Summer 8-12-2021

Comparison of Seven Extraction Kits for Detection of SARS-CoV-2 in Wastewater

Nicholas Sze
Ohio University

Catherine Pratt
University of Nebraska Medical Center

Bailey White
University of Nebraska Medical Center

Dylan George
University of Nebraska Medical Center

Michael Wiley
University of Nebraska Medical Center

Comparison of Seven Extraction Kits for Detection of SARS-CoV-2 in Wastewater

Nicholas Sze[1], Catherine Pratt[2], Bailey White[2], Dylan George[2,3], Mike Wiley[2]

[1]Ohio University, Athens, OH

[2]Department of Environmental, Agricultural, and Occupational Health, University of Nebraska Medical Center, Omaha, NE

[3]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE

Background

Wastewater-based epidemiology (WBE) has been used to trace outbreaks and monitor populations for pathogenic viruses such as poliovirus. SARS-CoV-2 has been identified as a candidate for WBE as the virus can survive for prolonged periods in wastewater. WBE can be used to predict clinical cases in populations and will be important as the COVID-19 pandemic progresses. Previously, methods such as ultra centrifugation, polyethylene glycol separation, and electrostatically charged membrane filtration have been used to concentrate the virus in wastewater. This study will investigate the performance of seven commercially available extraction kits on water of varying SARS-CoV-2 levels using less resource-intensive methods. In addition, solid and liquid components of wastewater will be compared for effectiveness in isolating SARS-CoV-2.

Materials and Methods

SARS-CoV-2-positive wastewater samples from Mississippi University wastewater treatment plant influent were categorized by cycle threshold (Ct) into high (Ct 25-30), medium (Ct 30-35), and low (Ct 35-40) groups. Seventeen samples were selected based on volume and pooled into high (n=7), medium (n=6), and low (n=4) bottles, mixed, and aliquoted. Three sample types were generated from the wastewater: soil (by centrifuging the wastewater and removing supernatant, assuming 1g of soil per 50 ml wastewater), liquid (the original wastewater) and supernatant (liquid removed after centrifuging the wastewater). Aliquoted samples underwent one freeze-thaw cycle to minimize viral degradation. Seven nucleic acid extraction kits were used to process the samples according to manufacturer protocol and RNA was stored at -80˚ C. Each sample was extracted in duplicate and ran in triplicate during qPCR. CT results were analyzed using pairwise comparisons and one-way ANOVA at 95% confidence.

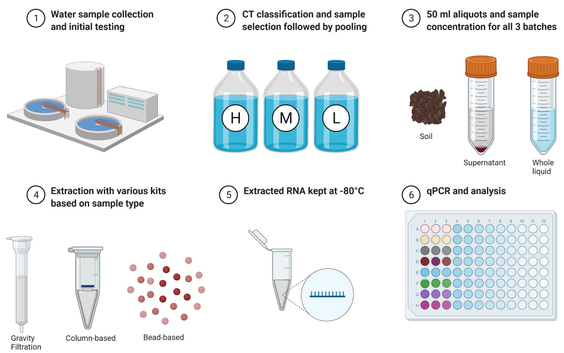

Fig 1. General overview of the strategy to group and process wastewater samples. Created with BioRender.com

Results

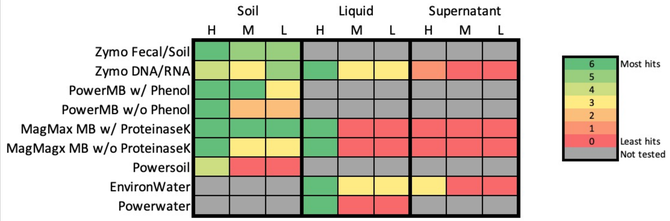

Fig 2 . Heatmap displaying kit consistency. The color scale represents the number of SARS-CoV-2-positive replicates (CT<40) for each kit: consistently positive (green), negative (red), not done (grey). MagMax Microbiome with proteinase K had positive results on all 6 replicates for all solids. The letters represent the viral concentrations: H (high), M (medium), L (low).

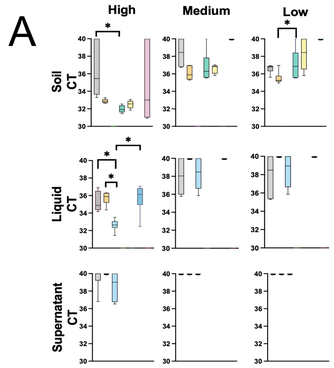

Fig 3A. Box and whisker plot visualizing the six cycle threshold replicates from each condition. The asterisk indicates significance at $p<.05$. Dashes represent kits which had a CT>40 for all 6 replicates. If no dash or bar is present the kit was not tested for the condition. Solids have significantly lower CT values than both liquids and supernatants, and liquids have significantly lower CT values than supernatants. MagMax without proteinase K and Qiagen Microbiome without phenol are not included as it was determined that proteinase K and phenol resulted in significantly lower CT values.

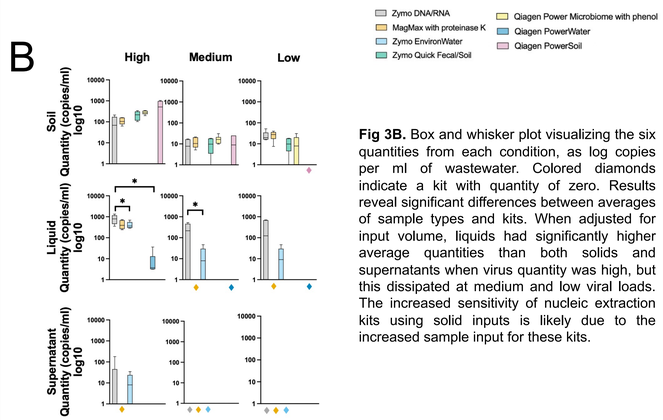

Fig 3B. Box and whisker plot visualizing the six quantities from each condition, as log copies per ml of wastewater. Colored diamonds indicate a kit with quantity of zero. Results reveal significant differences between averages of sample types and kits. When adjusted for input volume, liquids had significantly higher average quantities than both solids and supernatants when virus quantity was high, but this dissipated at medium and low viral loads. The increased sensitivity of nucleic extraction kits using solid inputs is likely due to the increased sample input for these kits.

Results

Kit	Soil (ml)	Liquid (ml)	Supernatant (ml)
ZymoBIOMICS™ DNA Miniprep Kit	12.50	0.75	0.75
MagMAX™ Microbiome Ultra Nucleic Acid Isolation Kit	12.50	0.50	0.50
Zymo Environ™ Water RNA Kit	-	4.00	4.00
Quick-RNA™ Fecal/Soil Microprep Kit	12.50	-	-
RNeasy® PowerMicrobiome® Kit	12.50	-	-
RNeasy® PowerWater® Kit	-	75.00	-
RNeasy® PowerSoil® Total RNA Kit	75.00	-	-

Fig 4. Table of input volumes for each kit based on sample type. Dashes indicate that the kit was not tested with the corresponding sample type.

- Soils should be used whenever possible, as they yield the most consistently positive results coupled with lower mean CT values. Zymo EnvironWater is a kit designed for liquids and performed significantly better with the liquid sample compared to kits like MagMax Microbiome, which is designed for solids.
- Pairwise comparison of CT values indicate a significant 3-way relationship between kit, sample type, and viral concentrations with a p-value of .031. MagMax Microbiome with proteinase K performs significantly better than ZymoBIOMICS DNA/RNA, Qiagen Powermicrobiome, Magmax Microbiome, and Qiagen Powersoil in soils across high, medium, and low concentrations. It is not as efficient at extracting liquid input compared to Zymo EnvironWater. Interestingly, Zymo DNA/RNA which is designed for either solid or liquid input, was the most versatile detecting SARS-CoV-2 across all sample types, although it was also highly variable.

Conclusion and Future Directions

- MagMax Microbiome with proteinase K should be used when soils are available as a sample source. It was least variable and consistently positive in this study. Zymo EnvironWater is a reliable source for liquid samples and ZymoBIOMICS DNA/RNA is a versatile kit with less reliability.
- MagMax Microbiome was the only kit to use bead-beating, indicating a potential difference in column vs. bead-beating techniques for extraction, which requires further investigation.
- Potentially new method for less-resource intensive virus concentration compared to previously established methods. Can make WBE more accessible at a larger scale.
- Future studies should focus on spiking known quantities of virus to determine kit efficiency and analyzing water from a variety of locations.

References

[1] Hamouda M, Mustafa F, Maraqa M, Rizvi T, Aly Hassan A. Wastewater surveillance for SARS-CoV-2: Lessons learnt from recent studies to define future applications. *Sci Total Environ*. 2021;759:143493. doi:10.1016/j.scitotenv.2020.143493

Acknowledgments

Thank you to the coordinators of the Summer Undergraduate Research Program, the College of Public Health, and the entire Wiley Lab for making this project possible.