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Detection of SARS-CoV-2 from Wastewater Samples Utilizing the Bead Ruptor Elite™ Bead Mill Homogenizer and chemagic™ Prime™ instrument

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Bead Ruptor Elite™ Bead Mill Homogenizer

Summary

The global pandemic caused by SARS-CoV-2 has highlighted the need for improved public health surveillance measures to track and predict the outbreak of disease in a community. While many innovative measures have been proposed in recent years to accomplish this task, one method gaining increasing traction for urban surveillance of SARS-CoV-2 transmission is wastewater testing for genetic evidence of the virus (Sherchan, 2020). SARS-CoV-2 is known to cause COVID-19 disease which is proven to be spread through both aerosolized respiratory secretions, as well as fecal shedding (Schmitz, 2021). Through regular sampling of a community's wastewater, public health officials are able to detect early signs of viral shedding with increased prevalence of SARS-CoV-2 RNA. These methods are proven to precede a spike in nasopharyngeal swabbing test positivity rates, as well as an increase in hospitalizations in a community for COVID-19 (McMahan, 2021). Utilizing wastewater testing allows for public health officials to have an insight into upcoming increases in disease burden to their community.

Currently, most wastewater surveillance programs are accomplished through regimented examination of filters put in sewage lines, or water samples acquired from wastewater treatment plants which are then prepared for nucleic acid extraction and RT-PCR amplification to detect the presence of known SARS-CoV-2 genetic targets. Standard methods for preparation of filters involve long processing times and incubations on ice which can be a burden to high-throughput workflows. Herein, we examine a streamlined methodology for filter homogenization utilizing the OMNI Bead Ruptor Elite™ Bead Mill Homogenizer followed by automated RNA extraction on the chemagic™ Prime™ instrument in preparation for RT-PCR detection of SARS-CoV-2 spiked wastewater samples. This high throughput methodology provides a robust and reproducible workflow for detection of SARS-CoV-2 RNA in wastewater samples.

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Materials and Methods

Equipment

- Bead Ruptor Elite™ Bead Mill Homogenizer (Cat # 19-042E)
- Bead Ruptor Elite™ Bead Mill Homogenizer 48 Position 2 mL Tube Carriage (Cat # 19-378)
- Bead Ruptor Elite™ Bead Mill Homogenizer 48 Position 2 mL Fingerplate (Cat # 19-370-248)
- 2 mL Hard Tissue Homogenizing Mix 2.8 mm Ceramic Beads (Cat # 19-628)

Procedure

The City of Houston (Houston, TX USA) Health Department provided the known SARS-CoV-2 positive wastewater samples for utilization in this workflow validation study. Herein, nitrocellulose filters with known SARS-CoV-2 exposure were taken from city wastewater plants from December 2021 to February 2022 to evaluate for the detection of SARS-CoV-2 nucleocapsid genetic segments via RT-PCR.

Filter Processing on the Bead Ruptor Elite™ Bead Mill Homogenizer

Intact nitrocellulose filters with known SARS-CoV-2 positive wastewater exposure were placed into respective 2 mL Hard Tissue Homogenization Mix Tubes (OMNI, Cat # 19-628) along with 600 µL of Lysis Buffer included with the chemagic Viral DNA/RNA 300 Kit (PerkinElmer, Cat # CMG-1433). Samples were then placed onto the Bead Ruptor Elite™ Bead Mill Homogenizer (OMNI, Cat # 19-042E) utilizing the 48 Position 2 mL Tube Carriage (OMNI, Cat # 19-378), and homogenized at 6.0 m/s for 60 sec. The samples were then centrifuged at 12,500 rpm for 4 min and transferred to the chemagic™ Prime™ instrument (PerkinElmer, Cat # CMG-404) for automated RNA extraction.

Automated RNA Extraction

Precisely 300 µL of homogenate was loaded into a 96 well plate (PerkinElmer, Cat # CMG-555-15) and processed on the chemagic™ Prime™ instrument (PerkinElmer, Cat # CMG-404) utilizing the chemagic Viral DNA/RNA 300 Kit (PerkinElmer, Cat # CMG-1433) per the manufacturer's instructions.

Viral Detection and Quantification

Five microliters of eluted RNA was loaded into a premixed RT-PCR reaction for the detection of SARS-CoV-2 RNA. The OPTI SARS-CoV-2 RT-PCR Test (IDEXX, Cat # 99-57004) was utilized for the RT-PCR detection and quantification of SARS-CoV-2 RNA in these samples. RT-PCR was conducted per the manufacturer's instructions without deviation.

Results

Utilizing the proposed method for wastewater detection of SARS-CoV-2 from filters, we were able to successfully amplify SARS-CoV-2 RNA in 100 % of the known positive samples, with the lowest detected concentration of virus being 6.4 viral copies/µL (Table 1). RT-PCR data demonstrates the reproducibility of our proposed workflow in SARS-CoV-2 detection from wastewater filters at various viral concentrations. When utilizing this methodology, it is worth noting that the average Ct observed, across all concentrations, was 30.30 with a standard deviation of 1.23, maintaining well below the United States Centers for Disease Control (US CDC) and World Health Organization (WHO) recommended cutoff Ct of 40 utilized in most SARS-CoV-2 detection assays.

Sample	Detection Date	Replicate	Ct	Average Ct	Viral Copies/µL
1	12/14/2021	1	29.66	29.46	80.44
	12/14/2021	2	29.25		105.21
2	12/14/2021	1	30.22	30.33	55.56
	12/14/2021	2	30.44		48.13
3	12/14/2021	1	29.29	29.20	103.90
	12/14/2021	2	29.12		115.02

4	12/14/2021	1	29.16	29.16	111.81
	12/14/2021	2	29.17		110.62
5	12/14/2021	1	31.96	31.63	17.63
	12/14/2021	2	31.29		27.39
6	12/14/2021	1	29.82	29.96	72.24
	12/14/2021	2	30.11		59.86
7	12/14/2021	1	29.70	29.60	77.92
	12/14/2021	2	29.50		89.21
8	12/14/2021	1	30.91	30.76	35.06
	12/14/2021	2	30.61		42.73
9	12/14/2021	1	29.83	29.71	71.61
	12/14/2021	2	29.60		84.30
10	12/14/2021	1	31.09	31.05	31.19
	12/14/2021	2	31.00		32.88
11	1/13/2022	1	29.03	29.07	108.00
	1/13/2022	2	29.11		102.18
12	1/13/2022	1	30.59	29.98	35.84
	1/13/2022	2	29.36		85.40
13	1/13/2022	1	29.58	29.23	72.97
	1/13/2022	2	28.88		121.61
14	1/13/2022	1	30.17	29.74	56.31
	1/13/2022	2	29.31		98.75
15	1/13/2022	1	29.85	29.60	60.41
	1/13/2022	2	29.35		86.33
16	1/27/2022	1	32.87	33.17	9.95
	1/27/2022	2	33.47		6.41
17	1/27/2022	1	29.49	29.79	93.73
	1/27/2022	2	30.09		62.54
18	1/27/2022	1	29.11	28.99	120.91
	1/27/2022	2	28.87		142.31
19	1/27/2022	1	29.07	28.97	123.92
	1/27/2022	2	28.86		143.12
20	2/11/2022	1	32.05	31.47	21.96
	2/12/2022	2	30.89		46.50
21	2/13/2022	1	31.60	31.77	29.31
	2/14/2022	2	31.94		23.46
22	2/15/2022	1	33.09	33.08	11.30
	2/16/2022	2	33.06		11.47
23	2/17/2022	1	30.74	30.92	51.53
	2/18/2022	2	31.11		40.36
24	2/19/2022	1	30.51	30.52	60.61
	2/20/2022	2	30.53		58.89

Table 1. Ct, Average Ct, and Viral Copies/µL obtained from SARS-CoV-2 identification from each wastewater filter processed following the proposed detection workflow.

Conclusions

In this application, we have demonstrated the robustness of a high throughput workflow utilizing the OMNI Bead Ruptor Elite™ Bead Mill Homogenizer and chemagic™ Prime™ instrument in SARS-CoV-2 detection from wastewater filters provided by the City of Houston Health Department. In this real-world application of our workflow, it was shown that homogenization and extraction processes allowed for consistent RNA yields which translated to accurate detection well below the standardized Ct detection cut off. Employing high throughput workflows, such as this, for SARS-CoV-2 detection will allow public health officials another critical tool in the tracking and preparedness of communities to combat the COVID-19 pandemic, as well as a road map for methods of detection for other viral diseases.

References

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