APPLICATION NOTE

Extraction of human coronavirus 229E from feces for RT-qPCR of the nucleocapsid gene using the Omni Bead Ruptor Elite bead mill homogenizer.

Summary

Human coronavirus 229E (HCoV-229E) is an alpha-coronavirus transmitted primarily via respiratory droplets and fomites [1]. However, this virus - as well as many other coronaviruses - has been detected in fecal samples of asymptomatic patients, indicating a potential for fecal-oral transmission after symptomatic infection subsides [2]. Additionally, some coronaviruses appear in higher viral titers in feces than they do in respiratory samples, which could prove to be a great source of viral analytes for extraction. As an enveloped, positive sense, single stranded RNA virus primarily infecting humans and bats, this virus is a strong model system for studying the novel SARS-CoV-2 in a BSL-2 setting [1]. Herein, we have used HCoV-229E to model RNA extraction procedures from fecal samples that have been spiked to clinically-relevant concentrations [3], demonstrating how the Omni Bead Ruport Elite bead mill homogenizer can be implemented into an extraction and RT-qPCR workflow for research studies investigating coronavirus infections.

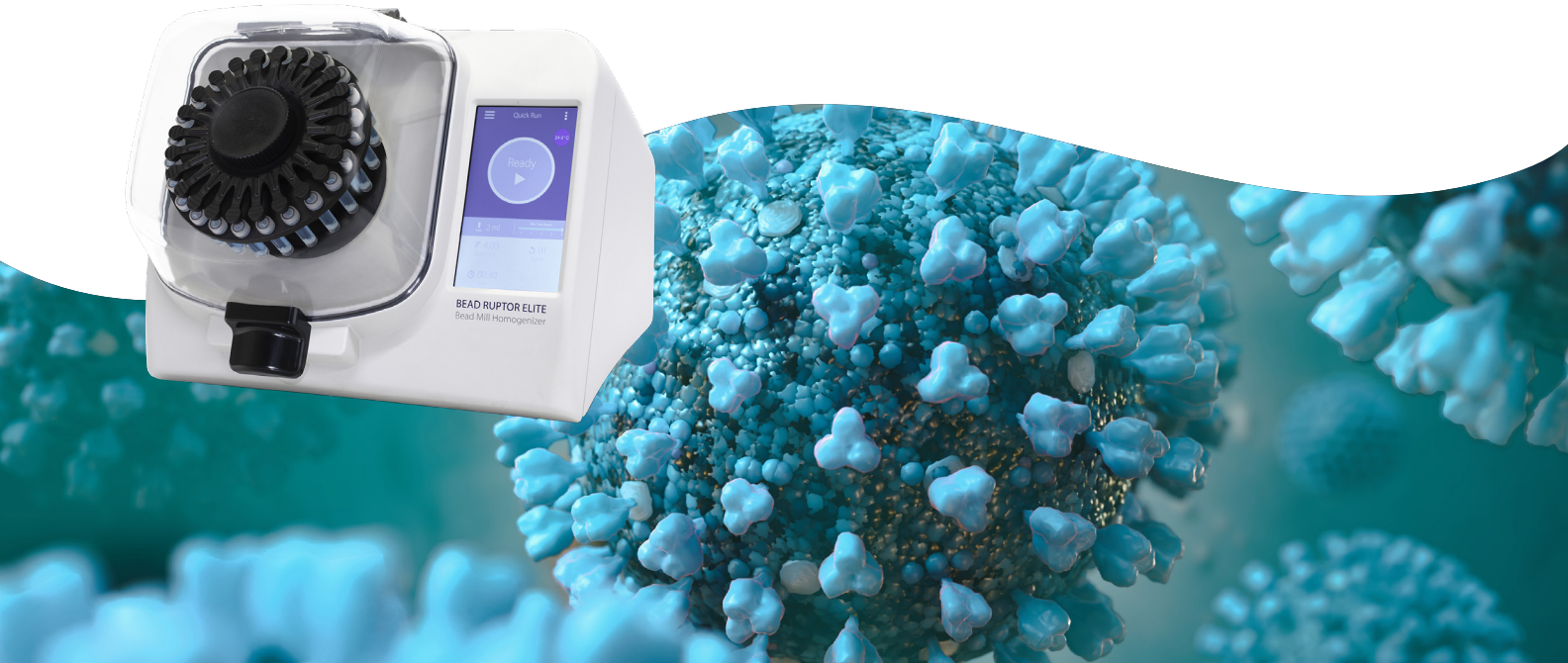
Authors

Caleb Proctor
Rodney J Nash, Ph.D.

Revvity, Inc.

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

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)
- 2 mL Tubes with Screw Caps (Cat # 19-647)

Methods

Cell culture and virus growth

Human coronavirus 229E (HCoV-229E) (ATCC, Cat. No. VR-740) was added at a multiplicity of infection (MOI) of 0.5 to a 85 % confluent T75 flask of MRC-5 cells, 48 hours after plating. The flask was maintained with DMEM (Fisher Scientific, Cat # 11-965-118) supplemented with 5 % heat inactivated fetal bovine serum (Gemini Bioproducts, Cat # 100-500), incubated at 37 °C with 5 % CO_2 [4]. The cell culture supernatant was harvested at 72 hours post infection (HPI) when 80 % cytopathic effect (CPE) was observed.

Virus Quantification

HCoV-229E was quantified with standard plaque assay protocols. HEK-293 cells were seeded at 2.0 x 10^5 cells per well in 6-well tissue culture treated plates (Costar, Cat # 3516) with 2 mL of DMEM (Fisher Scientific, Cat # 11-965-118) infused with 5 % heat inactivated fetal bovine serum (Gemini Bioproducts, Cat # 100-500). Once the cells achieved 85 % confluence, 200 µL of HCoV-229E stock was added to the media of the first well. The media was gently mixed and 200 µL of the infected media was transferred into the adjacent well. This was repeated to create stepwise dilutions throughout the plate. After 24 hours of incubation with the virally-infected media, the media was removed from each well and replaced with 2 mL of DMEM infused with 5 % heat inactivated FBS and 2 % agarose (Bio-Rad, Cat # 1613101). The plate was incubated for an additional 4 days at 35 °C with 5 % CO_2 and plaques were counted to determine viral concentration in plaque forming units/mL (PFU/mL).

Fecal sample virus spike

25 mg of fresh fecal sample from *Canis lupus familiaris* was placed into an empty 2 mL screw cap tube (Cat # 19-647) and spiked with 200 µL of HCoV-229E viral stock in different concentrations, mimicking the range of reported viral load as PFU's for SARS-CoV-2 in fecal samples [3]. The samples were spiked using 2.0 x 10^6, 2.0 x 10^5, or 2.0 x 10^4 PFU/mL of virus stock, prepared at 20 °C and allowed to incubate for 1 hour at 20 °C prior to processing for extractions.

Fecal sample viral extraction

250 mg of spiked fecal sample was used in each extraction following a commercially available fecal RNA purification kit per manufacturer's instructions. After combining samples and lysis buffer in the 2 mL tubes, the samples were homogenized at 4.2 m/s for 30 seconds on the Omni Bead Ruptor Elite (Cat # 19-042E), and then centrifuged at 10,000 rpm for 2 minutes. Finally, elution of the RNA from the column was completed using 50 µL of elution buffer. The buffer was placed on the column at 20 °C and allowed to sit for 5 minutes prior to centrifugation at 10,000 rpm for 2 minutes. The eluted sample was then used for downstream processing.

HCoV-229E RT-qPCR

HCoV-229E nucleocapsid gene (N gene) was selected as a target for RT-PCR from Vabret et al. [5]. The N gene was targeted with forward primer 5′-AGGCGCAAGAATTCAGAACCAGAG-3′ and reverse primer 5′-AGCAGGACTCTGATTACGAGAAAG-3′ [5] 1 µL of extracted RNA was added to create a final reaction volume of 20 µL using the proportions of primers, RNA, SYBR Green, RT, and DEPC laid out in the New England Biologics Luna RT-qPCR Kit (Cat # E3005S). The reaction was run for 44 cycles using the temperature and cycle settings outlined by the manufacturer [6].

Table 1. Sample homogenization summary

Sample	Lysis buffer volume (µL)	Speed (m/s)	Time (sec)	Cycles	Dwell time (sec)
250 mg Fecal material	475	4.2	30	1	0

Results

Using the Omni Bead Ruptor Elite bead mill homogenizer, we were able to successfully extract viral RNA from spiked fecal samples across the spectrum of reported clinically-relevant viral loads associated with coronavirus infections [3].

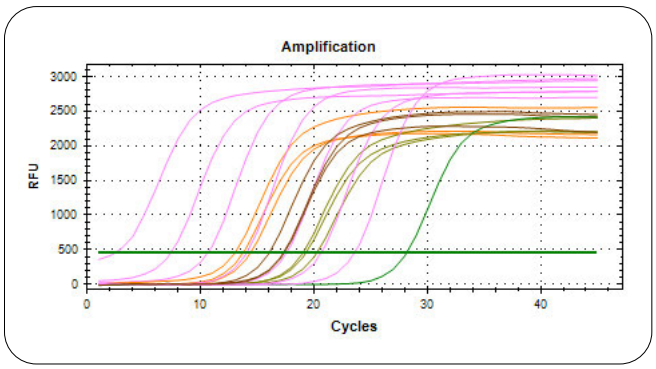


Figure 1. The results of RT-qPCR for HCoV-229E nucleocapsid gene (N gene) following RNA extraction from spiked fecal samples at varying concentrations visualized with the standard curve.

Pink: The standard curve of N gene starting with 1 ng of product at the furthest left line, moving to the right with logarithmic serial dilutions to a final concentration of 1 fg at the furthest right line. The standard curve was produced with an R2 value of 0.995.

1. **Orange:** Fecal sample extractions after being spiked with 2.0 x 10^6 PFU/mL of virus.
2. **Brown:** Fecal sample extractions after being spiked with 2.0 x 10^5 PFU/mL of virus.
3. **Olive Green:** Fecal sample extractions after being spiked with 2.0 x 10^4 PFU/mL of virus.
4. **Bright Green:** Negative controls for fecal samples run through the extraction process without any viral spike.

Table 2. Quantification of RT-qPCR results for N gene amplification from HCoV-229E spiked fecal samples. Results coordinate with the amplification visualization displayed in Figure 1.

1. Fecal sample extractions after being spiked with 2.0 x 10^6 PFU/mL of virus.
2. Fecal sample extractions after being spiked with 2.0 x 10^5 PFU/mL of virus.
3. Fecal sample extractions after being spiked with 2.0 x 10^4 PFU/mL of virus.
4. Negative controls for fecal samples run through the extraction process without any viral spike.

Sample name		Cq value	Cq mean
1	Fecal spike 10^4 – Sample 1	20.25	19.52
	Fecal spike 10^4 – Sample 2	19.28	
	Fecal spike 10^4 – Sample 3	19.04	
2	Fecal spike 10^5 – Sample 1	17.29	16.91
	Fecal spike 10^5 – Sample 2	16.07	
	Fecal spike 10^5 – Sample 3	17.36	
3	Fecal spike 10^6 – Sample 1	14.39	13.72
	Fecal spike 10^6 – Sample 2	13.74	
	Fecal spike 10^6 – Sample 3	13.05	
4	Negative control	28.18	

Conclusions

This study shows that the Omni Bead Ruptor Elite bead mill homogenizer can be used to extract viral RNA from fecal samples. As depicted herein, we were able to eliminate many of the incubation steps typically found in fecal nucleic acid sample preparation processes and replace them with a single mechanical homogenization step which requires only 30 seconds for simultaneous processing of up to 24 samples. This demonstrates a significant time-savings and increased throughput of samples. The data shows good RNA yield, acceptable for RT-qPCR across the range of clinically-relevant reported coronavirus fecal viral loads [3]. The Omni Bead Ruptor Elite bead mill homogenizer can be successfully implemented into any coronavirus fecal workflows to improve sample turnaround times while producing high-quality, purified RNA for downstream applications.

References

1. Lim YX, Ng YL, Tam JP, Liu DX. Human Coronaviruses: A Review of Virus-Host Interactions. Diseases. 2016;4(3):26. Published 2016 Jul 25. doi:10.3390/diseases4030026

2. Jevšnik M, Steyer A, Zrim T, et al. Detection of human coronaviruses in simultaneously collected stool samples and nasopharyngeal swabs from hospitalized children with acute gastroenteritis. Virol J. 2013;10:46. Published 2013 Feb 5. doi:10.1186/1743-422X-10-46

3. Pan Y, Zhang D, Yang P, Poon LL, Wang Q. Viral load of SARS-CoV-2 in clinical samples. The Lancet Infectious Diseases. February 24 2020; 20(4):411-412. Doi: 10.1016/S1473-3099(20)30113-4

4. Raabe T, Schelle-Prinz B, Siddell SG. Nucleotide Sequence of the Gene Encoding the Spike Glycoprotein of Human Coronavirus HCV 229E. Journal of General Virology, January 1990. Vol 71, Pg 1065-1073.

5. Vabret A, Mouthon F, Mourez T, Gouarin S, Petitjean J, Freymuth F. Direct Diagnosis of Human Respiratory Coronavirus 229E and OC43 by the Polymerase Chain Reaction. Journal of Virologic Methods, September 2001. Vol 97(1), Pg 59-66. Doi: 10.1016/S0166-0934(01)00343-3.

6. Giadone R. CReM In-House Laboratory Derived Test (LDT) SARS-CoV-2 (COVID-19) qRT-PCR Protocol. Boston University Center for Regenerative Medicine, 16 March 2020. http://www.bu.edu/dbin/stemcells/files/CReM%20In-House%20 Laboratory%20Derived%20Test%20(LDT)%20SARS-CoV-2%20(COVID-19)%20qRT-PCR%20Protocol.pdf

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com

For a complete listing of our global offices, visit www.revvity.com

OMNI International
935 Cobb Place Blvd NW
Kennesaw, GA 30144
(800) 776-4431
www.omni-inc.com