APPLICATION NOTE

RNA extraction from human coronavirus: a comparative study highlighting bead mill vs. manual methods

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Summary

Extraction of viral RNA from tissue samples is a critical method used in confirming the presence of a suspected viral infection. With viral infections on the rise globally, researchers and clinicians from all sectors have turned their focus to increasing research on virally induced pathologies.

Additionally, these samples were processed using the Omni Bead Ruptor Elite bead mill homogenizer, reducing the time needed for the initial homogenization steps of RNA kit extractions by half. This semi-automated sample preparation step increased throughput of traditional spin column RNA extraction protocols allowing for processing up to 24 samples in 30 seconds, where traditional methods require up to 24 minutes for the same procedure.

Use of the Omni Bead Ruptor Elite bead mill homogenizer to prepare viral lysates ahead of RNA extraction resulted in increased viral RNA product yield as detected by RT-qPCR, suggesting that low concentration samples may be detectable. Through amplification of the nucleocapsid gene of HCoV-229E from tissue culture supernatant, we demonstrated an increased efficiency and efficacy of bead mill homogenization over vortex homogenization when conducting viral RNA extractions using traditional spin column kits.

Omni Bead Ruptor Elite™ bead mill homogenizer

Materials and methods

Equipment

- Omni Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Omni Bead Ruptor Elite 2 mL Tube Carriage (Cat # 19-373)
- Hard Tissue Homogenizing Mix 2.8 mm Ceramic (2 mL) (Cat # 19-628)
- 2 mL Reinforced Tubes with Screw Caps (Cat # 19-649)

Cell culture and virus growth

Human coronavirus 229E (HCoV-229E) was added at a MOI of 1.4 to 60 % confluent MRC-5 (lung fibroblast) cells, 48 hours after plating. The flask was maintained with DMEM infused with 5 % heat inactivated FBS and 1 % L-Glutamine, incubated at 37 °C with 5 % CO_2. The cell culture supernatant was harvested at 72 hours post infection (HPI) when 70 % CPE was observed.

Viral RNA extraction from supernatant

300 µL of supernatant was added to 300 µL of either an empty 2 mL homogenization tube (Cat # 19-649) or a prefilled 2 mL tube containing 2.8 mm ceramic bead media (Cat # 19-628). Sample tubes were then homogenized by one of two methods: 1, using the Omni Bead Ruptor Elite bead mill homogenizer, 1 x 30s cycle at 4.2 m/s; or 2, vortexing for 60 seconds using a vortex mixer similar to the Vortex Mixer 24 (Cat # 28-101). After the initial homogenization step was completed, the remainder of the RNA extraction was carried out using a commercially available Viral RNA extraction kit per manufacturer's instructions. RNA was eluted from the spin column using DPEC water, allowing an on-column reaction/dissolution time of 5 mins prior to centrifugation.

HCoV-229E RT-qPCR

HCoV-229E nucleocapsid gene (N gene) was selected as a target for RT-qPCR from peer reviewed publications. The N gene was targeted with forward primer 5′-AGGCGCAAGAATTCAGAACCAGAG-3′ and reverse primer 5′-AGCAGGACTCTGATTACGAGAAAG-3′. 1 µL of extracted RNA was added, for a total reaction volume of 20 µL using the proportions of primers, RNA, SYBER, RT, and DPEC laid out in the New England Biologics Luna RT-qPCR Kit. The reaction was run for 44 cycles and the resulting amplicons were loaded into a 2 % agarose gel for product visualization.

Results

RT-qPCR was completed on the supernatant of MRC-5 tissue culture flasks, 72 HPI once 70 % CPE was observed. The efficacy of the 4 homogenization methods for processing these samples was evaluated using Cq values (Figure1) and confirmed with a 2 % agarose gel (Figure 2). The data demonstrates successful extraction of viral RNA from cell culture supernatant. Homogenization using the Omni Bead Ruptor Elite bead mill homogenizer increased extracted RNA yield in comparison to traditional vortex homogenization, as demonstrated by lower Cq values in samples processed using bead milling homogenization versus vortexing. Increased band intensity during amplicon visualization is seen from homogenization using the Omni Bead Ruptor Elite bead mill homogenizer, both with and without 2.8 mm ceramic bead media, in comparison to the bands representing vortex homogenization, both with and without 2.8 mm ceramic bead media. These results were confirmed via 2 % agarose visualization of amplicons as shown in Figure 2 and with quantified Cq values. In negative control replicates, N gene amplification on non-infected MRC-5 culture supernatant, the late rise was attributed to primer dimer formation in these samples.

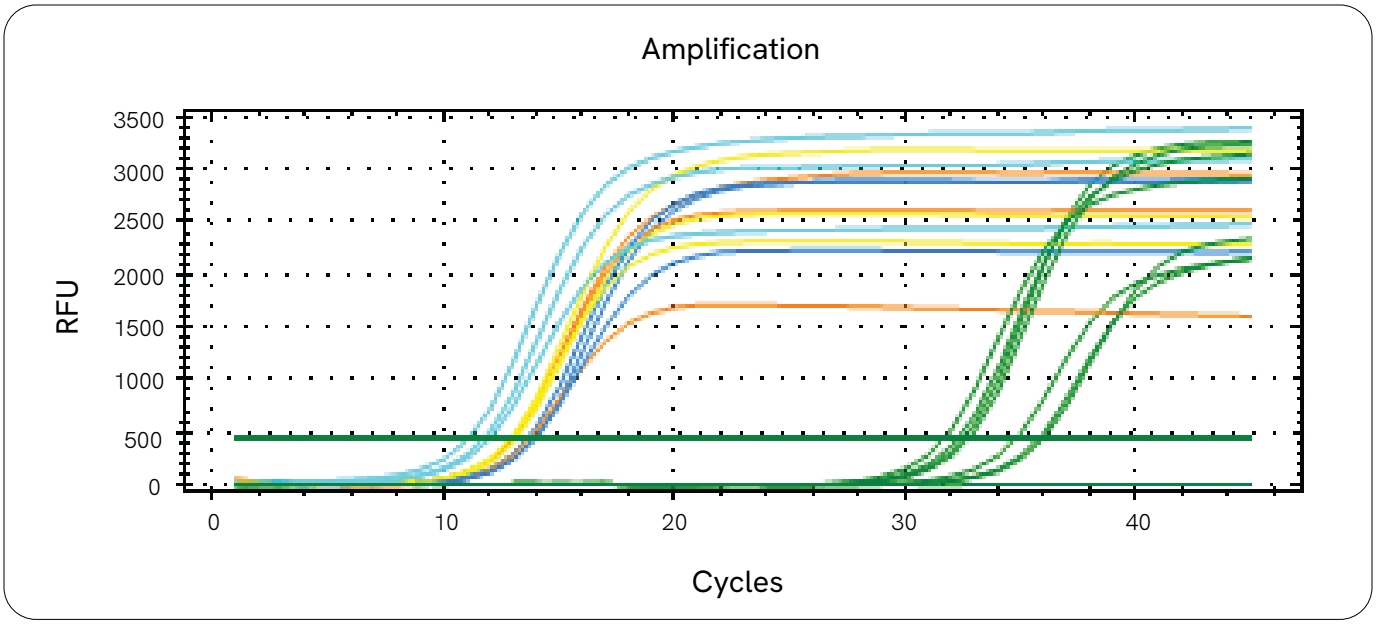

Figure 1: RT-qPCR Cq values for HCoV-229E N gene amplification visualization
Light blue: Omni Bead Ruptor Elite homogenization in an empty 2 mL reinforced tube (Cat # 19-649)
Orange: Vortex homogenization using an empty 2 mL reinforced tube (Cat # 19-649)
Dark blue: Omni Bead Ruptor Elite homogenization in pre-filled Hard Tissue Homogenizing Mix Tube (Cat # 19-628)
Yellow: Vortex homogenization using pre-filled Hard Tissue Homogenizing Mix Tube (Cat # 19-628)
Green: Negative control replicates, N gene amplification on non-infected MRC-5 culture supernatant.

Sample	Cq	Cq mean	Cq standard deviation
Bead Ruptor Elite -19-649 - Replicate 1	11.08	11.61	0.48
Bead Ruptor Elite -19-649 - Replicate 2	11.72		
Bead Ruptor Elite -19-649 - Replicate 3	12.03		
Bead Ruptor Elite -19-628 - Replicate 1	13.61	13.86	0.22
Bead Ruptor Elite -19-628 - Replicate 2	13.95		
Bead Ruptor Elite -19-628 - Replicate 3	14.02		
Vortex Mixer 24 -19-649 - Replicate 1	13.03	13.27	0.40
Vortex Mixer 24 -19-649 - Replicate 2	13.05		
Vortex Mixer 24 -19-649 - Replicate 3	13.73		
Vortex Mixer 24 -19-628 - Replicate 1	12.92	13.02	0.13
Vortex Mixer 24 -19-628 - Replicate 2	13.16		
Vortex Mixer 24 -19-628 - Replicate 3	12.97		

Figure 1B: RT-qPCR results for HCoV-229E nucleocapsid gene showing the quantified Cq results for each of the homogenization parameters tested in triplicate.

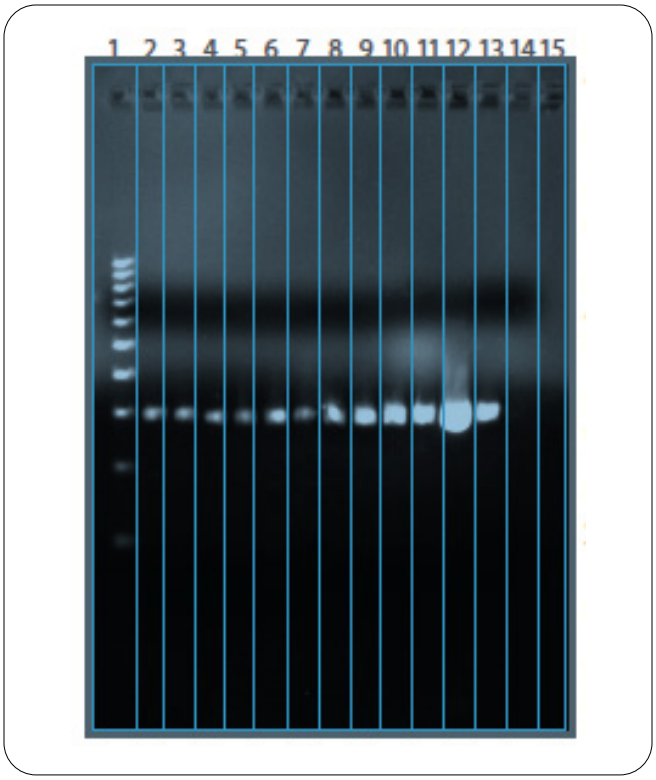

Figure 2: HCoV-229E N gene amplicon expression after RT-qPCR on a 2 % agarose gel

Lane 1: Bio-Rad 100bp DNA ladder

Lanes 2, 3, 4: MRC-5 cells at 70 % CPE supernatant viral RNA extraction using vortex homogenization with empty 2mL tubes

Lanes 5, 6, 7: MRC-5 cells at 70 % CPE supernatant viral RNA extraction using vortex homogenization with pre-filled Hard Tissue Homogenizing Mix

Lanes 8, 9, 10: MRC-5 cells at 70 % CPE supernatant viral RNA extraction using Omni Bead Ruptor Elite agitation with empty 2 mL tubes

Lanes 11, 12, 13: MRC-5 cells at 70 % CPE supernatant viral RNA extraction using Omni Bead Ruptor Elite homogenization with pre-filled Hard Tissue Homogenizing Mix

Lane 14: Empty

Lane 15: Uninfected MRC-5 culture supernatant RNA extraction using Omni Bead Ruptor Elite homogenization with pre-filled Hard Tissue Homogenizing Mix

Conclusions

Notably, when comparing the two homogenization methods for this extraction, homogenization using the Omni Bead Ruptor Elite bead mill homogenizer extracted more RNA than vortex homogenization of samples, as shown in the gel visualization of the amplicons of the HCoV-229E nucleocapsid gene. In addition to increasing the overall yield of extracted RNA, it is pertinent to note that the Omni Bead Ruptor Elite bead mill homogenizer also increased the throughput of extractions and decreased time required to complete them. The device can process 24 samples in a single run, more than traditional vortexing, while also reducing the time of the homogenization step in half from 60 seconds per sample (and up to 24 minutes per 24 sample batch) to only 30 seconds. The Omni Bead Ruptor Elite bead mill homogenizer is recommended as a medium throughput, semi-automated workflow for RNA extraction from virally-infected supernatant samples.

For research use only. Not for use in diagnostic procedures.

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