

Homogenization of nanopore membrane filters utilized in wastewater infectious disease surveillance.

Summary

Sparked by the COVID-19 global pandemic, there has been a renaissance of infectious disease surveillance techniques looking at tracking and categorizing disease as it spreads through a population. The utility of wastewater sampling for infectious particles gained popularity from local college campuses to country wide surveillance programs to help identify the presence of viral variants and predict oncoming surges in cases. While there are multiple methodologies proposed for these wastewater sampling techniques, many of them rely on filtration systems to capture the infectious particles on electronegative membranes or tissue culture grade nanopore filters. Once the particles have been captured on these membranes, the critical step in the process is to release as many of the particles as possible during processing to allow to accurate downstream detection. Herein, we demonstrate the utility of mechanical homogenization via bead beating technology for the complete disruption of these filters for downstream molecular analysis.

Bead Ruptor Elite™ bead mill homogenizer



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

Through complete substrate dissociation, bead beating homogenization allows the end user to maximize the number of particles released from any filter, whilst speeding up processing time and improving laboratory safety via infectious particle lysis. Bead beating technology has been shown to lyse greater than 96% of infectious viral particles in a sample, without disrupting downstream molecular analysis⁽²⁾.

The Bead Ruptor Elite bead mill homogenizer was shown to achieve full homogenization of a tissue culture grade electronegatively charged membrane filter after 2 minutes of processing, cycling at 6 m/s for 4, 30 second cycles.

Materials and methods

Equipment

- Bead Ruptor Elite bead mill homogenizer (Cat # 19-042E)
- Bead Ruptor Elite bead mill homogenizer 2 mL Tube Carriage (Cat # 19-373)
- Bead Ruptor Elite bead mill homogenizer finger plate (Cat # 19-370)
- Hard Tissue Homogenizing Mix 2.8 mm Ceramic Beads (Cat # 19-628)

Methods

The membrane filters (Pall, Cat # 66258) used were folded in half four times to fit into the 2 mL tube pre-filled with 2.8 mm ceramic beads (Omni International, Cat # 19-628). The tubes were filled with 1 mL of lysis buffer (Revvity, Cat # CMG-1033-S) and were then loaded into the Bead Ruptor Elite bead mill homogenizer and processed at 6 m/s for 4 cycles of 30 seconds, a total of 2 minutes of processing. Following homogenization, the complete homogenate was transferred to the appropriate media for downstream analysis.

References

1. Ahmed W, Angel N, Edson J, Bibby K, Bivins A, O'Brien JW, Choi PM, Kitajima M, Simpson SL, Li J, Tschärke B, Verhagen R, Smith WJM, Zaugg J, Dierens L, Hugenholtz P, Thomas KV, Mueller JF. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. *Sci Total Environ.* 2020 Aug 1;728:138764. doi: 10.1016/j.scitotenv.2020.138764. Epub 2020 Apr 18. PMID: 32387778; PMCID: PMC7165106.
2. Morehouse, Zachary P et al. "A novel two-step, direct-to-PCR method for virus detection off swabs using human coronavirus 229E." *Virology journal* vol. 17,1 129. 25 Aug. 2020, doi:10.1186/s12985-020-01405-y

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