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Remote Laboratory Management: Respiratory Virus Diagnostics

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Dear Editor,

Enclosed please find a manuscript entitled **"Remote Laboratory Management: Respiratory Virus Diagnostics"**, which we submit for the consideration in the Journal of Visual Experiment.

A number of recent epidemics such as Ebola, Zika, MERS, Influenza outbreaks uncovered the need for rapidly-deployable, laboratory facilities that address a multitude of issues from transportation, access, facilities, equipment, and communication. Off-grid capability is found to be crucial in rural, resource-constrained global settings. Therefore, we designed and built an innovative, off-grid laboratory facility. Here we present a study aimed to demonstrate capabilities of a novel modular and rapidly-deployable laboratory facility and provide a training guide for laboratory personnel working in remote, low-resource environments during an epidemic, natural disaster or other emergency relief situation. In this manuscript, we provide a protocol for a rapid respiratory virus diagnostic test in the proposed innovative, portable laboratory. We strongly believe that this work will be of significant value and interest for a broad audience including specialists in global health care, emergency and epidemics, rapid diagnostic tests in resource-constrained areas. Additionally, our approach and results could provide valuable assistance and reference for researchers with general interests in laboratory diagnostics and clinical methods in global health.

Author contributions: E.P. designed the validation method and study outline for the deployable laboratory facilities; V.A. and K.G. performed the RT-PCR diagnostic test of influenza in the deployable laboratories; S.M. developed the deployable, off-grid laboratory facilities and managed its manufacturing; P.P. designed workflow of respiratory virus PCR diagnostic tests in the deployable laboratories; S.A. conceived the idea, designed and developed portable, rapidly-deployable, off-grid laboratory facilities; E.P., V.A., and S.A. wrote the manuscript.

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Sincerely yours,

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TITLE:

Remote Laboratory Management for Respiratory Virus Diagnostics

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KEYWORDS:

Deployable, expandable, remote laboratory, resource-constrained area, safety requirements, diagnostic test, respiratory virus, influenza

SUMMARY:

A rapidly-deployable, off-grid laboratory has been designed and built for remote, resource-constrained global settings. The features and critical aspects of the logistically-enhanced, expandable, multifunctional laboratory modules are explored. A checklist for basic laboratory workflow and a protocol for a respiratory viral diagnostic test are developed and presented.

ABSTRACT:

An uptick in recent pandemics (Ebola, Zika, MERS, Influenza, *etc.*) underlines the need for a more ‘nimble’, coordinated response that addresses a multitude of issues ranging from transportation, access, facilities, equipment, and communication to provider training. To address this need, we have developed an innovative, scalable, logistics-enhanced, mobile, laboratory facility for emergencies and epidemics in resource-constrained global settings. Utilizing our background in clinical operations as an academic medical center, we designed a rapidly-deployable, modular BSL-2 and BSL-3 facility with user-friendly software for tracking and management of drugs and supplies in remote regions during epidemics and outbreaks. Here, we present our intermodal, mobile, expandable shipping-container laboratory units. The design of the laboratory facilitates off-grid usage by minimizing power consumption and

allowing alternate water sources. The unit's information communication technology (ICT) platform provides (i) user-friendly tablet-based documentation, (ii) enhanced tracking of patients and supplies, as well as (iii) integrated communication onsite with built-in telehealth capabilities. To ensure quality in remote environments, we have developed a checklist for basic laboratory workflow and a protocol for respiratory viral diagnosis using reverse-transcription polymerase chain reaction (RT-PCR). As described, our innovative and comprehensive approach allows for the provision of laboratory capability in resource-limited global environments.

INTRODUCTION:

Rapid diagnostics is a critical instrument in timely viral infection control, especially if early symptomatology is indistinguishable to a variety of infection diseases. The recent Ebola outbreak (2014-2015) in West Africa[1,2], Zika virus epidemics (2015-2016) in Asia and Latin America[3,4], the emergency of the Middle East Respiratory Syndrome (MERS), coronavirus infections[5,6], and the unusually deadly flu (influenza) epidemics (2017-2018) over the U.S.[7,8] uncovered the need for rapidly-deployable, laboratory facilities that address a multitude of issues from transportation, access, facilities, equipment, and communication.

Off-grid capability (autonomous power and water supply, *etc.*) is crucial in rural, resource-constrained global settings[9-11]. Our experience in clinical operations and global programs at Baylor College of Medicine was used to design and build a container-based mobile laboratory with capabilities for easy deployment, set-up, and multifunctional usage (BSL-2 and BSL-3). Images of this versatile, logistically-enhanced laboratory facility is shown in **Figure 1**.

This rapidly-deployable, laboratory facility has an expandable design similar to our previously described container clinic (the 'Emergency Smart Pod')[12-14], developed by Baylor College of Medicine and sponsored by USAID. A single packed unit (in Transport Mode) has the dimensions of 9'9" × 8' × 8' (**Figures 1A, 1B**), and expands to an area of 170 square feet (15.75 m^2) (**Figures 1C, 1D**). The unit can be deployed by two to four people in less than ten minutes.

The remote laboratory is built for a BSL-2 lab facility (**Figure 2A**) with a separate, modular, and attachable BSL-3 unit (**Figure 2B**) designed for work with infectious agents that may cause serious or potentially lethal disease through inhalation[15]. The connectivity of the two laboratory modules enables optimization of experimentation workflows, sharing of resources, and cost savings (**Figures 2C-2E**).

The modules are air-tight and water-tight to create a comfortable, energy efficient mobile shelter. Heating, ventilation, and air conditioning (HVAC) system is used for centralized and temperature-controlled units. In general, the design of the laboratory units minimizes power consumption by usage of their own alternate power sources such as solar panels and/or an independent electrical generator. Each unit includes a sink and eyewash station, electrical power and water connectors (**Figures 3A-3C**). The ICT platform delivers an optional, tablet-based documentation app for supply tracking and laboratory result documentation (**Figure 3D**) developed in partnership with Baylor's Information Technology (IT) research group who is wellexperienced in working in remote environments with limited connectivity. The system can function using cellular or wireless, and allows documentation without connectivity, with immediate back up or transmission to a secure-cloud based server when connectivity is re-established.

The laboratory has several key infection-control features including: (a) negative pressure air flow, (b) a glove box (GB) or biosafety cabinet, (c) a health risk management system: a germicidal ultraviolet (UVC) lighting system using 4 hierarchies of defense proven to eliminate 99.7% of pathogens that cause healthcare-related infections. The facility is easily disinfected using hydrogen peroxide or sodium hypochlorite (bleach) systems for efficient and effective decontamination[16].

The assurance of quality laboratory results depends on a commitment to assess all aspects of the entirety diagnostic testing process. Here, we present (1) a checklist for the BSL-2 and BSL-3 laboratory workflow, and (2) a protocol for rapid respiratory virus diagnostic test. The proposed diagnosis of viral diseases relies on the detection of viral RNA or DNA in specimen (nasal wash, blood, stool, and urine, *etc.*) through real-time reverse-transcription polymerase chain reaction (RT-PCR). The ability to rapidly estimate viral loads in a specimen makes PCR an efficient tool for viral disease screening[17,18]. The implementation of novel, molecular diagnostic assays allows expansion of diagnostic capabilities for viruses such as Ebola[19-21], Influenza[8,22], and Tuberculosis (TB)[23].

The goal of this work is to validate a novel modular and rapidly-deployable laboratory facility and provide a training guide for laboratory personnel working in remote, low-resource environments during an epidemic, natural disaster, or other emergency relief situation. Here, we present a protocol for respiratory influenza diagnosis in this innovative, portable laboratory.

Protocol:

1. Installation

Note: Only 2-4 people are needed to deploy the “Lego-like” laboratory unit. Optimally, 4 individuals would be used to deploy, but it is possible with only 2.

1.1. Utilize a fork lift truck (**Figure 1A, B**) or another suitable lifting device to handle the container. Use a fork lift truck with at least seven tons of lifting capacity to handle the two types of containers[24].

1.2. To set up a laboratory unit, select a patch of approximately 90 × 60 square feet (27.4 × 18.3 m^2) at flat landscape to ensure no obstructions hinder the proper layout. Select a site with well-drained soil to assure site drainage as this may cause potential problems with water dissipation after rainfall.

Note: The ideal site should have grounds that have been previously leveled and are hard compacted soil of a minimal compressive strength of 10 kN/dm^2. The surrounding area should permit access to equipment needed for unloading the unit from its conveyance device and accommodate the support equipment necessary to accomplish the task.

1.3. Place the unit or units in its 'Transport Mode' in the center of selected site and adjust the level of each unit with four leveling jacks. Keep a minimum elevation for the containers of ~ 6 inches to ensure the floor drains and discharge pipes work properly. Attach the support bracket to the arm of the jack. Ensure that the container is level by placing a bubble leveler at the center of each bottom rail. Do not expand the unit until it has been properly positioned.

Note: Each unit is equipped with four leveling jacks to allow deployment on a site that has a maximum grade of 6.5% (~4 degrees). Jacks should not be extended more than 12 inches.

1.4. Expand the unit by opening the panels for full functionality. First, locate the two piece support pole. Connect the support pole so that its height is almost as high as the container unit.

Note: The pole allows the user to open the panel and support the weight of the roof panel as the side doors are opened.

1.5. Remove the safety clip that acts as a pin to keep the panels locked, then lift and pull the cam lock pin from the hole. Place the pin behind the lever and out of the way of the cam lock lever located on the bottom (expandable) sides of the container.

1.6. Raise the roof (Panel 1, **Figure 4A**) considering that this panel has gas struts and once the panel doors are unlocked, the struts will release. This will allow for the user to raise the roof (Panel 1) using the two piece support pole. Move the support pole's tip under the roof panel to temporarily support it (**Figure 4A**).

1.7. While holding up the roof panel with the support poke, find the safety chain, located on top left side of the container. With the assistance of 2-3 individuals, carefully drag down Panel 2 until the safety chain is straight, holding the weight of the panel 2 and has engaged.

1.8. Connect the winch strap belt to the lug mount with hand by wrapping it around the exterior of the gas strut. If winch tool is not available, complete this step manually with at least two people on each side of the panel manually holding and lowering it.

Note: Exercise caution. The weight of the panel is 260 lb.

1.9. Ensure that there are no people or items in the way of Panel 2 and using the winch and drill, proceed to lower the panel (**Figure 4B**). When Panel 2 is completely lowered, disconnect the winch strap and reel it back into the winch. Remove the winch and place it on the opposite side of the container in preparation for use.

Note: Both sides of the unit are identical and follow the same steps above for the other side of the unit.

1.10. To complete deployment of the first side, reach down to the Panel 3 (which is currently the floor) and with at least two people on each side, manually lift Panel 3 upward into place as the door and front wall of the side (**Figure 4C**). Have two people remain to hold Panel 3, while the third person removes the support pole.

Note: No one should be inside the unit or under the roof until the Panel 3 wall is in place.

1.11. From the inside of Panel 3, locate the two latches and lock them into place using the safety strap. Make sure the black roof panel rubber gaskets are pulled out to face the inside of the units to prevent rain and other water penetration into the unit.

1.12. From the inside of the container, unlock Panel 4. Once unlocked, push out Panel 4 (**Figure 4D**) so that it swings open like a door. Lock the two safety latches on inside wall. Unlock Panel 5 and repeat the same steps for Panel 4. Secure this Panel with same internal safety latches. Once the entire unit interior has been locked, re-tighten the turnbuckle until the floor and end walls are sealed.

1.13. Once both sides of the container are safely expanded, check the jacks and make any necessary adjustments from shifting that may have occurred. Check shelter for level weekly. After extreme weather (rain or wind), inspect jacks on containers and adjust accordingly.

1.14. Expand the second container if the connectable laboratory modules are planned for use (**Figure 5**).

1.15. Connect the units to power source and water supply.

Note: The remote laboratory unit is now deployed. The inner non-collapsible volume of the unit allows to store minimum of the equipment and laboratory supply necessary for particular diagnostic tests. Installation of pressurization system for BSL-3 module has been previously described in detail[24] and requires additional quality control[24].

2. Checklist for Personal Protection and Basic Laboratory Workflow

Note: An error in any of the general safety and laboratory testing requirements phases may invalidate the results of the entire testing process.

2.1. Before preparing to enter the installed laboratory unit, ensure that all BSL-2 or BSL-3 safety requirements must be accounted for. Dress with proper personnel protective equipment (PPE), wash hands, wear gloves, and decontaminate any workspaces that may be used. 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).

2.2. Decontaminate all work space and supplies in the laboratory. If planning to use sodium hypochlorite solution (0.5%), known as liquid bleach, to decontaminate the work space and supplies, 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.

2.3. Before beginning to work in the laboratory unit, be familiar with its arrangement and layout. Strict rules apply for processing samples in the glove box (GB) room which has negative pressure. To operate a glove box, check the manufacturer instruction and multiple sources for detailed tutorials on glove box operation including video materials[25].

3. Rapid Influenza Virus Diagnostics by RT-PCR

Note: The purpose of this assay is to extract and purify ribonucleic acid (RNA) or deoxyribonucleic acid (DNA), if present, from specimens. The extracted RNA/DNA will be tested by a real-time RT-PCR to detect the presence or absence of targeted viral pathogens – Influenza (INF).

3.1. Receive and register samples in the BSL-2 laboratory facility.

Note: Apply the checklist for PPE (see step 2). INF is a Class 2 agent requiring BSL-2 practice. PPE appropriate for BSL-2 practice is required.

3.1.1. Receive a sample *via* pass-through window. According to World Health Organization (WHO) recommendations[26], use sterile Dacron or rayon swabs with plastic shafts for sampling from the respiratory tract.

Note: Cotton or calcium alginate swabs, or swabs with wooden sticks may contain compounds that inactivate some viruses and inhibit PCR testing[26,27].

3.1.2. In the pass-through window, totally submerge tubes containing samples in a hypochlorite bath for 1 min 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.

3.1.3. Register a sample within interactive tablet-based system or a laptop. Identify a sample with the following information: collection date, onset date, patient age and sex, specimen type (*e.g.,* nasal swab), unique identifiers, and other pertinent information.

3.1.4. Use barcodes for labeling tubes. If barcodes are not available, use an alcohol resistant marker. Always mark the vial itself, never the cap as this can get switched during handling.

3.2. Aliquot sample.

3.2.1. Use a certified Class II biosafety cabinet to handle specimens and take aliquots of samples. Use one probe for immediate examination and retain the others for reference purpose or retesting.

3.2.2. For specimens that arrive with nasal swab tip in the viral transport medium, stir up the swab tip in the medium for 30 s and squeeze it against the side of the vial before removing it from the medium and disposing it utilizing a biohazardous waste protocol (appropriately discard, autoclave or hang into 1/100 chlorine solution).

3.2.3. Store the medium with a minimum volume of 0.5 mL. Thus, divide a 3 mL sample into six aliquots (sub-samples). Use fresh sterile or disposable pipettes for each sample and discard them as biohazardous waste.

3.2.4. Place a barcode to identify a sample with the aforementioned information. Utilize one aliquot per specimen for immediate analysis and store others in freezer at -80 °C as it is a respiratory specimen.

3.3. Perform extraction and purification.

3.3.1. To assure quality of testing, move the barcoded PCR sample aliquots from specimen handling area to BSL-2 safety cabinet in extraction area which has a separate set of pipettes for handling of the sample.

3.3.2. Use the viral RNA mini kit (see **Table of Materials**) for extraction of RNA samples. Follow the step by step instructions for purification of viral RNA by spin protocol in the viral RNA mini kit instruction booklet.

3.3.3. Prepare the number of samples that need to be extracted. Label the 1.5 mL microcentrifuge tubes with the barcode numbers or unique identifier. Add 560 µL of the lysis buffer to 140 µL of the sample and pulse vortex. Incubate for 10 min at room temperature.

3.3.4. Add 560 µL of ethanol to the mix and vortex. Apply this mixture to the spin column and centrifuge it at 6000 × g, dispose the eluate and wash the spin column with two wash buffers. Finally elute the RNA by adding 100 µL of elution buffer.

3.4. Perform PCR amplification and detection.

3.4.1. Perform the PCR amplification of the viral target in a separate area designated for PCR using PCR protocol for one step procedure (see **Table of Materials**).

Note: A master mix is a kit that made using viral specific primers, probes, 2x RT-PCR buffer and RT-PCR enzyme.

3.4.2. Add the master mix to plates or tubes, then add and mix individual samples. Transfer the plate to the PCR machine and run according to the viral target amplification conditions. Once samples are loaded onto the PCR instrument it takes approximately 90 min to complete the run.

3.5. Perform regular and periodic maintenance after the equipment usage according to **Table 2**.

3.6. Receive and register samples in the BSL-3 laboratory facility.

Note: Apply the checklist for PPE and workflow BSL-3 (see step 2).

3.6.1. Receive a sample *via* pass-through window. Prior to being dropped at pass through window, disinfect tubes containing samples *via* submerging in a hypochlorite bath for 1 min.

3.6.2. Register a sample within interactive tablet-based system or a laptop. Identify a sample with the following information: collection date, onset date, patient age and sex, specimen type (*e.g.,* nasal swab), unique identifiers, and other pertinent information.

3.6.3. Use barcodes for labeling tubes. If barcodes are not available, use an alcohol resistant marker. Always mark the vial itself, never the cap as this can get switched during handling.

3.7. Aliquot sample.

3.7.1. Use a certified glove box to handle specimens and take aliquots of samples. Use one probe for immediate examination and retain the others for reference purpose or retesting. Make sure to decontaminate work space and supplies using bleach and then 70% ethanol to clean all areas exposed previously to bleach.

3.7.2. Receive specimens with nasal swab tip in the viral transport medium.

3.7.3. Take specimens and put one into each vial. Stir the swab tip in the medium for 30 s and squeeze it against the side of the vial before removing it from the medium and disposing it utilizing a biohazardous waste protocol (appropriately discard, autoclave or hang into 1/100 chlorine solution).

3.7.4. Store the medium at a minimum volume of 0.5 mL. Thus, divide a 3 mL sample into six aliquots (sub-samples). Use fresh sterile or disposable pipettes for each sample and discard them as biohazardous waste. Turn on UVC lighting.

3.7.5. Close vials with protection and remove from the glove box. Decontaminate glove box work space applying bleach for at least 1 min and 70% ethanol after bleach.

3.7.6. Place a barcode to identify a sample with the aforementioned information. Utilize one aliquot per specimen for immediate analysis and store others in freezer at -80 °C as it is a respiratory specimen.

3.8. Perform extraction and purification.

3.8.1. Move the barcoded PCR sample aliquots from specimen handling area to the glove box in the extraction area.

3.8.2. Use the viral RNA mini kit for extraction of RNA samples. Follow the step by step instructions for purification of viral RNA by spin protocol in the viral RNA mini kit instruction booklet.

3.8.3. Prepare the number of samples that need to be extracted. Label the 1.5 mL micro centrifuge tubes with the barcode numbers or unique identifier. Add 560 µL of the lysis buffer to 140 µL of the sample and pulse vortex. Incubate for 10 min at room temperature. Add 560 µL of ethanol to the mix and vortex.

3.8.4. Apply this mixture to the spin column and centrifuge it at 6000 × g (carefully close vials and take them out of glove box if a centrifuge is placed out of glove box), dispose the eluate and wash the spin column with two wash buffers. Finally elute the RNA by adding 100 µL of elution buffer.

3.9. Perform PCR amplification of the viral target using one-step PCR protocol in a separate area designated for PCR (use pass-through window). Prepare a master mix using viral specific primers, probes, 2x RT-PCR buffer and RT-PCR enzyme. Add the master mix to plates or tubes, then add and mix individual samples. Transfer the plate to the PCR machine and run the instrument according to the viral target amplification conditions.

3.10. Perform regular and periodic maintenance after the equipment usage according to **Table 2**.

Note: The overall diagnostic turnaround time is approximately 4 h. Extraction time and PCR setup time can vary depending on the number of samples, and the diagnostic test can take 4-5 h or more, correspondingly.

REPRESENTATIVE RESULTS:

The ultimate goal of this study is to demonstrate that the proposed mobile laboratory facilities BSL-2 and BSL-3 provide adequate environment allowing respiratory virus diagnostic tests with representative results identical to tests performed in high-quality stationary laboratories.

The laboratory facilities are designed to comply with the test requirements given in Occupational Health and Safety (ISO) recommendations. As soon as remote laboratory facility is deployed (**Figure 4**) and all equipment and supplies are installed (**Figure 5**), laboratory tests can be run.

In accordance with laboratory standard operating procedures, PPE (lab coats, protective shoes, gloves, advanced mask, protective eyewear, *etc.*) appropriate for BSL-2 practice is required. For BSL-3 practice, the PCR laboratory module of negative pressure is equipped with a certified glove box. The laboratory units are upgraded by external pass-through windows to protect personnel at the step of sample receiving. Registration process can be simplified with previously developed tablet-based application (**Figure 3D**). Other acceptable applications run on a laptop can be used as well.

This particular respiratory virus diagnostic test is planned to be performed in the connected laboratory modules to separate steps of the diagnostic procedure on purpose to avoid contamination or potential interference between biochemical reagents, which may affect the testing results. To maximize the quality of diagnosis, the rapid diagnostic test practice utilizes (i) both the basic laboratory BSL-2 and the traverse connected PCR room (Section 3.1-3.5) or (ii) the GB and PCR rooms connected by pass-through window (Section 3.6-3.10). The diagram of the proposed laboratory workflow is presented on **Figure 6** and emphasizes personal protection. The diagram recognizes importance of each indicated step for personnel protection, especially if laboratory staff in remote areas is minimally trained.

The rapid diagnostic test of Influenza is accomplished *via* RT-PCR technique. The procedure contains four main steps. Note that individual workspaces are assigned for each stage of the protocol. The first step is to obtain a sample and sub-divide it into several aliquots. The aliquots can then be marked with barcodes to improve effectiveness of data control and stored in the freezer for further investigations. The second step is to inactivate a sample in lysis buffer by centrifuging and heating. The first and second steps must be carried out in biosafety cabinets. Utilizing individual pipette sets and equipment is desirable. A PCR test can be performed in the PCR room, if available. The third step includes documentation of results. The last step is maintenance after equipment usage, and a reminder of personnel protection at the end of experiment.

If a specimen is expected to be classified as BSL-3+ (*e.g.,* Ebola, Zika, MERS, TB) the glove box facility must be used. In the remote laboratory, the GB room has its own pass-through window to receive specimens and a laptop or tablet for sample registration. The sample aliquot and virus inactivation must all be performed in the glove box chamber. UVC lighting is recommended to avoid contamination during procedure. After inactivation of a sample, further steps for protocol are similar to the basic laboratory BSL-2 and BSL-3 test and follows Checklist Part III (**Table 1, Figure 6**).

FIGURE AND TABLE LEGENDS:

Figure 1: **Laboratory facility prototype.** (**A, B**) Transport mode. **C.** Deployed mode: outside. **D**. Deployed mode: interior.

Figure 2: Schematics. **A.** The basic laboratory BSL-2. **B.** The BSL-3 module includes the glove box and PCR laboratories, which have a common pass-through window for protected specimen transfer. **C.** Connected laboratory facilities (**A**) and (**B**) with shared utilities. (**D, E**) Photographs of the connected units from opposite sides.

Figure 3: Schematics. **A.** Interior of the BSL-3 facility has a pass-through window (1), a sink and eyewash station (2) at inlet. **B.** Electrical power connectors. **C.** Water connectors. **D.** Tablet-based software for supply tracking and laboratory result documentations.

Figure 4: Deployment of the laboratory facility. Instruction for panels unfolding on one side of the unit as illustrated (**A-D**).

Figure 5: Schematics of the connectable laboratory. A. BSL-2 module 1. **B.** Glove box and PCR module 2.

Figure 6: Flow chart for a respiratory virus diagnostic RT-PCR test in the remote laboratory facility.

Table 1: Checklist for the PCR diagnostics workflow.

Table 2: Real-time PCR equipment maintenance.

Table 3: Minimum requirements for the RT-PCR respiratory virus diagnostic test BSL-2.

DISCUSSION:

The remote laboratory facility described above is logistically-oriented, expandable, rapidly deployable, multifunctional, and based on human-centered design concepts that have been geared to protect laboratory personnel and workspace efficiency. Our detailed protocol for quick laboratory set-up and safe respiratory virus isolation and diagnosis was developed and presented.

For optimal equipment functioning, the following conditions must 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.

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 onto the instrument components.

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.

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.

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%), or bleach, 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.

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 must 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.

As expected, workflow of diagnostic test depends on the disease and specimen. If it is recommended for virus identification to collect blood samples (*e.g.,* Ebola[19]), sample aliquots can be stored at -20 °C instead of -80 °C (necessary for respiratory viruses). It is always better to take more than one specimen when sampling from a patient than to subdivide specimens later. If possible, for each type of specimen at least two specimens have to be taken in separate specimen tubes. Specimens must be sub-divided if additional sampling is not possible.

If alternative specimens cannot be stored at appropriate temperatures (*e.g.,* no freezers are available), swabs should be stored in pure (100%) ethanol or 99% methylated spirit (methanol additives only). In this case, the swab tip has to be put into a vial with 1-2 mL of ethanol. Note that such specimens are suitable only for PCR. Also, note that well-established assay is necessary for each particular virus diagnosis[8,23], and unknown virus samples must be sent to assigned laboratories for further investigations[19-21].

Mandatory and recommended requirements to the list of laboratory equipment for respiratory virus diagnostic PCR tests have to be recognized. **Table 3** underscores basic and minimally advanced (recommended) equipment and requirements for the RT-PCR diagnostic test. For BSL-3 practice, extra negative pressure protection (*e.g.,* glove box) of personnel is crucial and necessary.

The connected laboratory modules are preferable to increase number of personnel involved in laboratory testing and speed up time required for a single test.

The future work will be focused on development of augmented reality (AR) and virtual reality (VR) trainings. The AR/VR glasses will be used to provide an interactive platform to teach requisite skills needed to become a well-trained laboratory worker. Helpful tips to perform some of the difficult, multistep procedures in laboratory diagnostic tests will be included in the software guide. This approach to personnel training should improve the quality of diagnostic test performance and management in remote laboratory facilities, especially remote and resource constrained areas.

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DISCLOSURES:
Baylor College of Medicine holds a U.S. provisional patent application for Mobile Clinics (U.S. Patent Application No. 15/523,126, # 620078924). The authors declare that they have no competing financial interest.

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Figure 1 - Remote Lab

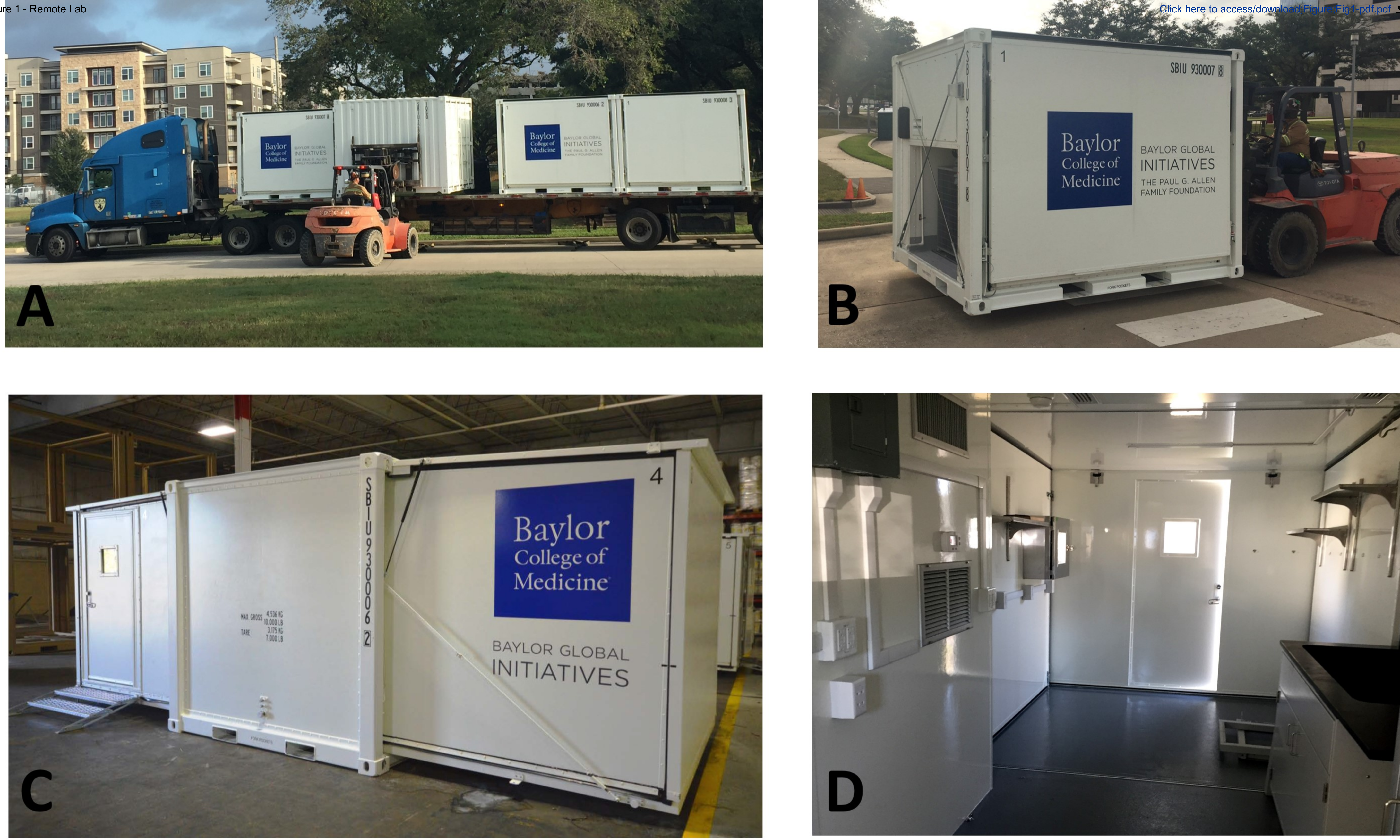

Figure 2 - Remote Lab

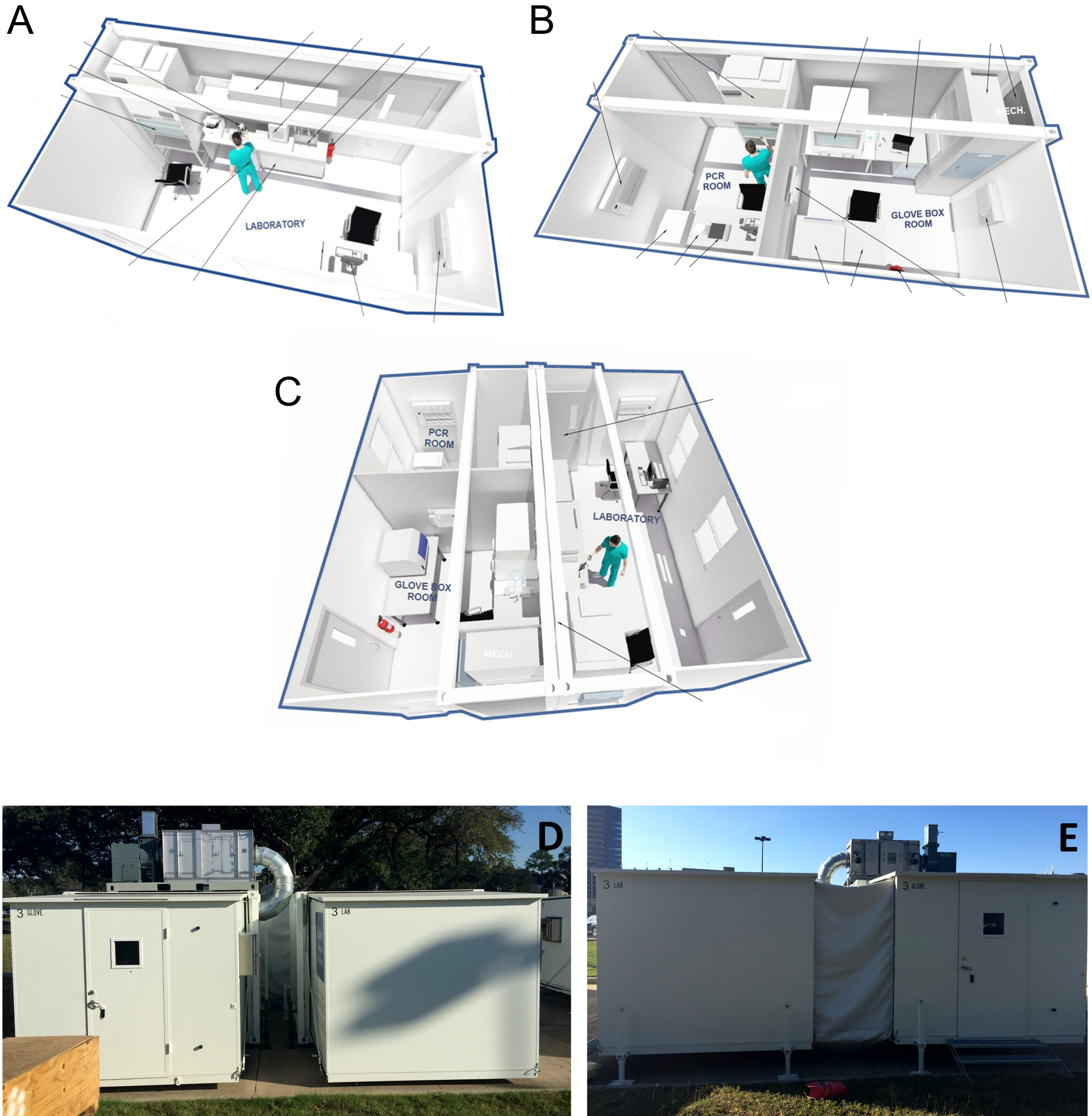

Figure 3 - Remote Lab

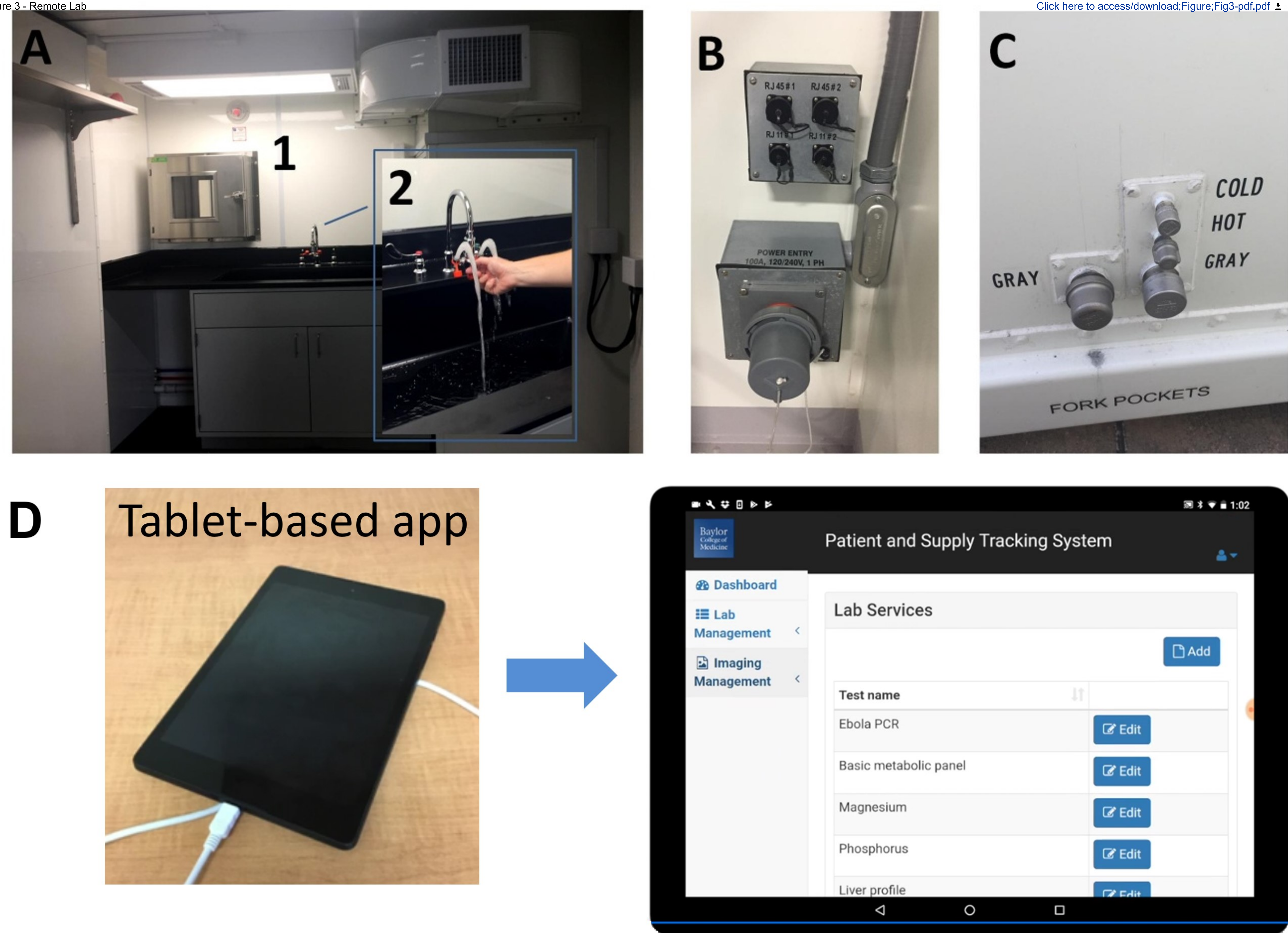

Figure 4 - Remote Lab

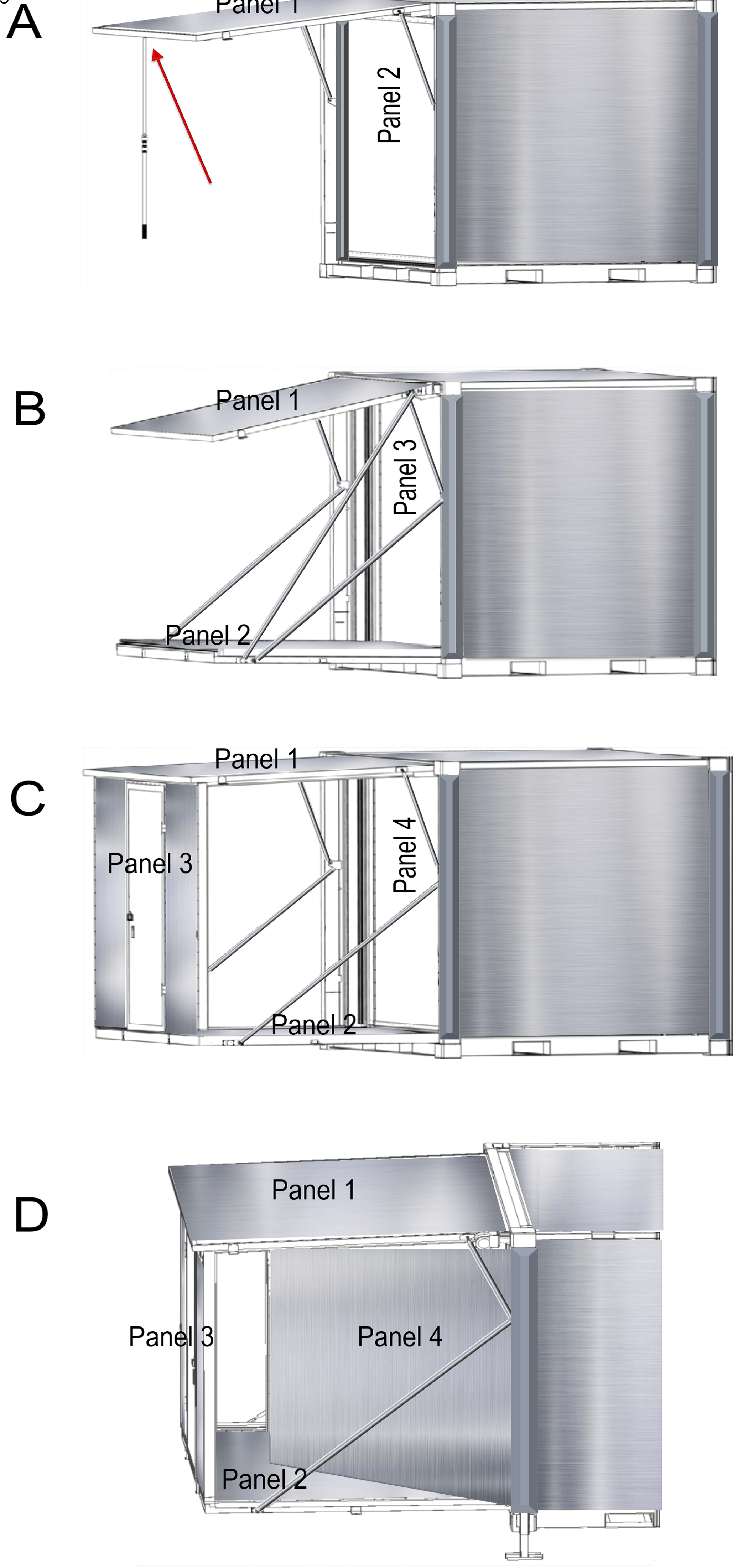

Figure 5 - revised

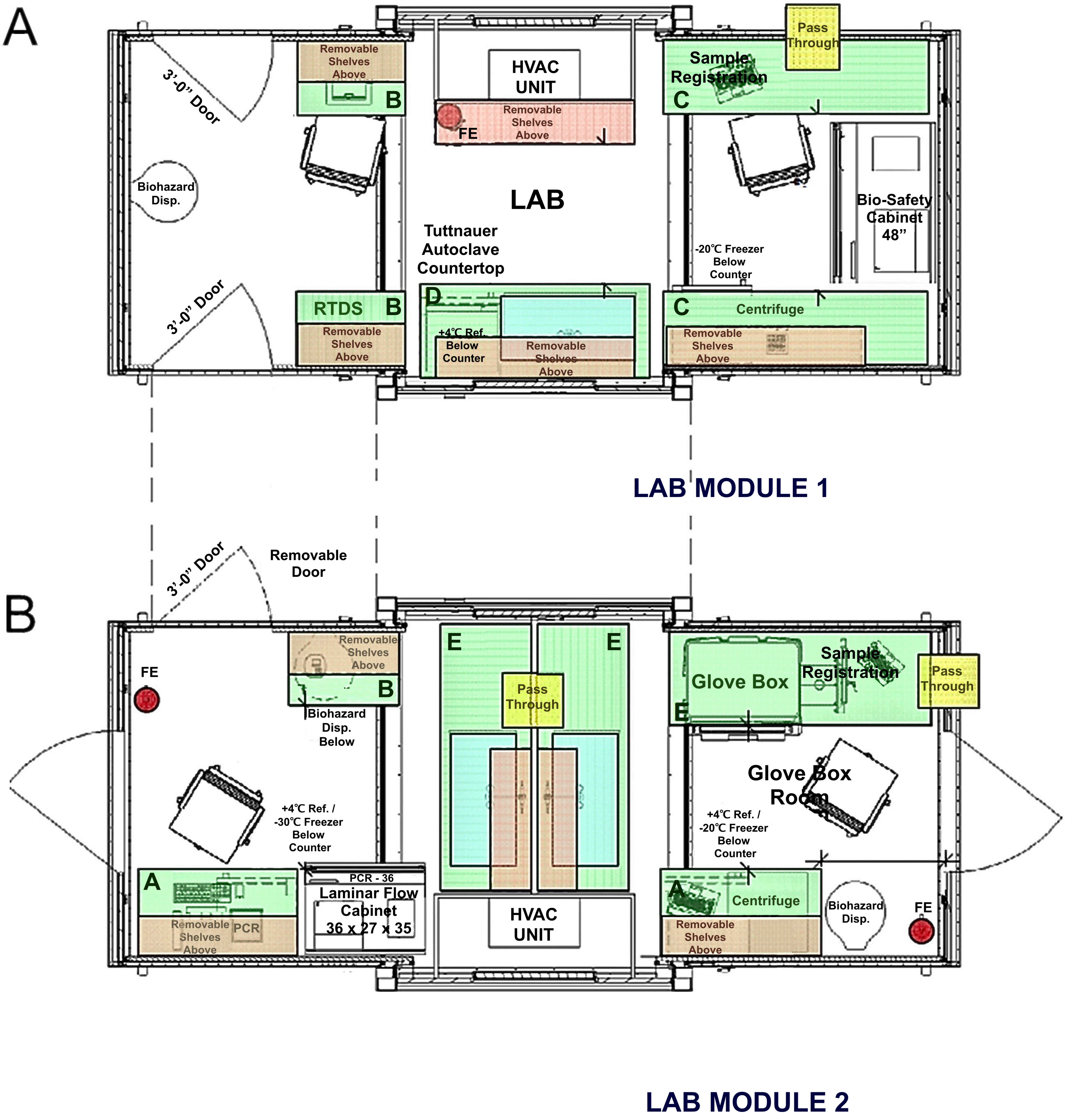

Figure 6 - revised

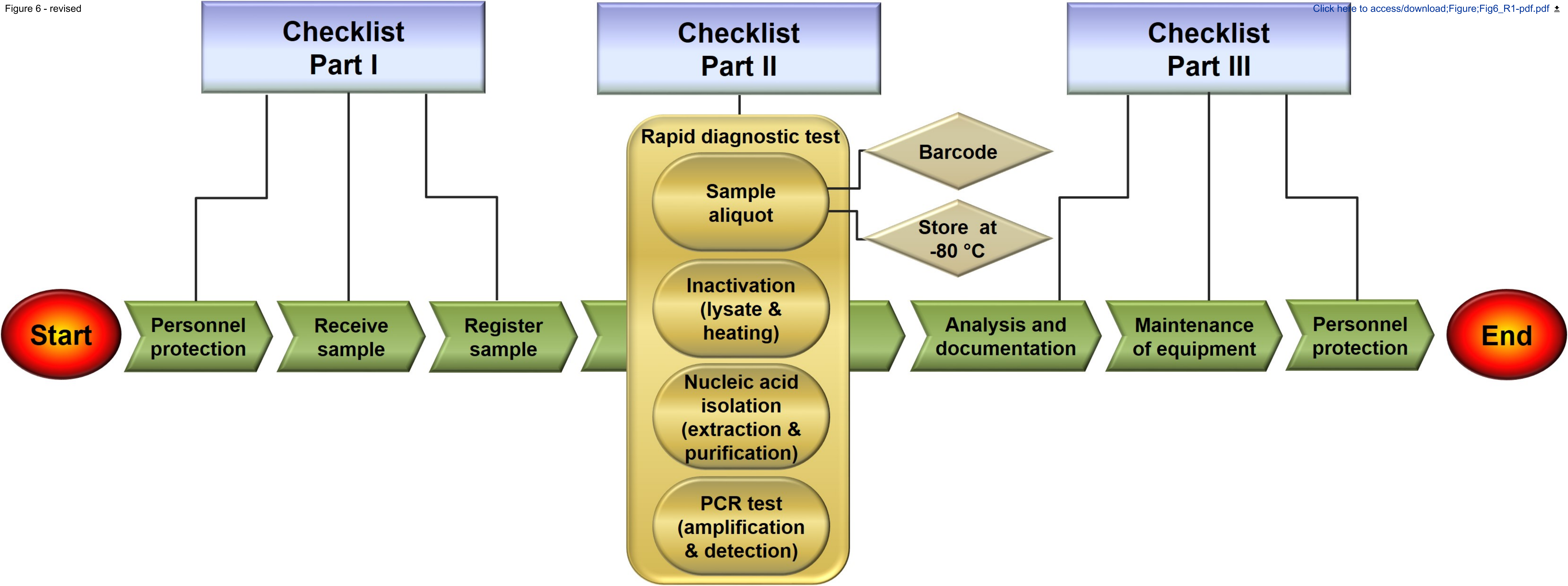

Table 1

Remote Laboratory BSL-2	Remote Laboratory BSL-3
Part I	**Part I**
i. Lab tech to enter through door labeled entrance and put on lab coat, which is hanging on rack next to entrance door. Open shoes are prohibited, advanced mask and protective eyewear are encouraged.	i. Lab tech to look into Glove Box window from outside of unit to insure negative pressure is activated. (Pink Ball should be visible in the unit to show the negative pressure is working).
ii. Lab tech to wash hands in sink, put on disposable gloves and begin with intake of samples.	ii. If negative pressure is working, lab tech to enter through only door and put on lab coat, which is hanging on rack next to entrance door. Open shoes are prohibited, advanced mask and protective eyewear are desirable.
iii. Samples that were dipped in hypochlorite bath prior to being dropped at pass-through window are sitting in pass through for lab tech.	iii. Lab tech to wash hands in sink, put on disposable gloves and begin with intake of samples.
iv. Received in sample reception.	iv. Samples that were previously dipped in hypochlorite bath prior to being dropped at pass through window are sitting in pass through for lab tech.
	v. Received in sample reception.
Part II	**Part II**
v. Depending on diagnostic procedure, specimens moved to biosafety cabinet and inactivated.	vi. Specimens inactivated in Glove Box.
vi. Specimens prepped for microscopy, centrifuge, or ROTs.	vii. Specimens executed for nuclei acid isolation.
vii. Appropriate diagnostic tests run.	viii. After extraction, specimens moved to pass-through window.
viii. Store specimens in 4 °C refrigerator or appropriate freezer.	ix. Lab tech enters through entrance in PCR side of unit (positive pressure).
Part III	x. Lab tech to put on lab coat from rack next to entrance, wash hands in sink, put on gloves.
ix. Use sink for staining & washing of items.	
x. Use laptop & counterpace to perform analyses and documentation.	xi. Receive samples from Glove Box room in pass-through window.
xi. Sterilize equipment by running autoclave.	xii. If necessary samples prepped in Laminar flow cabinet.
xii. Dispose of any biohazardous waste in biohazard waste container.	xiii. Appropriate diagnostics tests run.

xiii. Wash hands in sink. xiv. Hang lab coat back up on rack. xv. Exit through same door.	xiv. Store specimens in 4 °C refrigerator or appropriate freezer. **Part III** xv. Use sink for staining & washing of items. xvi. Use laptop & counterpace to perform analyses and documentation. xvii. Transfer vials into pass-through window to PCR room and sterilize equipment by running autoclave. xviii. Dispose of any biohazardous waste in biohazard waste container. xix. Wash hands in sink. xx. Exit through same entrance door.

Maintenance and calibrations

Real-time PCR systems	Monthly	Perform background calibrations every month
	18 months	Perform background, spatial and dye calibrations every 18 months
Centrifuge	1 year	Calibrate for revolutions per minute and temperature through external or internal calibration services
Glove box	Daily	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.
	6 months	Change the HEPA filter
	1 year	Calibrate the system
Autoclave	Weekly	Clean the water tank and racks using a mild non-abrasive detergent
	3 months	Calibrate timer and gauges
	1 year or every 50 cycles	Inspect, clean thoroughly, test and calibrate
Refrigerator and freeezer	6 months	Check fan motor, evaporator coils, vacuuming condensing coils and condensor filters and replace batteries as needed
	1 year	Calibrate freezer through internal or external calibration services

Mandatory	Recommended
Lab coat, protective shoes, gloves	Lab coat, protective shoes, gloves, masks, eyewear
Refrigerator 4 °C, freezer -20 °C	Refrigerator 4 °C, freezer -20 °C, freezer -80 °C
One set of automated pipettes	Three sets of automated pipettes
Centrifuge, shaker, thermocycler	Robotic system
RT-PCR machine, ice bath	RT-PCR with temperature control, ice-free cooler
Biohazard waste bags	Autoclave to dispose biohazard waste

Name of Material/ Equipment	Company
Class III Biological Safety Cabinet (Glove box)	Germfree Laboratories, Ormond Beach, FL, USA
Class II Biological Safety Cabinet	NuAire, Inc., Plymouth, MN, USA
Laminar Flow Cabinet	NuAire, Inc., Plymouth, MN, USA
RT-PCR machine 'Step-one plus'	Thermo Fisher Scientific, Carlsbad, CA, USA
Centrifuge - Microcentrifuge 17,000 x g	Thermo Fisher Scientific, Carlsbad, CA, USA
Pipettes automated 'Finnpipette'	Thermo Fisher Scientific, Carlsbad, CA, USA
Pipettes automated	VWR, Radnor, PA, USA
Mini Centrifuge	Thermo Fisher Scientific, Carlsbad, CA, USA
Vortex Mix	Thermo Fisher Scientific, Carlsbad, CA, USA
Barcode Scanner	Zebra Technologies ZIH Corp., Lincolnshire, IL, USA
Refrigerator	BioMedical Solutions, Inc., Stafford, TX, USA
Refrigerator	Thermo Fisher Scientific, Carlsbad, CA, USA
Freezer (30 °C freezer)	Thermo Fisher Scientific, Carlsbad, CA, USA
Cryo Coolers	VWR, Radnor, PA, USA
Autoclave Sterilizer 'BioClave'	Benchmark Scientific, Edison, NJ, USA
Chemicals	
SuperScript III Platinum One-Step qRT- PCR Kit	Invitrogen, Carlsbad, CA, USA
AgPath-ID One-Step RT-PCR Reagents	Applied Biosystems, Foster City, CA, USA
QIAamp Viral RNA Mini Kit	Qiagen, Hilden, Germany
Nuclease-free Water	Ambion, Inc., Carlsbad, CA, USA
Ethanol Koptec Pure 200 Proof	Decon Labs, Inc., King of Prussia, PA, USA
Disposable	
1000 µL Filter Tips	Phenix Research Products, Candler, NC, USA
200 µL Filter Tips	ART, Thermo Fisher Scientific, Carlsbad, CA, USA
20 µL Filter Tips	Multimax, BioExpress, VWR, Radnor, PA, USA
10 µL Filter Tips	Neptune, VWR, Radnor, PA, USA
MicroAmp Fast Optical 96-Well Reaction Plate	Thermo Fisher Scientific, Carlsbad, CA, USA
MicroAmp Optical Adhesive Film	Thermo Fisher Scientific, Carlsbad, CA, USA
MicroAmp Optical 8-Cap Strip	Thermo Fisher Scientific, Carlsbad, CA, USA
MicroAmp Fast Reaction Tubes (8 tubes/strip)	Thermo Fisher Scientific, Carlsbad, CA, USA
AB custom probes	Thermo Fisher Scientific, Carlsbad, CA, USA

Integrated DNA Technology (IDT) custom probes and primer	IDT
1.5 mL tubes	VWR, Radnor, PA, USA
Combitips	Eppendorf, Hauppauge, NY, USA

Supplies

Lab coat	N/A
Protective shoes	N/A
Gloves	Denville Scientific, Holliston, MA, USA
Masks	VWR, Radnor, PA, USA
Biohazard waste bags	VWR, Radnor, PA, USA

Catalog Number	Comments/ Description
#C-2937	Glove box, Portable, 36", Class III BSC. Dims: 36x20x23.75 in, Includes 2 interior outlets
NU-602-400	4 Ft. Class II Type A2 Cage Changing Biological Safety Cabinet, 12" Access Opening, HEPEX Pressure Duct
NU-126-300	3 Ft. Vertical Laminar Airflow Cabinet, 8" Access Opening, HEPA filter supply, 99.99%
4376598	Holds 96 samples, Dims: 9.7x16.8x20.2 in
75002440	Holds 24 x1.5 or 2 ml tubes, Dims: 8.9x9.6x13.8 in
4700880	Pipet 4-pack (2, 20, 200 and 1,000µL volume), Advanced Volume Gearing(AVG), Ultra durable
05-403-151	Pipet 4-pack (2.5,10, 100 and 1,000µL volume)
75004061	Dims: 4.1x5.0x6.0 in
88880017TS	Dims: 6.1x8.3x3.3 in
Symbol LS2208	Handheld, lightweight
BSI-HC-UCFS-0504W	Standard Undercounter Refrigerators & Freezers
05LRAETSA	To occupy 5.1 Cubic feet
Model ULT430A	To occupy 4.9 Cubic feet
414004-286	0.5 or 1.5 mL tube benchtop coolers
B4000-16	16 liter, Benchtop, Dims: 22x17.5x15.7 in, Fully automatic, Extremely Compact
11732-088	
4387391	
52906	
AM9906	
V1001	
TS-059BR	
ART, 2770	
MultiMax, P-3243-30X	
Neptune, BT10XLS3	
490003-978 CS	
4311971	
4323032	
4358293	
N/A	Custom probes

N/A	Custom probes
10025-726	
89232-972	
N/A	Customizable
N/A	Customizable
G4162-250	Small, meduim or large Nitrile or latex gloves
414004-663	Advanced protection mask
14220-046	20.3 x 30.5 cm Biohazard bags

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Date:	5/18/2018

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Title of Article: Remote laboratory management: respiratory virus diagnostics

Author(s): E. Petrova, V. Avadhanula, S. Michel, K.F. Gincoo, P.A. Piedra, S. Anandasabapathy

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Remote Laboratory Management: Respiratory Virus Diagnostics

Elena V. Petrova, Vasanthi Avadhanula, Sarah Michel, Karen E. Gincoo, Pedro A. Piedra, Sharmila Anandasabapathy

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 ℃ 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.

2. More clear labeling of figures is needed (especially Fig 5).
Figure 5 is improved and provided with higher resolution.

3. 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."

<u>Reviewer 2:</u>

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."