APPLICATION NOTE

Viral detection off swabs using the Omni Bead Ruptor Elite bead mill homogenizer in a novel two-step, direct-to-PCR technique.

Abstract

Currently, one of the most reliable detection methods for viral pathogens are polymerase chain reaction (PCR) based assays. These assays often involve procedures of swabbing a patient, processing the sample to lyse the virus, purify its nucleotides, and then process the purified genetic material via PCR for detection of a gene product needed to confirm the patient's suspected diagnosis. This process is time-consuming and is dependent on the availability of the reagents and plastics required to complete lysis, extraction, purification, and amplification procedures. Using human coronavirus 229-E (HCoV-229E) as our model system, we have developed a method to detect virus from an *in vitro* spiked swab using only mechanical lysis via the Omni Bead Ruptor Elite bead mill homogenizer and a direct-to-PCR methodology, bypassing the reagent-heavy and time-consuming extraction and purification steps.

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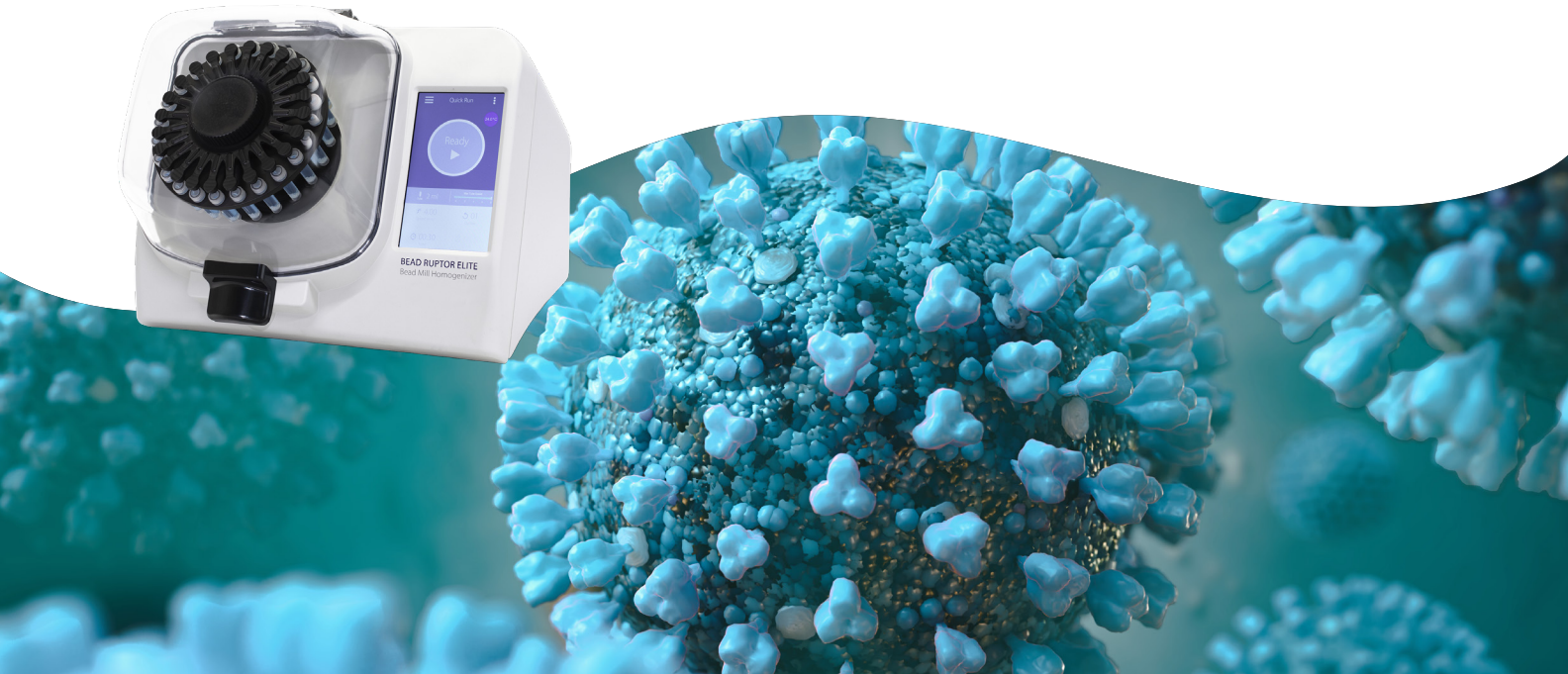
Omni Bead Ruptor Elite bead mill homogenizer



Materials and methods

Materials

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Omni Bead Ruptor Elite 2 mL Tube Carriage (Cat # 19-373)
- 2 mL Reinforced Tubes with Screw Caps (Cat # 19-648)

Methods

Swab viral spike

Sterile cotton swabs (Fisher Scientific, Cat # 22-029-488) were briefly submerged in HCoV-229E viral stock 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 2 mL reinforced screw cap tube (Cat # 19-648) pre-filled with 1 mL of viral transfer buffer. The handle 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 20 °C and then incubated for 1 hr at 20 °C prior to processing.

Omni Bead Ruptor Elite swab processing for viral lysis

The 2 mL screw cap tubes containing the virally-spiked swabs were homogenized on the Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E) for 1 cycle of 30 seconds at 4.2 m/s. This processing generated froth within the tube that was allowed to settle for 60 seconds prior to removal of 1 µL of sample lysate for RT-qPCR.

HCoV-229E RT-qPCR

HCoV-229E nucleocapsid gene (N gene was selected as a target for RT-qPCR from Vabret et al [1]. The N gene was targeted with forward primer 5′-AGGCGCAAGAATTCAGAACCAGAG-3′ and reverse primer 5′-A CAGGACTCTGATTACGAGAAAG-3′ [1]. 1 µL of sample lysate was added to create a final reaction volume of 20 µL using the proportions of primers, RNA, SYBR, RT, and DEPC treated H_2O laid out in the New England Biolabs Luna RT-qPCR Kit (NEB, Cat. No. E3005S). The reaction was processed for 44 cycles, and the resulting amplicons were loaded into a 2 % agarose (Bio-Rad, Cat. No. 1613101) gel for product visualization.

Results

Using our novel two-step method for viral detection, we have shown the methodology to detect viral sample off *in vitro* spiked swabs with a reliable lower limit of detection at 1.2e2 PFU/mL (Figure 1). Figure 1 shows the RT-qPCR results demonstrating a step-down pattern of detection, which follows the serial dilution scheme that the swabs were spiked with; these RT-qPCR results were also confirmed via amplicon visualization on a 2 % agarose gel (Figure 2). This confirms that our method can function as a robust mechanism of viral detection off swabs, while completely bypassing the extraction step used in traditional PCR-based viral detection.

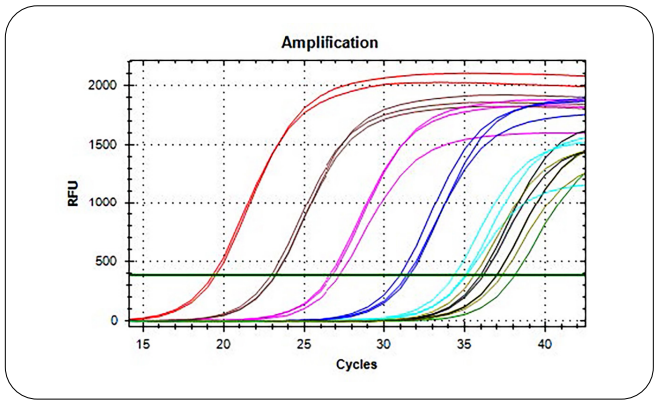


Figure 1: HCoV-229E N-Gene amplification via RT-qPCR following homogenization of virally-spiked swabs. **Red,** 1.2e6 PFU/mL spiked swab. **Brown**, 1.2e5 PFU/mL spiked swab. **Pink**, 1.2e4 PFU/mL spiked swab. **Navy**, 1.2e3 PFU/mL spiked swab. **Teal**, 1.2e2 PFU/mL spiked swab. **Olive**, 1.2e1 PFU/mL spiked swab. **Black**, 1.2e0 PFU/mL spiked swab. **Green**, negative control.

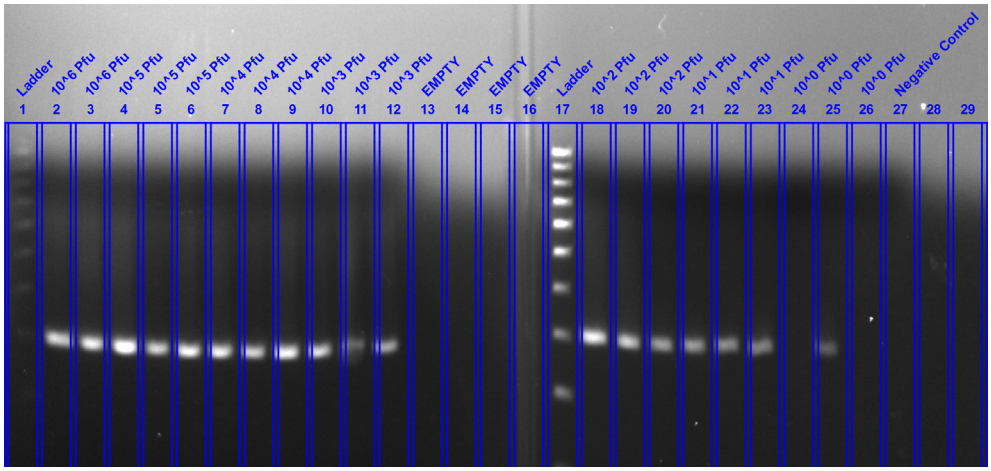


Figure 2: Amplicon visualization of the RT-qPCR results shown in Figure 1 on a 2% agarose gel.

Conclusions

We have successfully proven that use of the Omni Bead Ruptor Elite bead mill homogenizer for mechanical homogenization provides excellent viral lysis off swabs, where the resulting lysate can be used directly in PCR based assays for the detection of virus. The Omni two-step workflow benefits labs looking to reduce manual sample processing time to increase throughput of viral RNA testing. Sample prep time was reduced significantly: up to 384 samples can be prepared for RT-qPCR in less than 40 minutes. No reagents are required for this two-step RNA recovery technique. The two-step processes also meet Green lab standards, as the amounts of plastic consumables required for this technique is greatly reduced compared to conventional nucleic acid purification procedures. This research finding allows for decreased workflow run time and reduces the reagents and plastics required for each sample, ultimately reducing the potential cost and time of each viral test when compared to traditional PCR-based diagnostic methods.

References

1. Vabret A, Mouthon F, Mourez T, Gouarin S, Petitjean J, Francois F. Direct diagnosis of human respiratory coronavirus 229E and OC43 by the polymerase chain reaction. Journal of Virological Methods; 23 May 2001. 97(2001) Pgs. 59-66

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