In Clinic Feasibility Testing of a Point-of-Care Low-Cost COVID-19 RT-PCR Test:
A case study at MedStar Georgetown University Hospital Pediatric Clinic

Nuttada Panpradist[1], Jack Henry Kotnik[1], Qin Wang[1], Parker Ruth[1], Chunjong Park[1], Samuel Stewart[1], Annika Sahota[1], Charalampos Mystakelis[2], David Nelson[2], Sejal Bavishi[2], Ami Patel[2], Barry Lutz[1], Ruth Kanthula[2]

[1] University of Washington, WA [2] Georgetown University, Washington, DC

Corresponding author:
NP (nuttadap@uw.edu)

Introduction

A Key COVID Testing Hotspot: District of Columbia

Major Caveats (Standard Process):

Long Turnaround Time

Cumbersome Procedure

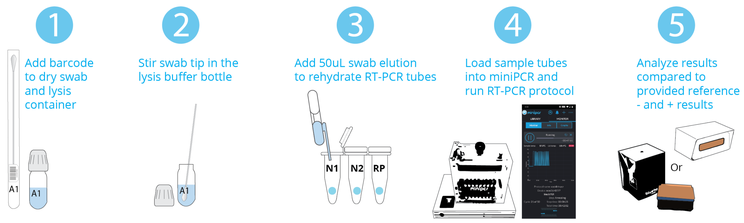
In the early pandemic, testing required sending specimens to **external laboratories for processing**. Results could take **up to a week**.

Impact: Improved medical decision making.

Solution: Development of a modified US CDC SARS-CoV-2 assay consisting of reverse transcription polymerase chain reaction (RT-PCR) and visual detection of SARS-CoV-2 viral RNA through end-point fluorescence signal in a low-cost reader.

Development of a Modified US CDC SARS-CoV-2 Assay

Our proposed solution: Simplified workflow by allowing sample eluate to be added directly to lyophilized reagents to avoid the need for RNA extraction and RT-PCR reaction set-up.



Evaluation of assay by Healthcare Workers in clinics

Healthcare Worker (HCW) Recruitment:

- HCWs without prior experience running point-of-care assays..
- HCW-led trials to inform test kit improvements.

Feasibility, Accuracy, and Precision Assessments:

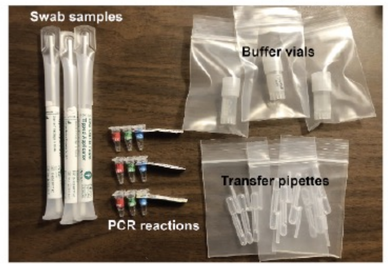
- Development of instructional videos and guidebooks to train HCWs on workflow.
- Blind specimen testing, containing varying concentrations of deactivated SARS-CoV-2 virus, independently performed by HCWs.
  - 0 copies/mL
  - 2000 copies/mL
  - 5000 copies/mL

Materials and Methods

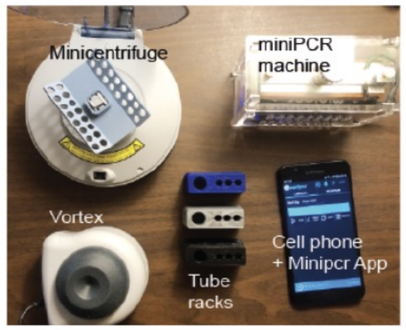
Step-wise protocols:

- **Elute swab** in provided buffer.
- Use disposable plastic pipette to **transfer eluate into three tubes** containing in-house **lyophilized RT-PCR reagents**.
- Subject tubes to a mini-PCR thermal cycler for RT-PCR processing.
- **Capture image** of reaction tubes using the mobile phone application
- Software analysis to **classify results (e.g., positive or negative**).

(A) Kit components prepared in the Lutz lab at the University of Washington



(B) Equipment required to operate our test



(C) Experimental set-up before RT-PCR

(D) Experimental set-up for post-PCR steps

(E) Data tracking system through barcodes

Figure 1. Feasibility of point-of-care RT-PCR COVID-19 test at a pediatric clinic. (A) Kit components (B) Low-cost equipment required to operate our assay including minivortex, minicentrifuge, miniPCR thermal cycler, a mobile phone, and tube racks. (C) and (D) images of the set-up areas to conduct this study in the clinic. (E) Data tracking system via barcodes. HCWs take picture of the barcodes on the tubes before taking images of the tubes to enable accurate data tracking.

Results and Discussion

Concordance Rate	True Positives	True Negatives	False Negatives	False Positives
93.6% (59/63)	45	14	1	3

- **False positives** were attributed to a **volume issue in RT-PCR**
  - These tubes had ~half the optimal reaction volume, causing a significant increase in salt concentrations and non-specific amplification.
- HCWs reported **improved comprehension** and **confidence** in workflow since Phase I trials
  - This was aided by the addition of **Aurora Red dye** to the rehydration buffer.

Conclusions

- Though the study was limited by the number of recruited HCWs, it holds promise as a pioneering attempt to transfer a laboratory-based **RT-PCR assay with minimal modifications to a clinical setting**.
- This assay could be valuable in future scenarios like '**tridemics**,' where multiple viruses (Influenza, RSV, and COVID) coexist during winter, especially for vulnerable populations **such as infants and young children**.

Acknowledgments

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Participants: Healthcare worker volunteers at MedStar Georgetown University Hospital Pediatric Clinics for participating in this study.

References

[1] Panpradist N, Wang Q, Ruth PS, Kotnik JH, Oreskovic AK, Miller A, Stewart SWA, Vrana J, Han PD, Beck IA, Starita LM, Frenkel LM, Lutz BR. **Simpler and faster Covid-19 testing: Strategies to streamline SARS-CoV-2 molecular assays**. EBioMedicine. 2021 Feb;64:103236. doi:10.1016/j.ebiom.2021.103236. Epub 2021 Feb 12. Erratum in: EBioMedicine. 2021 Apr;66:103296.

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