

Registration of Research Form

Arroyo Lab

Specimen Source

a) Clinical specimens will come from two different sources: a) untreated serum and plasma will be obtained from the university sanctioned biorepository currently being established by Antony Rosen and others in the hospital under proper IRB approval; b) inactivated oral fluid (gum swab + saliva, treated at 60° C for 30 min) will be obtained from the research group led by Christopher Heaney. The concentration of anti-SARS-CoV-2 antibodies in such samples will be measured via a novel point-of-care rapid diagnostic platform we have designed. The results of these measurements will be compared to the gold standard immunoassay to benchmark the specificity, sensitivity and precision of our technology. The objective will be to determine whether our new platform could be deployed for population-wide monitoring of SARS-CoV-2 spread.

Specimens will be clearly labeled and stored at -20° C. Specimens will be opened only inside a biosafety cabinet and not removed until they have been inactivated using detergent and heat.

b) SARS-CoV-2 is a highly contagious, airborne respiratory pathogen known to cause severe pneumonia in some individuals, with especially high rates of morbidity and mortality in at-risk populations. Particularly relevant co-morbidities include advanced age, type 2 diabetes, and hypertension. **No individuals who will be working with these samples are considered high risk.**

c) An extensive SOP is attached as **Appendix A** below. Briefly, serum, plasma and oral fluid samples will be opened inside a biosafety cabinet, and then be contacted by a test strip. The test strip will then be moved to a second tube containing invertase-fused antibodies, a third tube containing PBS/Tween for washing, and a fourth tube containing a sucrose solution. The glucose levels produced by invertase in the last tube will be measured using a glucometer. After heat inactivation at 95° C for 20 minutes inside the containment room, all tubes, strips, and instruments will be considered non-infectious and disposed of in biohazard bags that will be autoclaved at the end of the day, as described in the SOP. The SOP adheres to JHMI guidelines set out as of 3/28/2020.

d) SARS-CoV-2 is thought to be most efficiently transmitted as aerosolized droplets, but it is also believed that contact with contaminated surfaces can lead to infection. We will minimize potential risk by not opening samples outside of the biosafety cabinet and using aerosol barrier tips. All surfaces within the biosafety cabinet and benchtops will be thoroughly and frequently decontaminated with 10% bleach for 20 min and then 70% ethanol. Following use, the biosafety cabinet will be irradiated with UV-light for 20 min after bleach and ethanol cleaning.

e) Enhanced safety precautions and safety procedures for BSL-2 level pathogens will be utilized for samples that may contain SARS-CoV-2.

- **Safety practices:**

All samples will be retrieved from collaborators and transported to the containment room through approved laboratory transport routes in zip-locked bags contained within another zip-locked bag. These bags will be transported in a non-hermetically sealed hard-shelled plastic/Tupperware container.

Samples will be sprayed with 70% ethanol followed by thawing on ice in the biosafety cabinet (BSC). At least 250 ml of 10% bleach will be maintained in the BSC in case of spill so that arms and hands do not need to leave the hood for quick and effective decontamination. Bleach will be made fresh each day before starting to work with the samples.

Enhanced BSL-2 level practices will be employed when working with infectious samples, including 1) gowning with a single-use disposable, fluid impermeable lab coats that do not leave the designated SARS-CoV-2 containment room, 2) the use of and frequent changing of double gloves, 3) wearing of a face shield

until samples are heat inactivated, and 4) proper hand hygiene (immediate washing of hands before and after exit from the isolation room into the lab).

All waste generated inside the BSC will be treated with bleach for 20 min and placed inside a biohazard bag which is located inside the BSC. This bag will be tied up inside the BSC. Only heat-inactivated or bleached materials come out of the BSC, and these waste materials will be placed inside a second biohazard bag which is in a biohazard box.

If any material is spilled in the BSC, we will immediately apply 10% bleach solution or paper towels soaked in 10% bleach. The volume of 10% bleach will be similar to the volume of material spilled. We will wait 20 minutes to ensure complete destruction of the pathogen. We will wipe up, dispose in a biohazard box, re-wipe the area with 10% bleach, then wipe with water or 70% ethanol to remove residual bleach.

Any incidents including spills will be reported immediately to the supervisor (Dr. Netz Arroyo). In the unlikely event of an exposure, the affected area will be washed extensively with soap and warm water for at least 2 full minutes. If an eye splash occurs then eyes will be rinsed for at least 2 minutes using the closest eye wash station. Any clothing that has potentially been exposed will be removed and placed in a biohazard bag for autoclaving. If any exposure occurs, after the washing procedure is performed, 5-STIX (5-7849) will be called and consulted for further action. The supervisor and the Biosafety Office (biosafety@jhu.edu) will also be informed.

Individuals working with SARS-CoV-2 virus must monitor their health and immediately report any potential illness, especially fever, cough, shortness of breath, or flu-like symptoms to their PI/Lab Director and the Biosafety Office (biosafety@jhu.edu). Any individual experiencing symptoms noted above should self-quarantine.

The SARS-CoV-2 biosafety cabinet (BSC) will be clearly demarcated in SoM, Hunterian 310.

- **Equipment:** Samples will be handled only inside a biosafety cabinet. All materials and equipment required prior to sample inactivation will be kept inside the designated BSC in SoM, Hunterian 310.
 - **Facilities:** Sample handling will take place exclusively within SoM, Hunterian 310, a room which has been designated for use ONLY in SARS-CoV-2 related research during the pandemic. Only after samples have been heat inactivated will they be brought outside into the main lab area for disposal.
 - **Training:** Personnel handling these samples will undergo bloodborne pathogen training annually, in addition to standard lab safety training.
- f) Virus-containing samples (serum, plasma and oral fluid) will be heat inactivated at 95° C for 10 min following platform deployment. Working surfaces will be disinfected with 10% bleach solution after each set of samples is processed. All contaminated materials will be treated with 10% bleach for 20 min, placed in biohazard bags, and closed up after each set of samples is processed. At the end of the day, heat-inactivated and bleached materials in tied biohazard bags will be removed from the BSC and placed in a second biohazard bag in a biohazard box.

APPENDIX A

JHU Office of the Provost Funded Research Response to SARS-CoV-2

Adapting Commercial Glucose Sensors to Population-Scale Measurement of SARS-CoV-2 Antibodies.

Arroyo Lab

Last updated: 4/25/20

Principle:

The fastest approach to producing a point of care device that can be delivered at population-scale to clinics, hospitals and community centers is to adapt already existing, commercially available technologies to the measurement of virus-targeting antibodies. Following this reasoning, we propose here to adapt widely available commercial glucose meters to the measurement of Covid-19-binding antibodies. The approach of adapting glucose meters to the detection of other metabolic or physiologic markers was demonstrated and previously validated. The proposed measurement scheme combines a sandwich immunoassay with electrochemical detection via a glucometer.

Specimen and Organism Types:

The glucometer-based detection assay can use serum, plasma, whole blood, or oral fluid as the biological matrix. The preferred specimen types, specimen requirements and methods are summarized in Table 1.

Table 1. Specimen Requirements and Methods.

Requested Test	Specimen Type	Specimen Volume	Specimen Storage and Stability	Processing Methods
Glucometer-based detection test	Serum, plasma, or oral fluid	100 uL	Frozen at -20°C	Glucose strip analysis

Quality Control:

Samples will include both known positive SARS-CoV-2 and known negative sample to benchmark the precision of the glucometer-based test. Equipment and materials required for this protocol are summarized in Table 2.

Table 2. Equipment and Materials

Reagents / Kits	Consumables	Equipment
Test strip containing immobilized recombinant spike protein		
Diluted serum or plasma sample	1.5 ml microcentrifuge Tubes 200 uL pipet tips	Tube racks 200 uL pipettor Freezer/Refrigerator
Solution of invertase conjugated with anti-human IgM/IgG antibodies	1.5 ml microcentrifuge Tubes	Tube racks

2M Sucrose solution	1.5 ml microcentrifuge Tubes Red biohazard bag for waste	Tube racks
70% Ethanol / 10% Bleach Sprays		Tissue Culture Hood

Procedure:

Prior to the start of the glucometer-based test, disinfect and decontaminate all consumables and equipment listed in Table 2 using a combination of 10% bleach, 70% ethanol, and UV light as required.

Start of Day Decontamination Procedure: (10% bleach- 5 minutes, then water, then 70% ethanol) then 30 minutes of UV prior to starting procedure. Obtain ice and place all reagents/kits needed that day to thaw prior to sample retrieval.

1. Specimen thawing and Sample dilution
2. Glucometer-based test iteration
3. Clean up

Step 1: Specimen Thawing and Sample Dilution

- 1.1 Transport all samples from -20C on ice and spray with 70% ethanol followed by thawing on ice in biosafety cabinet (BSC).
- 1.2 Into individual 1.5mL eppendorf protein lobind tubes, add 198 uL of PBS. Add 2 uL of serum for a 100x dilution. Keep on ice in TC hood until needed.
- 1.3 Residual samples should be sprayed with 70% ethanol and immediately stored at -20C.

Change gloves before proceeding to step 2.

Step 2: Glucometer-based test iteration

- 2.1 Insert the SARS-CoV-2 spike protein-functionalized test strip into a tube (for t ~ 15 min) containing the patient sample. If the sample contains anti-Covid-19 IgG/IgM antibodies, these will bind to the strip-immobilized spike protein.
- 2.2 Transfer the strip into a second tube containing invertase conjugated with anti-human IgG/IgM (for t ~ 15 min), to allow binding of antibodies against the conserved region of Covid-19 antibodies.
- 2.3 Insert the strip into a third tube containing buffer (for t ~ 5 mins) to wash off any excess invertase-conjugated anti- IgG/IgM complex.
- 2.4 Insert the strip into a fourth tube (for t ~ 15 mins) containing a solution of sucrose. There, any invertase-antibody complexes bound to the strip will catalyze the conversion of sucrose to glucose. The amount of glucose produced will be directly proportional to the concentration of IgG/IgM antibodies in the sample.
- 2.5 Measure glucose generated using a commercial glucometer, providing a quantitative measure of extent of infection in the sample.

2.6 Heat inactivate all tubes by incubating them on a heat block at 95° C for 10 mins to inactivate any potentially remaining infectious virus. Treat all contaminated materials with 10% bleach for 20 min. Wipe down heat block with 10% bleach followed by 70% EtOH. Samples are now considered non-infectious. Place all materials in a red biohazard bag and tie the bag.

Change gloves before proceeding to step 3.

Step 3: Clean up

3.1 Dispose of any waste in the tied biohazard bag in the BSC into a second biohazard bag in a biohazard box, which will be immediately sealed for pickup.

3.2 If done for the day: Decontaminate the lab bench, hood and all equipment inside and outside of hood.

End of Day Decontamination Procedure: (10% bleach- 5 minutes, then 70% ethanol) then 30 minutes of UV exposure for items within the BSC hood. All surfaces including bench tops, draws, lab equipment and furniture and lab plastics outside within the red/orange zones should be decontaminated: (10% Bleach- 5 minutes, then water, then 70% ethanol) All biohazard bags should be tied shut, placed into a new biohazard bag also tied shut and sealed for pickup.

IN THE CASE OF A SPILL

Room 310 has been tested for air pressure differential to the hallway and found to be negative to the hallway. This means that aerosols generated by a spill of virus containing material outside the BSCs should be contained in the room. Although serum and plasma are not expected to contain much virus, oral fluid may do.

In the event of a spill, steps must be taken to minimize the number of staff who may be exposed to the virus. To do this, **the door to the room should remain closed.** The spill should immediately be decontaminated with a 10% bleach solution. **The staff in the room should wait a minimum of 15 minutes before leaving the room.** If the door is opened prior to this hold time, there is a chance that aerosols from the room will be released into the hallway either by the pumping action of the door or by being entrained in the “wake” of a departing person.

This response differs from what one might instinctively do, but it makes the most sense for the situation. Anyone in the room at the time of the spill has already been potentially exposed. To minimize any additional staff exposures, you should stay put and allow the ventilation system to clear the air of aerosols. Waiting 15 minutes should result in a significant reduction of any aerosol load generated by the spill.

Please also note that clothing that may have been exposed to spilled material, pants, shoes, socks, etc. if spilled on the floor, should be bagged and handled as biohazardous material.

Finally, please store materials that may contain SARS-CoV-2 in clearly labelled secondary containers such as Tupperware-type boxes or ziplock bags or such to help ensure folks know the risks prior to handling materials either for research purposes or moving materials in the event of a freezer failure.

Acknowledgment of Standard Operating Procedure Guidelines and Policies

I certify that I have read and understood the contents of this document and that I will abide by all the policies and guidelines described in it.

Lab Member Name

Signature

Date