

Submission ID #: 62379

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Project Page Link: <https://www.jove.com/account/file-uploader?src=19025643>

Title: Swabbing the Urban Environment—A Pipeline for Sampling and Detection of SARS-CoV-2 From Environmental Reservoirs

Authors and Affiliations:

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Author Questionnaire

1. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

3. Interview statements: Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one.**

☒ Interviewees wear masks until videographer steps away (≥ 6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. Filming location: Will the filming need to take place in multiple locations? **No, Same building 3rd and 4th floor**

Current Protocol Length

Number of Steps: 17

Number of Shots: 28

Introduction

1. Introductory Interview Statements

REQUIRED:

- 1.1. **Maria-Isabel Rojas**: This study presents a novel workflow to detect SARS-CoV-2 on surfaces that are rarely cleaned or disinfected in the urban environment such as gas pump handles, playgrounds, and ATMs.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.
- 1.2. **Maria-Isabel Rojas**: In a pandemic, supplies and reagents are scarce. We use easily obtainable materials and reagents and equipment available in basic laboratory settings. We use an extraction that preserves the RNA without a cold-chain and a detection method resistant to inhibitors.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

- 1.3. **Maria-Isabel Rojas**: This protocol is of public health interest and a framework for the assessment of environmental viral reservoirs for the current CoVID-19 pandemic, and other infectious agents during future global outbreaks.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Protocol

2. Sampling the urban environment

- 2.1. Recruit citizen scientists using a direct and clear call-to-action released via local and social media [1]. Create a social media handle to connect the topic across social media content [2-TXT].
 - 2.1.1. SCREEN: 62379_screenshot_1.0:00-0:28
 - 2.1.2. SCREEN: 62379_screenshot_1.0:31-0:42 **TEXT: e.g., #swab4corona**
- 2.2. Create a link to the SMP, providing a multilingual plugin to enable navigation in multiple languages [1], for individuals to apply to participate in the environmental sampling effort by answering biosafety-related questions specified in an online form [2].
 - 2.2.1. SCREEN: 62379_screenshot_2.0:00-0:14
 - 2.2.2. SCREEN: 62379_screenshot_2.0:16-0:43
- 2.3. Include in the sampling section graphic and audiovisual protocols in English and Spanish [1].
 - 2.3.1. SCREEN: 62379_screenshot_3.0:00-0:22
- 2.4. Visualize geospatial data using a geospatial application programming interface facilitated by a cloud computer service provider [1]. Store the data submitted to the LIMS through the SMP to facilitate centralized storage, tracking of processing workflows, and management of the logistics [2].
 - 2.4.1. SCREEN: 62379_screenshot_4.0:00-0:16
 - 2.4.2. SCREEN: 62379_screenshot_4.0:21-0:31
- 2.5. Pre-load information such as Sample Kit ID, Sample ID, date, time, and Global Positioning System coordinates to enable data-type compliance and minimize the error [1].
 - 2.5.1. SCREEN: 62379_screenshot_5. 0:10-0:25
- 2.6. Include a Sample Pickup Request link for the participants, which they can use once they have collected all samples [1].
 - 2.6.1. SCREEN: 62379_screenshot_5. 0:35-0:48
- 2.7. Build a kit that contains all the sampling supplies, including the necessary personal protective equipment like mask, gloves, a sampling protocol, and biosafety relevant information [1].
 - 2.7.1. All the kit components distinctively displayed on the table *Videographer: This step is important!*

- 2.8. Swab rarely disinfected surfaces in households and the urban environment by wetting a 1 centimeter-square polyester absorbent swab with a detergent [1-TXT] and swabbing a surface of 10 centimeters-squared [2]. Aided by a toothpick, submerge each sample swab in the prelabeled tube containing 200 microliters of guanidinium thiocyanate [3].
 - 2.8.1. Talent wetting the absorbent swab detergent **TEXT: Detergent e.g., 0.5 % SDS**
 - 2.8.2. Wide: Talent swabbing the surface *Videographer: This step is important!*
 - 2.8.3. Talent submerging the sample swab in a tube *Videographer: This step is important!*
- 2.9. Wear the provided mask and a new pair of gloves for the collection of each sample to avoid cross-contamination [1]. After finishing the sampling, use the provided hand sanitizer [2].
 - 2.9.1. Talent changing the gloves.
 - 2.9.2. Talent using hand sanitizer.
- 2.10. Store tubes at 4 degrees Celsius until they are transported to the laboratory [1]. Once the samples arrive in the laboratory, store them at minus 80 degrees Celsius [2].
 - 2.10.1. Talent storing the tubes in ice-pack filled container
 - 2.10.2. Talent storing the tubes in -80 degree.

3. SARS-CoV-2 detection

- 3.1. To increase the speed of the screening, process the samples in pools. If a pool is positive, extract the RNA of each sample independently. Combine the samples from each sampling kit into 2 pools by pooling 50 microliters of each of the 8 samples into a microcentrifuge tube and saving the remaining samples at minus 80 degrees Celsius [1].
 - 3.1.1. Talent pooling the samples in 2 pools of 8 samples *Videographer: This step is important!*
- 3.2. Add 80 microliters of chloroform and vortex for 15 seconds [1], then incubate for 20 minutes at 4 degrees Celsius [2]. Centrifuge at 13,000 times g for 20 minutes at 4 degrees Celsius [3].
 - 3.2.1. Talent vortexing the tube
 - 3.2.2. Talent incubating the tube at 4 degrees
 - 3.2.3. Talent putting the tubes in the centrifuge
- 3.3. Transfer the aqueous layer into a new microcentrifuge tube [1]. Store the remaining interface and pink liquid in the minus 80-degree Celsius freezer. These fractions contain DNA and proteins [2].

- 3.3.1. Talent transferring the aqueous layer into tube
- 3.3.2. Talent storing the tube in -80 degrees **NOTE: Don't use tk 1 – tk3**
- 3.4. Extract RNA from the recovered aqueous layer using a guanidinium thiocyanate-based RNA crude extraction protocol [1].
 - 3.4.1. Talent adding isopropanol to the tube. *Videographer: This step is difficult and important!*
- 3.5. Prepare the RT-LAMP (*pronounce 'r-T-lamp'*) reaction mix at room temperature with 10% excess volume to account for pipetting loss [1].
 - 3.5.1. Talent preparing RT-LAMP reaction mix **NOTE: Don't use tk 1**
- 3.6. Add 5 microliters of RNA to the sample reaction and 5 microliters of RNA plus 2.5 microliters of synthetic SARS-CoV-2 RNA to the spiked reaction [1]. Add 2.5 microliters of synthetic SARS-CoV-2 RNA to the positive control [2] and 5 microliters of water to the negative control. Mix well, and spin down the reactions [3].
 - 3.6.1. WIDE: All the reaction tubes with their labels
 - 3.6.2. Talent adding RNA to the tubes labelled for positive reaction
 - 3.6.3. Talent spinning down the tubes
- 3.7. For colorimetric observation, a negative result is indicated by pink and a positive result is indicated by yellow [1-TXT]. After RT-LAMP, perform gel electrophoresis [2].
 - 3.7.1. Tubes with two different colors either pink or yellow **TEXT: Pink: pH = 8.8, Yellow: pH = 5**
 - 3.7.2. A gel running

Results

4. Results: Sampling and Detection of SARS-CoV-2 From Environmental Reservoirs

- 4.1. The distribution of sample collection sites is shown here [1]. A majority of kits were complete and the corresponding data was uploaded to the LIMS [2].
 - 4.1.1. LAB MEDIA: Figure 4A
 - 4.1.2. LAB MEDIA: Figure 4A *Video editor: Please highlight the second pie chart of orange color with 91.1 % written in bold*
- 4.2. The limit-of-detection at a frequency of 100% was 500 copies per 25 microliters of reaction [1]. In the colorimetric RT-LAMP, positive samples changed color from pink to yellow due to a pH shift from 8 to 5.5 [2]. At low-copy numbers, samples were run on an agarose gel to confirm the positives with the resulting ladder-like pattern [3].
 - 4.2.1. LAB MEDIA: Figure 3A and 3B
 - 4.2.2. LAB MEDIA: Figure 3B
 - 4.2.3. LAB MEDIA: Figure 3C
- 4.3. RT-qPCR methods were tested with environmental samples. All master mixes were sensitive to inhibitors at low-copy-number concentrations of the positive control [1].
 - 4.3.1. LAB MEDIA: Figure 5A
- 4.4. At low concentrations of the template, traditional RT-PCR methods showed false positives [1] and false negatives [2].
 - 4.4.1. LAB MEDIA: Figure 5B *Video editor: Please highlight the first gel image with N1 and N2*
 - 4.4.2. LAB MEDIA: Figure 5B *Video editor: Please highlight the second gel image with E1*
- 4.5. Lastly, a technique called rolling circle amplification detected small quantities of the target sequence. However, it showed amplification of the probe in the absence of an RNA template [1].
 - 4.5.1. LAB MEDIA: Figure 5C

Conclusion

5. Conclusion Interview Statements

- 5.1. **Maria-Isabel Rojas:** It is vital to release a call-to-action that reaches all sectors of the community, so the sampling truly represent the risk of exposure ranges of all members of the community
 - 5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.1.1 and 2.1.2*
- 5.2. **Maria-Isabel Rojas:** Following this protocol, the collected samples can be tested for viral infectivity. The presented framework detects SARS-CoV-2 genetic material. Tissue culture studies on its viability are the next step.
 - 5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera