**Editorial comments:**  
  
1. There have been edits made to the manuscript.   
  
Changes to be made by the Author(s):  
  
1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

We thank you for the copyediting that was provided. We also proofread the manuscript for additional errors and made minor corrections.  
  
2. Each Figure Legend should include a title and a short description of the data presented in the Figure and relevant symbols. The Discussion of the Figures should be placed in the Representative Results. Details of the methodology should not be in the Figure Legends, but rather the Protocol. The Figure Legends should not be longer than the Representative Results.

We thank the editor for pointing out these requirements. We added short titles to the legends (in bold) and move almost the entire content of figure legend 1 into the representative results. Figure Legend 3 was also shorted. However, we could not shorten figure legend 2 as it deals entirely with explanations for labels in the figure.

3. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. “This figure has been modified from [citation].”

We appreciate the concern of the editor. Figures 1-3 were reproduced or modified from a previous publication, reference 5, that we published. As government employees, or contractors to the government, copyright was not transferred, i.e., we still hold the copyright for those figures and therefore can re-use them. We refer the editor to lines 549, 576, and 584-585 for attribution.  
  
4. Note for step 3.2.2: If anesthetized NHPs are to be filmed, the method of anesthesia used must be included.

We appreciate the concern of the editor. However, we will not film an anesthetized NHP, but instead will be using a dummy subject. Due to the risk associated with Risk Group 4 pathogens (e.g., Ebola virus), no pathogen will be involved in the filming, and all procedures will be done with a mock control on a dummy subject to not endanger the film crew.  
  
**Reviewers' comments:**  
  
**Reviewer #1:**   
Aerobiology procedures for a BSL-4 laboratory.   
  
This is a revision work from a previously submitted manuscript that demonstrates the procedures and performance characteristics of a complete inhalation exposure system within a Biosafety level 4 laboratories operated by the US National Institute of Health, NIAID. The facility, located at Fort Detrick, Maryland, is referred to as the Integrated Research Facility (IRF).   
  
This manuscript is a detailing and listing of the materials and associated hardware/electronics/software required to safely expose nonhuman primate species to high consequence pathogenic organisms by the aerosol modality. The purpose of the article was not hypothesis driven research, rather, it provides a step-by-step accounting of the procedures associated with this laboratory operation, and details the materials and engineering controls required to operate at this biosafety level.  
  
The article is clearly written, and has been revised to appropriately to describe completely to the uninitiated reader the complexities associated with this operation. This article, however, does not serve as a 'how-to' accounting of inhalation challenge, rather it is provided as a general guide on the particular operations within the IRF and the unusual amount of care that is taken to work with this group of pathogens at this level of biocontainment.

We thank the reviewer for the positive assessment of our manuscript.  
  
The following comments are provided for the authors to consider to possibly improve the further comprehension of the content of this article.  
  
\* Although the modality of exposure was aerosol (to the NHP species), there is no mention of sampling and/or aerosol characterization in the description of procedures. How are the aerosols characterized in the exposure? How are the aerosols sampled during the exposure? Are they sampled through a filter or an impinger during the actual exposure? How does one know what the animals are exposed to (in terms of dose) without understanding of the aerosol concentration that is generated into the exposure chamber? In addition, is there any confirmatory analysis of particle size distribution that has been generated by some sort of aerosol characterization device run concurrently to the aerosol exposure (or even before the exposure in a sham exposure)? Is there any proof that the particle size distribution is in fact what the device (the CENtag) is generating? The authors should consider revision to address these two issues.

We agree with the reviewer that we should have provided some more details. Steps 4.3.1., 4.3.13., and 4.3.15. describe the biosampler. We now added step 4.3.7. to describe the aerosol particle analyzer that we used to verify generated particle sizes.  
  
\* Although the authors do mention that this article was produced in a 'general' fashion to accommodate all high-consequence pathogens and the associated methodology surrounding this type of exposure, there still are some areas where important details are left out of the story with apparently no explanation. An example is the detailing of the anesthetic used for the NHP species in this study. Is it ketamine? Or another drug of similar consequence? Why leave out this detail when a species such as a NHP is in use?

We appreciate the reviewer’s concern. We added more detail on the types of anesthetics used on lines 151-156. However, we would like to point out that we will not be anesthetizing an actual NHP during the actual filming due to both safety and security concerns, but will use a dummy subject instead.  
  
\* In contrast, there are some rudimentary details (e.g, placing trash into the biohazard trashcan) that probably are a bit too simplistic for a scholarly publication - is this really necessary? The authors may want to consider rephrasing in a general statement for all 'waste materials' generated from the procedure as disposed of via autoclave, etc. This should suffice as explanatory for the entire publication.

The purpose of this publication is to describe the biosafety procedures that are in use during an aerosol challenge of NHPs with a high-consequence pathogen. We added lines 183-187 to more clearly define the focus of the article. Part of ensuring the biosafety of the laboratory staff is waste disposal. We consider the level of detail to match the importance of proper waste disposal in ensuring that laboratory-acquired infections do not occur.  
  
\* There is no detailing of material transfer in and out of the class III BSC (e.g., virus transfer). A detailing of these procedures and the associated precautions taken would be insightful to the reader as to the methodology used to safely transfer virus/pathogen into/out of the Class III BSC.

We agree with the reviewer. We revised step 1.1.4. to include more details on pathogen transport into the cabinet laboratory. Step 4.3.12. describes pathogen disposal.

\* Regarding the Class III BSC. There are Class III BSC units that are 'winged' wherein each side is operable and opens during times of sterility/when not in use. These units are built by Baker Corporation and are in use in other BSL-4 operations (the GNL at UTMB in Texas). It may be important to make this distinction in this article by detailing the manufacturer of the Class III BSC in use at the IRF and word the description accordingly to make the reader understand that no all Class III BSCs are built exactly to these standards rather the units in use at the IRF are built to this standard.

We agree with the reviewer. We added a sentence to indicate that other Class II BSCs are in use at other institutions (JoVE requirements do not permit the use of brand names or the manufacturer).

**Reviewer #2:**   
*Manuscript Summary:*   
This manuscript describes methods for aerosol challenge of non-human primates under biosafety level 4 containment. The methods are presented to emphasize the state-of-the-art safety procedures that protect the laboratory staff and environment from the infectious aerosol used to challenge non-human primates for vaccine and therapeutic studies. Overall the methods are clearly presented and appropriately highlighted.

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

I have one minor comment for clarification:  
  
*Major Concerns:*  
1. A discussion of alternative practices was basically entirely lacking.

While we appreciate the valid concern of the reviewer that practices differ among BSL-4 laboratories, the focus of this article is to describe biosafety procedures that are specific to the IRF-Frederick. We refer the reviewer to lines 89-92, 542-543, and 564-569. In addition, we added wording to indicate that the practices are specific to the IRF-Frederick, lines 134-137 and 183-184 (note that the focus on the IRF-Frederick was also emphasized in the accompanying JoVE articles 1 and 2 that address entry, exit, and general procedures in the BSL-4).

2. Step 3.1.1 references administering additional anesthesia during the procedure, but more detail could be provided. For example, what is done if anesthesia is needed while the animal is in the class III BSC; if used in the BSC, what all is brought into the BSC in advance (syringes, anesthesia bottles), where are these supplies kept and how are the sharps discarded from the class III BSC?

We thank the reviewer for pointing out missing information. We added lines 154-156 to describe transport of additional anesthesia supplies to the cabinet laboratory. We added lines 292-294 to describe administration of additional anesthesia to step 3.2.1. Step 4.3.2 outlines administering additional anesthesia when the animal is in a Class III BSC. We added step 4.3.11. to describe disposal of sharps, and we revised step 4.3.12 to include greater detail on the composition of materials placed in the biohazard bag.

*Minor Concerns:*  
N/A  
  
*Additional Comments to Authors:*  
N/A