Shaking Things Up: Direct-to-PCR Viral Detection Off Swabs Using Shaker-Mill Homogenization

Zachary P Morehouse, M.S., OMS-III[1,2], Caleb Proctor, B.S.[2,3], Gabriella Ryan, M.S.[2,3,] Angel A Rivera, M.D., Ph.D.[4], Rodney J Nash, Ph.D.[2,3,5]

1. Michigan State University College of Osteopathic Medicine, East Lansing, MI
2. Omni International Inc, Kennesaw, GA
3. Department of Biology, Georgia State University, Atlanta, GA
4. Quorum X Diagnostics, Tucker, GA
5. Jeevan Biosciences, Tucker, GA

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College of Osteopathic Medicine
MICHIGAN STATE UNIVERSITY

Background

As the number of viral diseases are on the rise, it is critical to continue to innovate and advance diagnostic, treatment, and surveillance methods surrounding viral infections. Currently, one of the most reliable methods for viral infection detection are polymerase chain reaction (PCR) based assays. These tests often involve procedures of swabbing a patient, processing the sample to lyse the virus, extract, and purify its nucleotides, and then run the purified genetic material via PCR for detection of a gene product needed to confirm the patient's suspected diagnosis. This process requires time to complete and is dependent on the availability of the reagents and plastics required to complete the lysis, extraction, purification, and amplification procedures. Herein, we have developed a method to detect virus off a swab using solely shaker-mill based mechanical lysis and the transfer of the viral lysate directly to a PCR based assay, bypassing the reagent heavy and time-consuming extraction and purification steps.

During the COVID-19 pandemic, a global strain on the supply chains providing the reagents and plastics needed for traditional PCR-based viral diagnostic testing was seen. Additionally, large volumes of samples are pushing testing laboratories past their capacity, inciting delays in receiving critical diagnostic information for clinicians and public health officials. In the proposed diagnostic workflow, we have used human coronavirus 229E (HCoV-229E) to demonstrate a novel approach to mechanically lysed virus off swabs and a direct-to-PCR, extraction-less, workflow that allows for 24 samples to be prepared in 30 seconds and loaded directly into the PCR based assay for virus detection. This process reduces total time required for each test, while also reducing the amount of plastics and reagents needed to complete them – both of which thee authors felt would provide benefit in increasing the overall testing capacity and access to care in the face of this global pandemic.

Methods

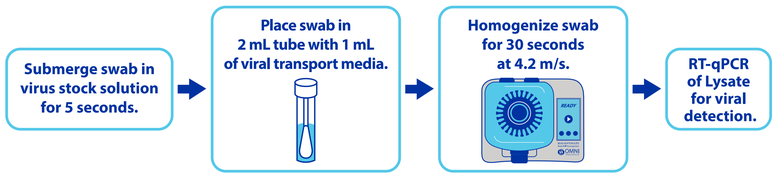


Figure 1. Graphical depiction of the experimental design for proof-of-concept in vitro testing of HCoV-229E detection using the novel direct-to-PCR viral detection protocol. This method was used to generate all of the data displayed on this poster.

Cell Culture and Virus Growth

Human coronavirus 229E (HcoV-229E) (ATCC, Cat. No. VR-740) was added at a multiplicity of infection (MOI) of 1.0 to an approximately 85% confluent T75 flask of MRC-5 cells (ATCC, Cat. No. CCL-171), 48 hrs after plating. The flask was maintained with DMEM (Fisher Scientific, Cat. No. 11-965-118) supplemented with 5% heat inactivated fetal bovine serum (Gemini Bioproducts, Cat. No. 100-500) and 1% L-Glutamine (Gemini Bioproducts, Cat. No. 400-106), incubated at 37°C with 5% CO2. The cell culture supernatant was harvested at 72hr post infection when 80% cytopathic effect (CPE) was observed.

Swab Viral Spike

Sterile cotton swabs (Fisher Science, Cat. No. 22-029-488) were submerged for 5 seconds in viral solutions ranging from 1.2 x 10^6 to 1.2 x 10^0 PFU/mL . The swabs were exposed in a serial dilution pattern, with three swabs being exposed at each concentration log to evaluate the detection capabilities of this method. The saturated swabs were then placed in a 2mL screw capped tube (Omni International, Cat. No. 19-648) prefilled with 1 mL of viral transfer buffer. The stem of the swab was then broken off at a level even with the top of the tube to allow for the cap to be screwed on for transporting and processing. The samples were prepared at 23°C and then incubated for 1 hr at 23°C prior to processing (Figure 1).

Shaker-Mill Swab Processing for Viral Lysis

To maintain optimal levels of biosafety, the following shaker-mill processing was completed in a biosafety cabinet to protect the user from any potential aerosol production during processing. Twenty-four 2 mL screw cap tubes containing the virally spiked swabs were processed on the Bead Ruptor Elite (Omni International, Cat. No. 19-040E) for 30 s at 4.2 m/s. This processing generated froth within the tube which was allowed to settle prior to removal of 1 µL of lysate for RT-qPCR (Figure 1).

HcoV-229E RT-qPCR

HcoV-229E nucleocapsid gene (N gene) was selected as a target for RT-PCR from Vabret et al. The N gene was targeted with forward primer 5'-AGGCGCAAGAATTCAGAACCAGAG-3' and reverse primer 5'-AGCAGGACTCTGATTACGAGAAAG-3'. 1µL of sample lysate was added to create a final reaction volume of 20µL using the proportions of primers, sample, SYBR, RT, and DEPC-treated H2O as laid out in the New England Biologics Luna RT-qPCR Kit (NEB, Cat. No. E3005S). Amplification of lysate was performed for 44 cycles and the resulting amplicons were loaded into a 2% agarose (Bio-Rad, Cat. No. 1613101) gel for product visualization. Out of abundance of caution, the loading of the PCR plate with viral lysate should be completed in a biosafety cabinet to protect the user from any potentially viable virus particles remaining following shaker-mill homogenization.

Results

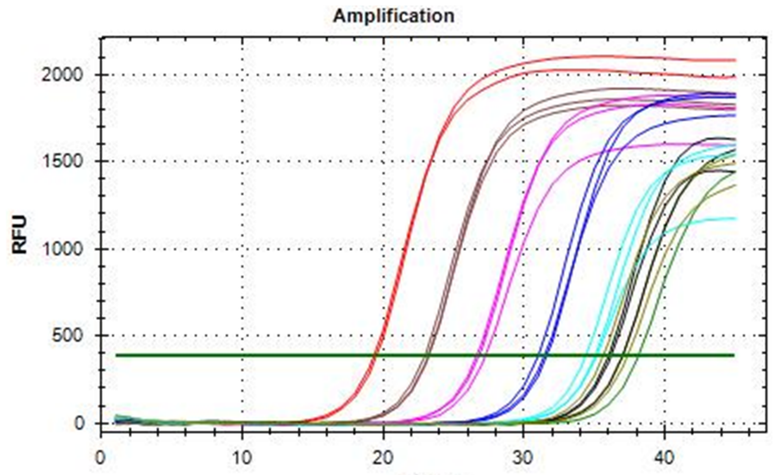
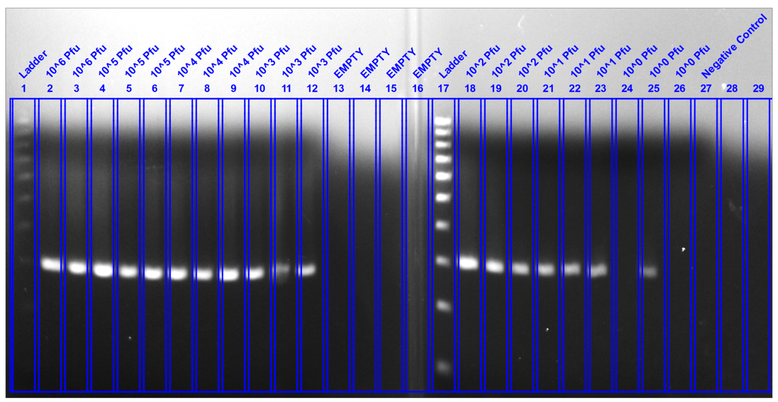


Figure 2. Lower limit of detection testing following the proposed direct-to-PCR method. Serial dilution of viral concentration spiked onto swabs for limit of detection testing. Red lines represent 1.2 x 10^6 PFU/mL spiked swabs. Brown lines represent 1.2 x 10^5 PFU/mL spiked swabs. Pink lines represent 1.2 x 10^4 PFU/mL spiked swabs. Navy lines represent 1.2 x 10^3 PFU/mL spiked swabs. Teal lines represent 1.2 x 10^2 PFU/mL spiked swabs. Olive lines represent 1.2 x 10^1 PFU/mL spiked swabs. Black lines represent 1.2 x 10^0 PFU/mL spiked swabs. Green lines represent virus free, negative control swabs.

Sample	Cq	Gel Confirmed Positive	Cq Mean	Cq Standard Deviation
10^6 PFU/ml	19.31	Yes	19.40	0.125
10^6 PFU/ml	19.49	Yes		
10^5 PFU/mL	23.21	Yes	23.11	0.142
10^5 PFU/mL	23.18	Yes		
10^5 PFU/mL	22.95	Yes		
10^4 PFU/mL	26.77	Yes	26.87	0.324
10^4 PFU/mL	26.60	Yes		
10^4 PFU/mL	27.23	Yes		
10^3 PFU/mL	31.00	Yes	31.33	0.299
10^3 PFU/mL	31.57	Yes		
10^3 PFU/mL	31.41	Yes		
10^2 PFU/mL	35.04	Yes	34.84	0.426
10^2 PFU/mL	34.35	Yes		
10^2 PFU/mL	35.12	Yes		
10^1 PFU/mL	35.58	Yes	36.63	0.918
10^1 PFU/mL	37.31	Yes		
10^1 PFU/mL	36.98	Yes		
10^0 PFU/mL	35.99	No	36.38	0.537
10^0 PFU/mL	36.99	Yes		
10^0 PFU/mL	36.15	No		
Neg Control	38.10	No	N/A	N/A

Table 1 (left). Corresponding Cq values for the lower limit of detection testing demonstrated in Figure 2 using the direct-to-PCR workflow off in vitro spiked swabs with HCoV-229E shown in Figure 1. Additionally, this table shows if the viral concentration detected via RT-qPCR was also confirmed via amplicon visualization in Figure 3.

Figure 3 (below). Confirmatory amplicon visualization of HCoV-229E N gene detected via RT-qPCR shown in Figure 2 and Table 1. Amplicon visualization was used to ensure positive RT-qPCR results were positive for viral transcript and not primer dimerization for the lower viral concentrations tested. .



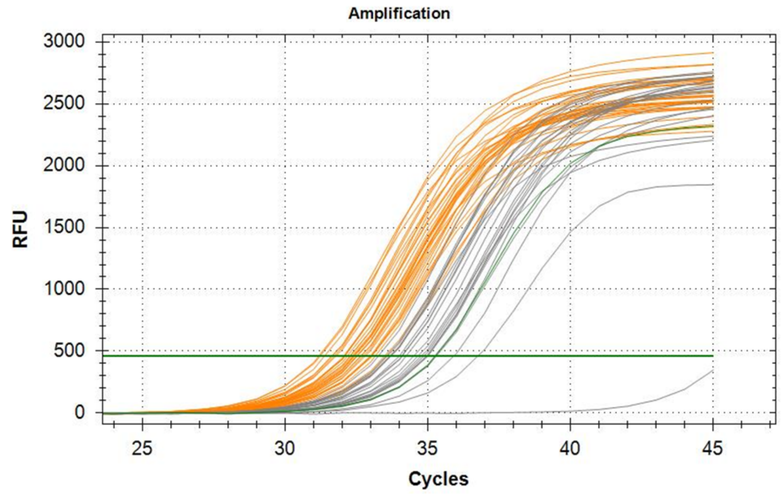


Figure 4. Viral transcript detection using RT-qPCR and the direct-to-PCR workflow shown in Figure 1. HCoV-229E was spiked at the suspected lower limits of detection following the initial range of detection runs illustrated in Figures 2 and 3 and Table 1. N gene transcripts were successfully detected using 1.2 x 10^3 PFU/mL in vitro spikes of HCoV-229E (orange lines) and 1.2 x 10^2 PFU/mL in vitro spikes of HCoV-229E (grey lines). The green line represents a negative control, virus-free swab. All results were confirmed with gel visualization of amplicons in the same manner as Figure 3. Gels are not shown on this poster due to size constraints but are available upon request from authors.

Conclusion

We have successfully proven that shaker-mill homogenization provides sufficient viral lysis off swabs, where the resulting lysate can be used directly in PCR based assays for the detection of virus. This finding allows for decreased run time in traditional PCR based diagnostics and reduces the reagents and plastics required for each sample, ultimately reducing the cost and time of each viral test when compared to traditional PCR based methods.

Following testing of over 100 *in vitro* spiked swabs at the lower limit of detection, we determined using our novel direct-to-PCR approach for HCoV-229E detection we can confidently state our limit of detection is 1.2 x 10^2 PFU/mL with 96.30% sensitivity *in vitro*.

Below are the major benefits to this novel approach for virus detection off swabs:

- Process 24 samples in 30 seconds and load directly into PCR plate for detection
- Reduce time and cost of processing by eliminating standard extraction-based protocols while maintaining accuracy in virus detection – ultimately increasing access to vital diagnostic testing
- 96.30% sensitivity in vitro when spiked with sub-clinical levels of HCoV-229E

Future directions:

- Expand testing to look at clinically derived samples
- Test this protocol using additional clinically relevant virus groups
- Apply to alternate sample types such as saliva or blood for virus detection

Sources

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