Initial evaluation of a mobile SARS-CoV-2 RT-LAMP testing strategy

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Article Summary:
RT-LAMP performed in a point-of-need mobile laboratory is useful for increasing SARS-CoV-2 testing capacity

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Abstract

SARS-CoV-2 control in the United States is hampered by limited testing. We evaluated a simple, outdoor, mobile RT-LAMP assay workflow where self-collected saliva is tested for SARS-CoV-2 RNA. 494 volunteers were tested over 16 days. Testing results were 99.8% concordant with qRT-PCR, with all but two people testing SARS-CoV-2-negative.

Body

More than 50,000,000 SARS-CoV-2 tests have been performed in the United States, yet it is estimated that between 80-95% of infected individuals are not tested [1,2]. Because transmission can occur before symptoms manifest, delays in reporting prevent timely isolation of infected individuals. The current testing regimen fails to effectively identify and notify infected individuals and reduce transmission, creating a major barrier to safe returns to workplaces and schools.

We obtained Institutional Review Board approval (Protocol #2020-0855) to prototype a simple, mobile, non-diagnostic SARS-CoV-2 testing workflow. Replication-competent SARS-CoV-2 is rarely recovered from individuals whose nasopharyngeal swab viral loads are less 1E+6 copies / mL, while peak respiratory viral loads exceeding 1E+10 copies / mL have been observed [3]. Furthermore, detection of virus in saliva is correlated with presence of virus in nasopharyngeal swab samples [4]. Therefore, we reasoned that a cost-effective testing strategy that detects at least 1E+6 copies / mL in saliva and provides near real-time results could be effective in identifying those at greatest risk of infecting others, encouraging isolation and follow-up diagnostic testing.

After providing informed consent, volunteers self-collect at least 50 µL of saliva into a 1000 µL pipette tip placed inside a 1.5 mL “safe-lock” microcentrifuge tube. The volunteer disinfects the outside of the tube thoroughly with a pre-moistened disinfectant wipe. The sample is heated to 65°C for 30 minutes to inactivate SARS-CoV-2 [5] and then to 98°C for 3 minutes to improve nucleic acid detection and inactivate saliva enzymes. The inactivated saliva is then centrifuged for two minutes in a benchtop centrifuge. 50 µL of the supernatant is added to 50 µL of 1x phos-
phate buffered saline, and 3 µL of this mixture is added in duplicate to 17 µL of a colorimetric reverse-transcriptase loop-mediated isothermal amplification (RT-LAMP) reaction mix containing SARS-CoV-2-specific primers, building on an approach described in [6]. The LAMP reactions are then heated for 30 minutes at 65°C.

Reaction vessels are photographed with a smartphone before and after this incubation. A colorimetric change from pink/orange to yellow is scored relative to inactivated SARS-CoV-2 that is directly added as a positive control in each batch of reactions at a concentration of 1,000 copies per reaction (corresponding to a calculated saliva viral load of 6.6E+5 copies / mL). These ready-to-run positive control standards must be stored at -80°C; storage at -20°C led to inconsistent performance in two early runs which were resolved after changing storage conditions.

From July 16 to July 31, this testing approach was used 12 times at 4 different locations in the Madison, WI area. 494 adult volunteers were tested across the 4 locations (each separate consent document constitutes a volunteer; some individuals volunteered more than once during the study). 487 of the 494 volunteers also consented to additional research testing of residual saliva leftover after RT-LAMP; 7 of these samples contained no residual saliva. Additional research testing of residual samples was conducted in pools of 6 for samples that appeared unambiguously negative by RT-LAMP, and individually for those that appeared positive or ambiguous. RT-LAMP detected very low levels of SARS-CoV-2 nucleic acids in one pool of negative samples (460 copies/ml, well below the limit of detection for RT-LAMP), but none of the other pools had detectable SARS-CoV-2 RNA. Three samples were flagged as positive by RT-LAMP; qRT-PCR identified these as two positives (one with low viral loads at 8.58E+03 copies/ml, one at 2.15E+07 copies/ml), and one was negative by qRT-PCR, suggesting a false positive RT-LAMP result (Figure 1).

The excellent performance of this RT-LAMP workflow suggests that it could be extremely useful for non-diagnostic, mobile, “winnowing” testing of places at high risk for close-contact, indoor SARS-CoV-2 transmission: schools, workplaces, places of worship, prisons, etc. Rapid, sub-2-hours availability of results enables people with potential SARS-CoV-2 infections to self-isolate.
quickly and then obtain diagnostic testing, while the low per-test cost allows for short-interval serial testing to identify incident infections and reduce the duration of their exposure to others. While the RT-LAMP workflow is not as sensitive as diagnostic PCR tests in laboratory testing, its detection threshold is sufficient to identify most individuals who have viral loads consistent with live virus shedding who likely pose the greatest risk of transmission [7,8]. Replicating the performance of this initial testing may be challenging with less skilled operators and in other settings; nonetheless, this approach offers a promising alternative to PCR-based testing that can be easily deployed in various environments to test the hypothesis that frequent, rapid turnaround testing can enable safe returns to work and schools.

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Biographical Information

Dr. Christina Newman is an associate research scientist in the Pathology and Laboratory Medicine department in the School of Medicine and Public Health at the University of Wisconsin-Madison. Her research interests include emerging infectious diseases, in particular, emerging viruses.

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Supplemental Materials

RT-LAMP protocols and information about mobile testing are available at https://openresearch.labkey.com/wiki/Coven/page.view?name=field-testing

Regulatory oversight

This work was performed under approved UW-Madison Health Sciences IRB Protocol #2020-0855.

Figure legend

Figure 1: Samples positive by RT-LAMP. Two samples (numbers 13 and 27) were positive by qRT-PCR, though sample 13 was a low positive. One sample (number 78) was negative by qRT-PCR, suggesting that this was a false positive result by RT-LAMP. A clear color change to yellow in either or both replicates was established as a threshold for positivity a priori but may be revised in the future if discrepant replicates are not consistently qRT-PCR positive.

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Figure 1. Samples positive by RT-LAMP

Before  

After 30 min incubation  

Viral load by qRT-PCR

sample 13:  
8.58E+03 copies/ml

sample 27:  
2.15E+07 copies/ml

sample 78:  
negative by qRT-PCR  
(false positive by RT-LAMP)