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Viral Detection off Swabs Using the Bead Ruptor Elite in a Novel Two-Step, Direct-to-PCR Technique

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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 Bead Ruptor Elite and a direct-to-PCR methodology, bypassing the reagent heavy and time-consuming extraction and purification steps.

Materials and Methods

Materials

- Bead Ruptor Elite (Cat. No. 19-040E)
- Bead Ruptor Elite 2 mL Carriage Kit (Cat. No. 19-010-310)
- 2 mL Empty Sample Reinforced Tubes with Caps (Cat. No. 19-648)

Bead Ruptor Elite

Methods

Swab Viral Spike

Sterile cotton swabs (Fisher Scientific, Cat. No. 22-029-488) were briefly submerged in HCoV-229E viral stock solutions ranging from 1.2×10^6 to 1.2×10^0 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 (Omni International, Cat. No. 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.

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Bead Ruptor Elite Swab Processing for Viral Lysis

The 2 mL screw cap tubes containing the virally spiked swabs were homogenized on the Bead Ruptor Elite (Omni International, Cat. No. 19-040E) 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 CAGGACTCTGATTAC-GAGAAAG-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 1.2e2PFU/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.

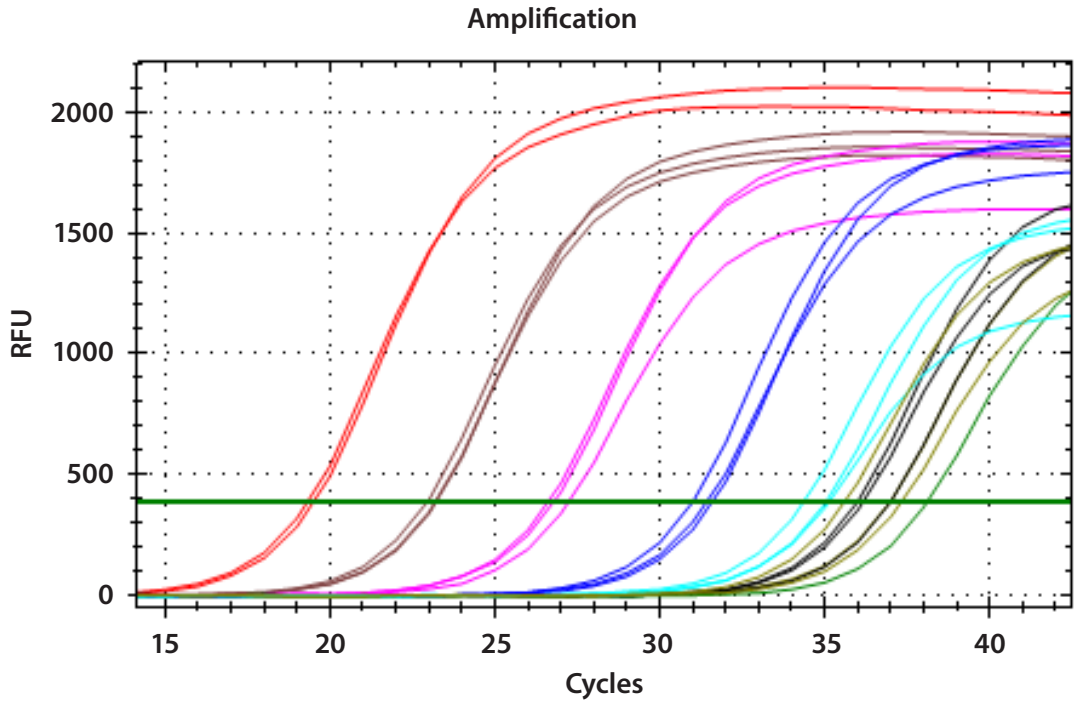

Figure 1. HCoV-229E N-Gene amplification via RT-qPCR following homogenization of virally spiked swabs.
Red, 1.2e6 PFU/mL spiked swab. **Brown**, 1.2e5 PFU/mL spiked swab. **Pink**, 1.2e4 PFU/mL spiked swab. **Navy**, 1.2e3 PFU/mL spiked swab. **Teal**, 1.2e2 PFU/mL spiked swab. **Olive**, 1.2e1 PFU/mL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

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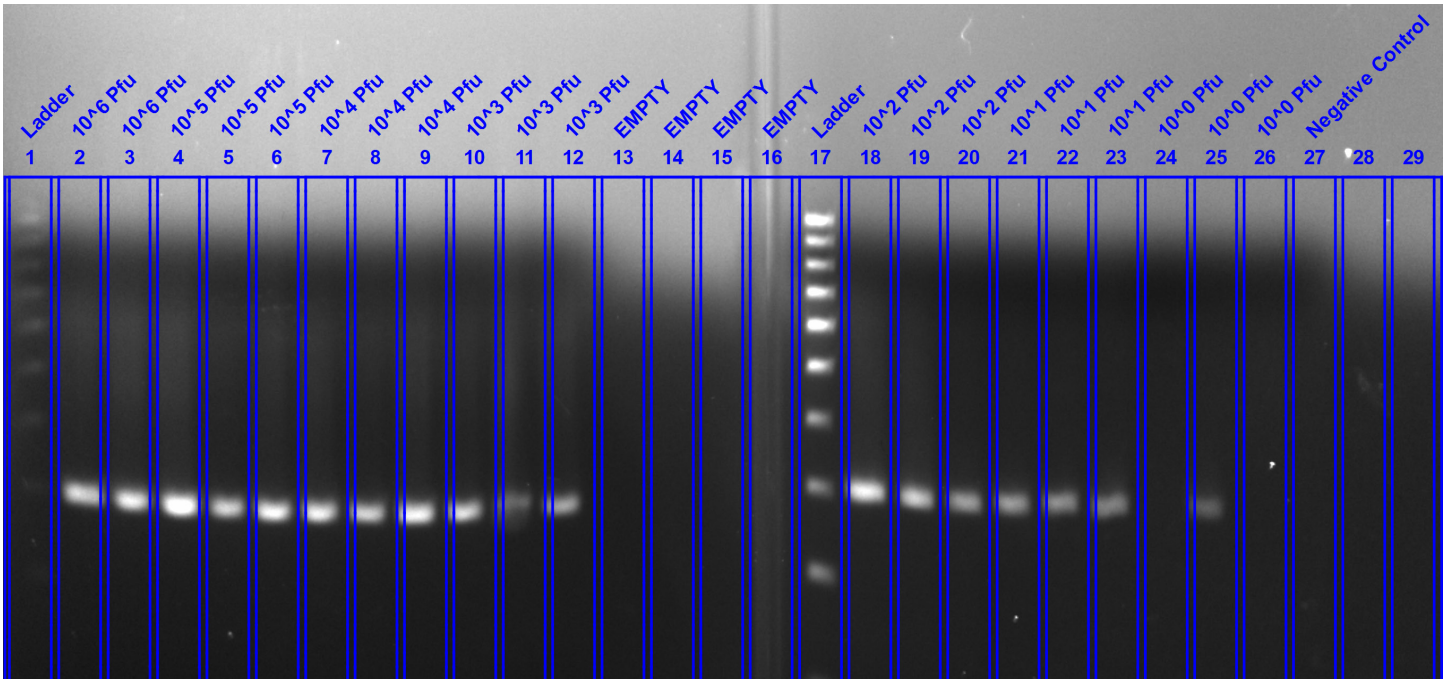

Figure 2. Amplicon visualization of the RT-qPCR results shown in Figure 1 on a 2% agarose gel.

Conclusions

We have successfully proven that use of the Bead Ruptor Elite 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 meets Green lab standards, as the amounts of plastic consumables required for this technique is greatly reduced compared to conventional nucleic acid purification procedures. This finding allows for decreased workflow run time 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 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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Ordering Information

To Extract Fecal RNA in Your Laboratory, Contact Your Local OMNI International Representative for:

- Bead Ruptor Elite (Cat. No. 19-040E)
- Bead Ruptor Elite 2 mL Carriage Kit (Cat. No. 19-010-310)
- 2 mL Reinforced Sample Tubes with Caps (Cat. No. 19-648)

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