Sperian suit repair kit | Honeywell Sperian | CC0199621 |
| Sperian Protection Suit Integrity Test Lo | NA | NA |
| Sperian positive pressure suit | Honeywell Safety Products | BSL 4-2 |
| Soapy water | NA | NA |
| Outer suit gloves (canners) | Fisher | 19-019-601 |
| Outer suit gloves (MAPA) | Fisher | 2MYU1 |
| Inner nitrile suit gloves | Fisher | 19-050-592 |
| Zip Lube | Amazon | B000GKBEJA |
| Scrubs | Cintas | 60975/60976 |
| Socks | Cintas | 944 |
| Duct Tape | Pack-N-Tape | 51131069695 |
| Towels | Cintas | 2720 |
| O-rings | O-ring warehouse | AS568-343 |
| Overshoes | Amazon | B0034KZE22 |
| Plastic dunk tanks | Fisher | 14-831-113 |
| Entry and exit logbook | NA | NA |
| Baby powder | Amazon | 44230 |
| Ethanol | Fisher | BP2818500 |

**Comments**

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1) Please take this opportunity to thoroughly proofread your manuscript to ensure that there are no spelling or grammar issues. Your JoVE editor will not copy-edit your manuscript and any errors in your submitted revision may be present in the published version.

We are grateful to the editor for pointing out this issue. We have carefully proofread the manuscript and corrected minor mistakes.

2) Please note that reviewer # 2 have raised some concerns about aspects of your manuscript. Please thoroughly address or rebut each comment to further strengthen and clarify your submission.

We very much appreciate the thoroughness of all three reviewers, as their suggestions have improved the manuscript. We address all of the reviewers' concerns below point by point. Changes made to the manuscript in response to the reviewers' suggestions can be seen in the submitted track-changed version.

**Reviewers' comments:**

**Reviewer #1:**
This manuscript provides procedures for entry and exit of BSL-4 laboratories at the NIH Integrated Research Facility. The procedures are written in an outline format that is easy to follow.

There are no major concerns.

We thank the reviewer for the positive assessment of our work.

Minor comments:
Lines 262-263, bullet 2.3.6. "for approximately 5 minutes. After 5 minutes,..." is redundant.

We agree with the reviewer. We changed the sentence to

> "for approximately 5 minutes and…"

Section 2.4 Should you check the inside of the face mask to see if it needs to be cleaned/windexed?

We agree with the reviewer that we overlooked this important aspect of donning suits. We added the following sentence to section 2.4.1.:

> "If necessary, clean the inner face shield with glass cleaner for increased visibility"

Lines 298-299, Please indicate that the APR door must fully engage and seal inflate before activating the second APR door.

We agree with the reviewer that this is a very important point to be raised. We changed part of section 2.4.4. to

> "To pass through the chemical shower room, close the APR door to the suit room, wait for the bladder to re-inflate and fully seal the door frame, and activate the second APR door leading to the laboratory."

Line 304, I do not understand "activate the chemical shower to disinfect the room" - what room? Do you mean the chemical shower? Shouldn't it automatically activate as per section 5.2 that states the chemical shower automatically activates?

We agree with the reviewer that we should have been clearer. We reworded section 2.4.6. to clarify that the chemical shower will disinfect the suit plus the chemical shower area and that the chemical shower would be automatically activated:

> "Before proceeding further, close the APR door leading to the laboratory and ensure that chemical shower is automatically activated to disinfect the chemical shower area and the suit. Once disinfected, the APR door in chemical shower room can be opened again from the suit room. If a cycle does not start, contact facility management personnel."

Lines 308-309, May be helpful to indicate that the over boots/shoes should fit correctly or it is a safety risk/hazard.

The reviewer raises a very important point that we have overlooked. We added the following sentence to section 2.4.7.:

> "To avoid additional risk/hazards, check that the overshoes are form fitting and snug."

Section 3 is very thin and lacking details. Is there another article that can be referenced regarding working within the BSL-4 laboratories?

We are delighted to see the interest of the reviewer in further elaborations on BSL-4 research procedures. The purpose of this particular article is to address basic safety concerns regarding BSL-4 containment laboratories, as well as entry and exit requirements. This article is the first of a planned series of articles, each of which will describe particular aspects of BSL-4 research. To keep this paper and video within of the journal-prescribed length limits, we have therefore chosen not to focus on performing experiments in this paper. To clarify the focus of this paper, we replaced "we will describe the practices and procedures" with

> "The focus of this particular article is to address basic biosafety concerns and describe the entrance and exit procedures for the BSL-4 laboratories at the NIH/NIAID Integrated Research Facility at Fort Detrick."

in the long abstract.

Section 5.2 Does the deluge handle literally release a deluge of water/disinfectant or can you run a manual shower cycle?

The reviewer raises an interesting question. You cannot run a manual cycle. The showers are automatic. In case they do not start, staff must use the disinfectant deluge. We reworded section 5.2 for clarification of this issue:

> "Once the APR door is closed, the chemical shower cycle will automatically activate to release an adequate mixture of detergent disinfectant cleaner to properly disinfect the suit and the chemical shower area. If the cycle does not start automatically, pull on the deluge handle. When the shower is deemed complete after approximately 1 minute of contact while scrubbing the suit, push the deluge handle back into place to prevent fully draining the chemical disinfectant tanks. A manual shower cycle is not available."

Sections 5.3 and 5.4 should be combined into a single section 5.3. It is distracting and breaks up flow for the discussion about the glove leak to be in a separate bullet.

We agree with the reviewer and combined both points.

Section 5 May be helpful to indicate how to decontaminate/shower in case of an emergency such as a fire?

We agree with the reviewer that a comment is necessary. We added a new bullet point, 5.6.:

> "In the event of an emergency, such as a nonlife-threatening cut or fire, follow the same procedure outlined in 5.2."

Section 6.1 Do you really towel off before unzipping? Doesn't that deplete the air supply/cause a vacuum if you bend over while drying the suit?

The reviewer asks an interesting question. At the IRF-Frederick, a large towel is used to dry the exterior of the suits. At that point in time, the air line has already been disconnected (in the chemical shower room, section 5.4). Not much air is lost during the process and on can always reconnect to the air lines located in the suit room. We added "large towel" to section 6.1.

**Reviewer #2:**

*Manuscript Summary:*

This manuscript describes the standard operating procedures of the IRF Frederick BSL4 facility for entering and exiting their BSL4 laboratory. While the manuscript is very well written, there is extremely little (if any) scientific benefit to its publication.

We thank the reviewer for commending our writing. We disagree, however, with the reviewer's assessment that there is little scientific benefit to the publication of our manuscript. While our work does not describe experiments or accumulated data, BSL-4 research is always under a magnifying glass by the concerned public. Recent events, i.e., lapses in biosafety and biosurety at CDC, FDA and elsewhere, emphasize that good practices in regard to research with high-consequence pathogens are of paramount importance. Even more so, it is crucial that these good practices are demonstrated not only to inspectors, but also to collaborators and any other interested party, including the public. Our article therefore serves two purposes by demonstrating: 1) part of the difficulties and impediments of maximum-containment research to collaborators who would like to get "things done quickly," and 2) how very well thought-through this type of research is, how many safety precautions are in place to ensure that nothing goes wrong, or how that proper containment is in place if indeed something does go wrong. In our eyes, communication of scientific conduct to a general audience is worth as much as communication of specific data to a specific audience, as both audiences are interconnected at the political, cultural, and financial levels.

*Major Concerns:*
Operating BSL4 facilities and the relevant standard operation procedures is a very sensitive and highly political topic. This reviewer agrees that transparency and communicating the extensive safety measures that are taken in operating these facilities to the (scientific) public is extremely important; however, I do not believe that publishing detailed standard operating procedures (SOPs) is the right way to do so. There certainly is no scientific benefit to this, since there are very few BSL4 facilities operating, all of which have these SOPs already implemented, and even fewer facilities can be expected to become operational in the foreseeable future, and have other means for obtaining this information. While the authors argue that it is of interest for non-BSL4 facilities to obtain a better understanding of the work in a BSL4 facility, due to the fact that there is a lot of collaborative work taking place in these laboratories, again a detailed protocol is not required to obtain this understanding - rather, a better understanding of the concepts underlying the operations of a BSL4 laboratory is required for this.

We understand the reviewer's point. It is important to point out here that our manuscript is not a review article (of which there several), but a script for a video to be filmed at the facility. JoVE requires a detailed protocol to serve as a script. Consequently, the individual steps shown in the SOP will be shown in the video, which is the main interface with the "reader" of the journal. The video will only be 5-10 minutes long (per JoVE requirement), but we will ensure that all steps necessary for entry and exit into the BSL-4 environment are shown – and these steps are those outlined in the SOP. Communicating to the public that SOPs exist in the first place, and also what these SOPs contain, is of utmost importance for increasing collaborator and public understanding of our facility's primary mission. We point out that this manuscript is the first of a planned series of video articles, all of which will detail individual procedures following entry/prior to exit. Finally, existing collaborator networks change constantly, and as science becomes ever more interdisciplinary, new collaborators who often have not even worked with any pathogens find themselves in discussions and projects with BSL-4 researchers. Having videos available that already have been cleared for public distribution (through publication for instance in JoVE) will be immensely helpful as informational tools.

In addition, there are a number of specific concerns that should be addressed:

Line 406: "We outlined the BSL-4 entrance and exit procedures that are required when working with highly hazardous (Risk Group 4) pathogens." This is not true, the authors described procedures that are used in their specific facility; other facilities (that have a much longer history of successful operations than the IRF Frederick) might have opted for different procedures that are equally effective. This statement should be changed to reflect this, e.g. "We outlined BSL-4 entrance and exit procedures that allow safe working with highly hazardous (Risk Group 4) pathogens." or "We outlined BSL-4 entrance and exit procedures used at the IRF Frederick for working with highly hazardous (Risk Group 4) pathogens."

We agree with the reviewer that we cannot repeat enough the fact that procedures outlined are specific for our facility. We therefore followed the reviewer's advice and changed the sentence to

> "We outlined the BSL-4 entrance and exit procedures used at the NIH/NIAID Integrated Research Facility at Fort Detrick for working with highly hazardous (Risk Group 4) pathogens".

Line 417: "As the integrity of the positive-pressure suit is of utmost importance in preventing potential pathogen exposure, staff is required to check for suit leaks before entrance and after exiting from BSL-4 laboratories." This statement (i.e. the "utmost importance") is not entirely true, safe work practices and primary containment (all work in a BSL4 laboratory is performed in a biosafety cabinet) are equally (if not even more) important. This is exemplified by the fact that some of the first laboratories working on BSL4 agents operated without positive pressure suits, without laboratory-acquired infections. While positive-pressure suits are an important safety measure, it is wrong (and even potentially dangerous for newly trained personal) to cultivate the impression that they are the only or the most important safety measure, and the authors should avoid doing so.

We absolutely agree with the reviewer and apologize for having created a false impression through our writing. We changed the sentence accordingly:

> "As the integrity of the positive-pressure suit is one of several important primary barriers for preventing potential pathogen exposure…"

Line 337-356: It is advisable to disconnect the breathing air line both during the chemical and the water cycle to disinfect the connection, which was exposed to the laboratory air but can otherwise not be reached by the microchem, and to rinse it of any microchem, respectively. This should be added to this section.

We agree with the reviewer and reworded section 5.5 (now 5.4):

> "Disconnect the breathing air line, previously exposed to laboratory air, both during the chemical and the water cycle to disinfect the connection on the suit. Rinse the air line connection of any detergent disinfectant cleaner. Without disconnection, the detergent disinfectant cleaner cannot otherwise clean the air line connection."

Given the sometimes sensitive nature of information regarding BSL4 laboratories, the authors should include a statement that they have the necessary permissions to publish their SOPs in this form, and from whom they obtained this permission.

Please see comments to next concern.

*Minor Concerns:*
What are the contributions of the 11 (!) authors to a manuscript describing a relatively simple standard operating procedure?

We understand the reviewer's concern. As the reviewer points out above ("Operating BSL4 facilities and the relevant standard operation procedures is a very sensitive and highly political topic"), numerous permissions were obtained to publish materials such as SOPs or to film in a highly secure and therefore sensitive environment. In addition, challenging coordination efforts have to be undertaken to allow for the filming without negatively influencing ongoing studies. Finally, a BSL-4 suite will have to be disinfected and therefore effectively be taken out of commission for a while to allow for filming without risk to the film crew.

These efforts, some of which are already underway and some which will be initiated upon acceptance of the article, justify the number of authors in our opinion. Michael Holbrook is the facilities high containment supervisor and therefore is responsible for timing, taking the suite off line, and coordinating other studies around this effort. Jason Barr is the facility's chief (government) biosafety officer and will therefore ensure that all steps shown in the video are in concordance with approved SOPs. He is also primarily responsible for the SOP itself and forgiving permission to publish the SOP. Peter Jahrling, Lisa Hensley, and Linda Coe are the government leadership of the facility (Peter Jahrling is the director). Ultimately, they ensure that everything described and filmed has proper approval. Linda Coe, in particular, will be the interface between the film crew and campus security/Biosurety. Krisztina Janosko and Matthew Lackemeyer will be the "faces" in the video. Jens H. Kuhn conceived the video article series, is the primary contact with the journal, and coordinates all the efforts between the various involved parties. Together with the medical writer, Laura Bollinger, and Krisztina Janosko and Matthew Lackemeyer, he assisted in writing the manuscript and will provide illustrations for the film. Finally, Je T'aime Newton and Corrie Ntiforo are liaisons from a different BSL-4 facility (UTMB at Galveston, Texas) who are heavily involved in producing BSL-4-related educational material for training courses. Together, we developed the protocol and script that is facility-specific when necessary to ensure that this article can be used for BSL-4 training purposes in class settings.

Line 318: "vacuum" in this context is wrong, it should be replaced with "underpressure" or a similar term.

We agree with the reviewer and replaced "vacuum" with "negative pressure".

Line 349: extraneous "."

We agree with the reviewer and removed the redundant period.

**Reviewer #3:**
*Manuscript Summary:*

In this manuscript the authors have outlined a method for safely entering and exiting a newly established BSL4 facility in Ft. Detrick, MD. The authors describe the inherent challenges for working safely in such an environment, and the extent to which training and evaluation must occur before an individual can access and work in the laboratory. The authors give an overview of the entire entry/exit process, and place specific focus on the positive pressure suit - both its use and maintenance. The message of the paper is that working in a BSL4 environment has physical, engineering, and logistical challenges that all must be taken into consideration when planning scientific experiments.

*Major Concerns:*
No major concerns. Given the scope of the paper, they do a thorough job outlining the steps of entry and exit.

We thank the reviewer for the positive assessment of our work.

*Minor Concerns:*
Because the authors spend a considerable amount of time describing the positive pressure suit, there are a few items that would make the paper and video more help to the viewer/reader:

1. 2.3.7/2.3.8. PLEASE if at all possible show what a leak looks like in the video. Checking for leaks during an integrity test (or just a daily check) can be challenging, and often user "fatigue" will set in after repeated successful uses of a suit. If you could show some common areas where leaks are likely to occur (by videotaping the soap bubbles around the gloves or something like that), and then how to fix it, it would be very helpful to the viewer.

The reviewer raises makes an excellent suggestion. We will ensure that demonstrations of leaks are part of the video.

2. 5.3. Can the authors please clarify what "checking for leaks" looks like when preparing to exit the lab. Are there different color nitrile gloves under the outer gloves? Or another mechanism to tell if liquid is under the outer glove? This would also be very helpful to show in the video.

We agree with the reviewer that we should have been clearer. We will ensure that demonstrations of leaks are part of the video. In addition, we reworded section 5.3 to further address leaks:

> “Check for glove and foot leaks by placing gloved hands and feet in the plastic tub containing detergent disinfectant cleaner solution kept inside the chemical shower. Look for liquid or wetness underneath the outer glove and check for tears, rips or weak portions on the outer glove. If a leak is found in one of the outer gloves, wait until the shower cycle is complete, remove outer suit glove and the outermost inner glove, and leave the outer glove in the plastic tub.”

3. Can the authors clarify (if possible) the type of suit they are describing (Dover, or Sperian?). If only describing one type of suit it would be good to mention there are others out there that do have

differences, just so the reader/viewer is aware.

The reviewer raises an important point. The types of suits used were emphasized in the original version of the manuscript. Unfortunately, JoVE requires that we refrain from using any type of commercial language and we therefore we cannot truly differentiate the type of suit we are using in the body of the article. The specific suit is listed in the materials list that is included in form of an Excel table with the publication. To address the reviewer's concern, we added the following to the introduction:

> "White suits made of polyester fabric with polyvinyl chloride coating are used at the NIH/NIAID Integrated Research Facility at Fort Detrick. Facilities that use other types/commercial brands of positive-pressure suits may require different operational procedures for entering and exiting the BSL-4 laboratory than those outlined here. Facilities need to account for these differences prior to training."

During filming, we will be using a particular suit (Sperian) and will mention that other options are available.