**Editorial comments:**  
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.