Journal of Visualized Experiment, manuscript # JoVE58493

**Remote Laboratory Management: Respiratory Virus Diagnostics**

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**Response to the reviewers’ comments.**

The authors would like to express gratitude for the extremely valuable feedback provided by both reviewers and the editor! In the revised version of the manuscript we made every effort to accommodate the editorial and reviewers’ comments and suggestions, resulting in significant improvement of the manuscript’s quality.

Below we provide the detailed response to the editorial and reviewers’ comments. The editorial and reviewers’ comments are in the **Bold Text**, and our response is in *Italics*. Also, in attachment please find the revised version of our manuscript modified in accordance with our response to the reviewers’ comments (the changes are in RED color).

**Editorial comments:**

**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 proofread the manuscript carefully to ensure that text doesn’t contain spelling or grammar issues. The changes were done in Line 63, 70, 79, 85, 88, 93, 114, 128, 145, 164, 167, 170, 198, 275, 279, 313, 326, 362, 365, 370, 384, 394, 407, 413, 422, 549.*

**2. Please revise lines 140-142, 160-163, 165-170, 172-175, 177-179, 182-184, 185-188 to avoid previously published text.**

*We agree that some words and/or phrases were repeated in the mentioned lines and we revised text in these sentences properly. On the other hand, we believe that clarity of the instruction is our highest priority and some of those repetitions help us to avoid misinterpretations.*

**3. Figure 5: Please provide a figure with higher resolution.**

*We thank the editor for the comment and provide Figure 5 with higher resolution in the attachment.*

4. **Figure 6: Please include a space between -80 and °C. Please delete the space before “lysate” and the space after “heating”.**

*Figure 6 was revised. A space between -80 and °C was included, and the space before “lysate” and the space after “heating” were deleted. Please find the attached pdf-file with this figure.*

**5. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example: QIAamp Viral RNA Mini Kit (Qiagen), Applied Biosystems, StepOnePlus, etc.**

*We removed all commercial language from text of the manuscript.*

**6. Please revise the protocol so that all text in the protocol section is written in the imperative tense as if telling someone how to do the technique (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” However, notes should be concise and used sparingly. Please include all safety procedures and use of hoods, etc.**

*We ensured usage of imperative tense in the protocol and avoidance of phrases as “should be.” Regarding safety procedures and use of hood, we added the following text, correspondingly, to 1) Section 2 and 2) Table2:*

*1). “To operate a glove box, check the manufacturer instruction. Multiple sources provide detailed tutorials on glove box operation including video materials.25”*

*2). “Visually inspect elements, particularly for damage to the exposed surfaces of the HEPA filters, gloves, o-rings and hoses. Make sure duct clamps are tight and in place. Perform leak pressure test. Test the pressure alarm.”*

7. **Lines 200-206, 222-223, 270-279: Please write the text in the imperative tense.**

*The mentioned text was rephrased in the imperative tense and now just reads:*

*Line 200-206: “Before preparing to enter the installed laboratory unit, ensure that all BSL-2 or BSL-3 safety requirements must be accounted for. This includes dressing with proper personnel protective equipment (PPE), washing hands, wearing gloves, and decontaminating any workspaces you plan to use. Follow the checklist in Table 1 which contains safety requirements for personal protection during tests run in the lab BSL-2 and the BSL-3 module (the assembled glove box room – negative pressure and PCR room – positive pressure). Decontaminate all work space and supplies in the laboratory. Note if you plan to use sodium hypochlorite solution (0.5%), also known as liquid bleach, to decontaminate your work space and supplies, you MUST plan to also use 70% ethanol to clean all areas exposed to bleach, as bleach can mix with other chemicals in the workspace to create toxic fumes. Dispose all bleach products into their own designated waste bin. Before beginning to work in the laboratory unit, be familiar with its arrangement and layout.”*

*Line 222-223: “According to World Health Organization (WHO) recommendations,26 use sterile dacron or rayon swabs with plastic shafts for sampling from the respiratory tract.”*

*Line 270-279: “Perform the PCR amplification in a separate area designated for PCR. Carry out the PCR amplification of the viral target using PCR protocol for one step procedure. Note, a master mix is a kit that made using viral specific primers, probes, 2X RT-PCR buffer and RT-PCR enzyme. Add the master mix to plates or tubes and then add and mix individual samples. Transfer the plate to the PCR machine and run according to the viral target amplification conditions.”*

**8. Lines 222-225: Please include text in these lines as “Note”.**

*We included text in these lines as “note” and now one can read “Note, cotton or calcium alginate swabs, or swabs with wooden sticks may contain compounds that inactivate some viruses and inhibit PCR testing..26,27”*

**9. Please include single-line spaces between all paragraphs, headings, steps, etc.**

*Single-line spaces were included between all paragraphs, headings, and steps.*

**10. There is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. Remember that non-highlighted Protocol steps will remain in the manuscript, and therefore will still be available to the reader.**

*We revised the highlighted text to ensure its limit of 2.75 pages.*

**11. Please ensure that the highlighted steps form a cohesive narrative with a logical flow from one highlighted step to the next. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.**

*A logical flow in highlighted text was reviewed and the text was changed accordingly.*

**12. References: Please do not abbreviate journal titles. Please include volume and issue numbers for all references.**

*References were corrected to replace abbreviated journal titles with full journal titles. Volume and issue numbers were verified and added.*

**13. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.**

*We removed commercial symbols from the Table of Equipment and Materials.*

**14. Section 1: rework the text marked in red.**

In Section 1, subsections 1.4 – 1.9 are reworked in accordance with the request.

**Reviewers’ comments:**

We thank the reviewers for very detailed analysis of our manuscript and greatly appreciate the provided feedback!

**Reviewer #1:**

**General comments**

**The manuscript by Petrova et al. nicely describes logistical and operational requirements for deploying and managing a mobile laboratory with molecular diagnostic capability, adhering to BSL-2/3 safety conditions. This will be received with particular interest by agencies involved in infectious disease outbreak response efforts.**

*We deeply appreciate the review’s analysis of our manuscript, the provided questions and comments.*

**This manuscript (and the accompanying video) will be most useful to laboratory scientists who intend to operate a mobile "field" laboratory in a resource-constrained environment. It may therefore be helpful to include a few additional operational details, such as:**

**1. What is the estimated daily power usage of the laboratory with the equipment/protocols described? What size of generator is recommended to accommodate this load? This would be helpful to estimate the daily fuel requirements for the generator, which is a major operational cost for a field lab.**

*We thank the reviewer for the great observation and comment. It resulted in the following four paragraphs being added to the Discussion section starting on line 437.*

*“ Energy consumption is one of the most important parameters for management of an off-grid laboratory. For core laboratory equipment, the power efficiency can differ 15-40%; however, average energy consumption is estimated here to deliver an appropriate service. The highest power rate (1500-2000 W) relates to air conditioner, glovebox system, PCR machine, autoclave sterilizer. Considering 8 hours of intensive work carrying out the protocol and 16 hours of the laboratory environment control, the daily energy consumption of laboratory units is approximately 36 kWh/day for BSL-2, about 43 kWh/day for BSL-3, and 73 kWh/day for the connected BSL-2/BSL-3+ facilities. For a single unit, we recommend providing a source of electrical power with capacity of running/continuous power ≥8000 W, surge/starting power ≥ 10,000 W; for the connected facility, running/continuous power ≥12000 W, and surge/starting power ≥14,000 W. Note, in the BSL-3 laboratory facility, a backup energy source is strongly recommended to avoid accidental power outage and guarantee steady work of the glove box and negative pressure system during a diagnostic test.*

*A gasoline powered electric generator is a cost-effective solution for emergency energy supply. Assume that fuel efficiency of a gasoline generator is approximately 1.5 gallons per hour at 100% load. Then, if the average daily energy consumption is 8 hours of 40% load and 16 hours of 10% load, the laboratory unit BSL-2 or BSL-3 requires 7-9 gallons of fuel per day, correspondingly, and the connected facility needs ~15 gal/day.*

*The remote laboratory units are designed to fit capabilities of off-grid solar panel systems. It is prominent that solar panels do not require additional fuel and can be operated with high productivity in the tropical and subtropical regions of Africa, Asia and Latin America due to the high solar irradiation. Currently, one unit of a commercially available solar panel system allows a daily power usage of up to 44 kWh/day.*

*Regardless of the selected type of alternative electrical energy source, dirty electricity filters are strongly recommended and preinstalled in the laboratory facilities to improve power quality and to protect laboratory equipment. Keep the PCR system away from sources of strong and unshielded electromagnetic radiation because strong electromagnetic radiation may interfere with the proper operation of the device. It is also important do not use the PCR system in close proximity to strong vibration sources, such as a centrifuge or pump because excessive vibration will affect instrument performance. The laboratory equipment may only be installed in an environment that has nonconductive pollutants such as dust particles or wood chips. Ensure the room is away from any vents that could expel particulate material on the instrument components.’*

**2. What is the estimated daily water usage? What quality of water is needed? Does the water need to be pumped for pressure? Pre-filtered?**

*Please find the explanation below in a paragraph added to the manuscript starting on line 470:*

*“ The laboratory water usage depends on number of diagnostic tests running daily and number of laboratory technicians working in the facility. Nuclease free water is required for preparation of mixers during diagnostic procedure including extraction and PCR test and must be delivered in advance as other supplies and chemicals. At least 50 mL of nuclease free water is needed to run one diagnostic test; the required volume of nuclease free water depends on work load, i.e. on number of samples. Distilled water is needed to run the autoclave sterilizer. Autoclave water consumption in one cycle is 160-180 mL; the autoclave is recommended for daily use. Most of the plastics (tubes, pipette tips, etc.) are disposable, but some are re-usable and need to be washed (large containers, racks, etc.). Regular running water is used for washing hands between procedures and its minimal volume is estimated to be 15-20 L daily. The water needs to be pumped for pressure; sediment pre-filter system is recommended to protect the water appliances from the damaging effect of sediment and to improve quality of running water.”*

**3. What ambient temperature needs to be maintained in the lab for optimal equipment functioning?**

*Please look at the text starting on line 433:*

*“ For optimal equipment functioning, the following conditions have to be maintained in the laboratory units: ambient temperature of 21±2 °C, permissible temperature of 5 to 40 °C, humidity of 14±5% RH, permissible maximum relative humidity of 80% RH (noncondensing), and an altitude between 0 and 2000 m above sea level.”*

**4. Additional detail on specimen and laboratory decontamination would be helpful.**

*We are thankful for this comment and elaborate on laboratory decontamination in the following added paragraphs:*

*In Section 2:*

*“Decontaminate all work space and supplies in the laboratory. Note if you plan to use sodium hypochlorite solution (0.5%), also known as liquid bleach, to decontaminate your work space and supplies, you MUST plan to also use 70% ethanol to clean all areas exposed to bleach, as bleach can mix with other chemicals in the workspace to create toxic fumes. Dispose all bleach products into their own designated waste bin.”*

*In subsection 3.1.1:*

*“In the pass-through window tubes containing samples must be totally submerged in a hypochlorite bath for one minute in order to provide adequate decontamination before they enter the laboratory unit. Following the submersion, the lab technician inside the unit will open the pass-through window and collect the samples from the bleach container to be registered.”*

*In subsection 3.6.1:*

*“Note, prior to being dropped at pass through window, tubes containing samples must be disinfected via submerging in a hypochlorite bath for one minute.”*

*In subsection 3.7.1:*

*“Make sure to decontaminate your work space and supplies using a) bleach, and b) 70% ethanol to clean all areas exposed previously to bleach.”*

*In subsection 3.7.5:*

*“Decontaminate glove box work space applying bleach for at least one minute and 70% ethanol after bleach.”*

*In Discussion:*

*“ Laboratory decontamination includes several levels: cleaning -> antisepsis -> disinfection -> sterilization. Simple cleaning can be performed using soap and water while scrubbing with a gloved hand or brush. Antisepsis includes washing with liquid antimicrobial chemical in order to inhibit the growth and multiplication of germs. Alcohol solutions (70%) can be used as an antiseptic liquid. Disinfection is the application of a liquid chemical to eliminate nearly all pathogenic microorganisms (except bacterial spores) on work surfaces and equipment. Chemical exposure time, temperature, and concentration of disinfectant are important. Sodium hypochlorite solution (0.5%) is an effective disinfectant on a large scale for surface purification and water purification. Ultraviolet germicidal irradiation is another method of disinfection. A germicidal lamp produces UVC light and leads to the inactivation of bacteria and viruses. Sterilization employs a physical or chemical procedure to destroy all microbial life -- including highly resistant bacterial spores. Sterilization can be performed with an autoclave sterilizer.”*

**5. Additional detail on cold storage requirements would be helpful (e.g. reagent storage) and volume of refrigerator/freezer space.**

*Please find an according paragraph starting on line 484:*

*On cold storage requirements at least one 5.1 cubic feet refrigerator (+4 °C) and one 4.9 cubic feet (-20 °C to -30 °C) freezer are required in each laboratory unit to store samples/ RNA.*

**6. Waste disposal is not well described. A more detailed description of liquid and solid waste management would be helpful - e.g. use of an autoclave, incinerator, etc. This is a major operational requirement with implications for power usage, space needs, safety, etc.**

*We agree with the reviewer and provide detailed description of waste management starting on line 501:*

*“ All laboratory waste has to be segregated at the point of generation. Place solid, non-sharp, infectious waste in leak-proof waste bags marked as biohazard. If generated waste is sharp, it has to be placed in puncture-resistant containers. Collect potentially infectious liquid waste in properly labeled biohazard containers for liquids. Containers and bags should not be filled more than 2/3 the volume. The disposal of all bleach products must be sorted into their own designated waste bin. Laboratory waste must be handled gently to avoid generating aerosols and breakage of bags/containers. Collection bags/bins with biohazard waste must be sealed and external surfaces decontaminated after use with 0.5% sodium hypochlorite solution. Sterilize all laboratory waste in autoclave at 121 °C for 30 minutes prior to incineration. Refer to functioning manual for the proper use of an autoclave. If possible, add a chemical or biological indicator to the autoclave to ensure proper sterilization. All autoclaved solid and liquid waste has to be clearly labeled as sterilized with setting, date, time, and operator. The labeled waste must then be placed in a secure, separate area prior to incineration.”*

**7. Table 2 provides brief equipment maintenance recommendations. This could be expanded to include more specific recommendations for all required equipment/instruments (thermocycler, centrifuge, glove box, refrigerator, freezers, etc.)**

*We expanded specific recommendations for the major equipment. Please find the revised Table 2 in the attachment.*

**8. Table 3 lists required equipment. More detailed equipment specifications and recommended models could be provided, taking into account size dimensions, ruggedness for field deployment, ease of maintenance, etc.**

*We thank the reviewer for this note. More detailed equipment specifications are provided in the Table of the Equipment and Materials.*

**Other comments/suggestions:**

1. **Figure and table legends were not included in the manuscript I was provided.**

*We have provided figure and table legends at the first submission of the manuscript.*

1. **More clear labeling of figures is needed (especially Fig 5).**

*Figure 5 is improved and provided with higher resolution.*

1. **An estimate of overall diagnostic turnaround time should be provided.**

*We appreciate the reviewer’s valuable comments and add two new paragraphs 1) on line 298, and 2) on line 375:*

*1). “Once samples are loaded onto the PCR instrument it takes approximately 90 minutes to complete the run.”*

*2). “The overall diagnostic turnaround time is approximately 4 hours. Extraction time and PCR set up time can vary depending on the number of samples, and the diagnostic test can take 4-5 hours or more, correspondingly.”*

**Reviewer 2:**

**Manuscript Summary:**

**Petrova et al. present a very detailed and thorough description of how to set up mobile BSL2 and BSL3 labs using their innovative off-grid laboratory. They describe the setup of a laboratory, and the use of the laboratory to run a sample diagnostic test. While it is noted that a fair amount of know-how would be needed to have someone not skilled in the art replicate producing the laboratory (eg. producing the panels), if all materials are available the instructions are thorough enough to replicate. In addition, the protocol is extremely thorough for running the influenza test.**

**Major Concerns:**

**None**

**Minor Concerns:**

**Only 1 - line 160,/line 138 1.4, clarify if 2 or 4 people are needed or add more description about how it is harder with 2 people, as in resource limited environments I imagine this could be rate limiting.**

*We appreciate the reviewer’s analysis of our manuscript, observation and recommendation. We agree with the comment on line 160/line 138. A new sentence was added to the Protocol section (starting on line 138), which clarifies this question:*

*“Optimally, 4 individuals would be used to deploy, but it is possible with only 2.”*