DNA Isolation from Wastewater: Experimentation from a SARS-CoV-2 Optimized Workflow

Kristina M. Babler[1], Mark E. Sharkey[2], Ayaaz Amirali[1], Samuel Comerford[2], George S. Grills[3], Brian D. Reding[4], Christopher E. Mason[5,6,7,8], Krista Ryon[5,6,7,8], Mario Stevenson[2], and Helena Solo-Gabriele[1]

[1] Department of Chemical, Environmental, and Materials Engineering, University of Miami, Coral Gables, FL USA
[2] Department of Medicine, University of Miami Miller School of Medicine, Miami, FL USA
[3] Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, Miami, FL USA
[4] Environmental Health and Safety, University of Miami, Coral Gables, FL USA
[5] Department of Physiology and Biophysics, Weill Cornell Medical College, New York City, NY USA
[6] The HRH Prince Alwaleed Bin Talal Bin Abdulaziz Alsaud Institute for Computational Biomedicine, Weill Cornell Medicine, New York, NY, USA
[7] The WorldQuant Initiative for Quantitative Prediction, Weill Cornell Medicine, New York, NY, USA
[8] The Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, New York, NY, USA

Abstract

Wastewater-based surveillance (WBS) has become a non-invasive, epidemiological strategy for assessing severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) within communities. RNA of SARS-CoV-2 is detectable in wastewater from pre-symptomatic, symptomatic, or asymptomatic individuals infected with the virus. A long-standing SARS-CoV-2 WBS program was established at the University of Miami (UM) starting in September 2020, and from routine methodology focused on RNA isolation, modifications were developed to isolate and quantify DNA of alternative pathogens from wastewater samples. The concentration method for isolating SARS-CoV-2 from wastewater was electronegative (EN) filtration, with the coupling of sample pretreatment (addition of magnesium chloride and hydrochloric acid) to improve binding affinity of viral particles to negatively charged membranes. Saturated EN membranes were placed in DNA/RNA shield which released the viral RNA into the lysate for subsequent RNA extraction and molecular assessment. For alternative targets, DNA was isolated through a bead bashing (BB) technique by first concentrating the microbes via size exclusion using 0.45 µm pore size membranes (GN-6 Metricel); as many DNA carrying microbes are larger than this size, the adaptation of the chosen membrane excluded the need for additional pretreatment, simplifying the initial processing. Although these GN-6 filters were also placed in DNA/RNA shield, mechanical lysis was necessary since many DNA carrying microbes have cell walls which reduce the overall effectiveness of DNA/RNA shield's lysis capability. A ZymoBIOMICS DNA Miniprep kit, and corresponding protocol, were selected for use with BB, as this product was optimized for extracting DNA from stool samples as well as sediment and biofilms – which are of similar composition to wastewater. Results of including saturated GN-6 membranes within bashing tubes, rather than strictly including the DNA/RNA shield lysate (EN process), ameliorated the DNA isolation process by improving the DNA concentrations (ng/µL) following extraction by roughly 2-3 times. Quality assessment for nucleic acid concentrations were performed for each experimental sample branching from the SARS-CoV-2 EN process and illustrated concentrations of roughly 25 – 45 ng/µL in 100 µL eluates, and DNA fragment sizes measuring between 4500 – 8000 bp. Overall, with slight modifications to current-standing WBS programs methodology, alternative pathogens beyond SARS-CoV-2, can readily be detected and monitored.

Comparison of Methodology Workflows

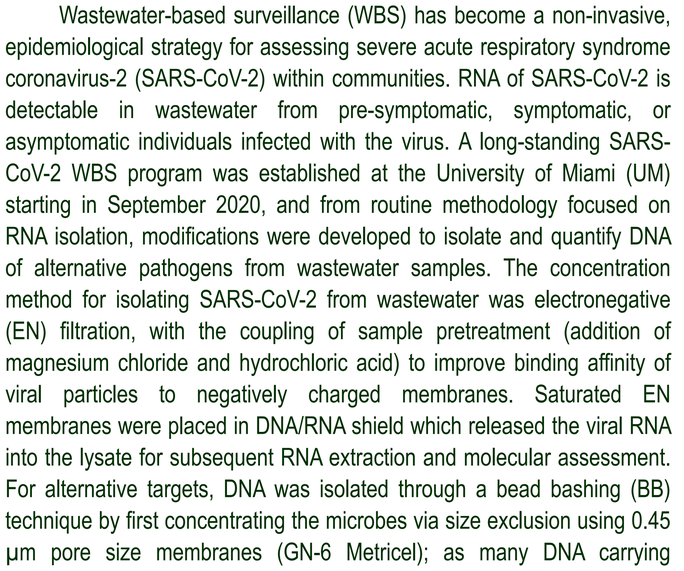


Figure 1. Illustrated comparison of SARS-CoV-2 optimized sample processing workflow vs. optimized DNA isolation sample processing workflow, minor differences in workflow include pretreatment, membrane selection, tube selected for wastewater concentrate creation, and commercial kit utilized.

Results – Bead-Bashing vs. No Bead-Bashing

Figure 2. Percent recovery experimentation between hospital and wastewater treatment plant (WWTP). Bacterial recovery control utilized was *Mycobacterium smegmatis*, and viral recovery control utilized was human coronavirus-OC43. Results illustrated that the inclusion of bead-bashing (BB) can impact % of recovered bacterial DNA but had little impact on viral RNA recovery by qPCR.

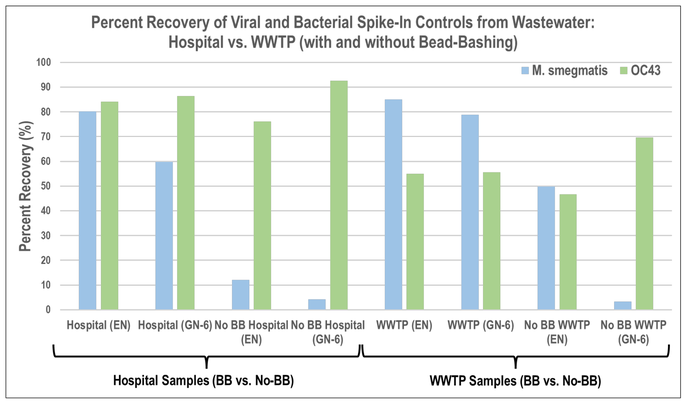


Figure 3. Percent recovery experimentation of WWTP samples processed under two conditions: 1) immediately, and 2) 20-Hrs Post-Spike-in. Results provided minimal degradation of bacterial and viral recovery control targets following 20-hour hold. Inclusion of BB provided more robust % recovery for bacterial control by qPCR, however, viral control did not benefit from its inclusion.

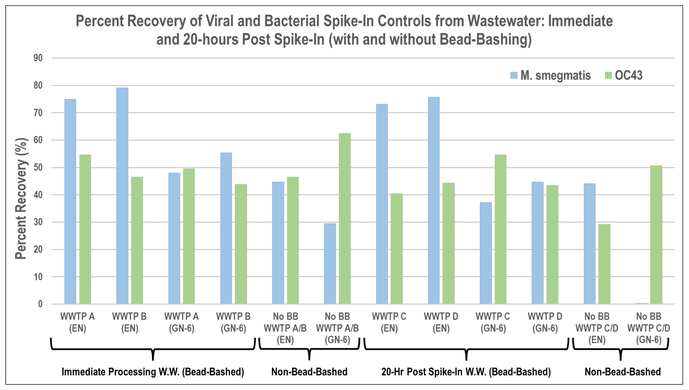
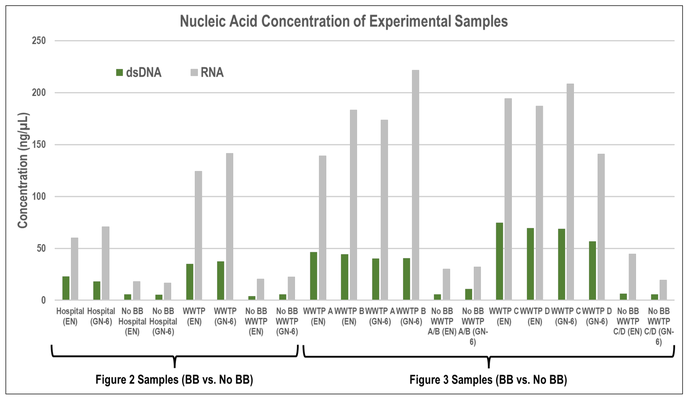
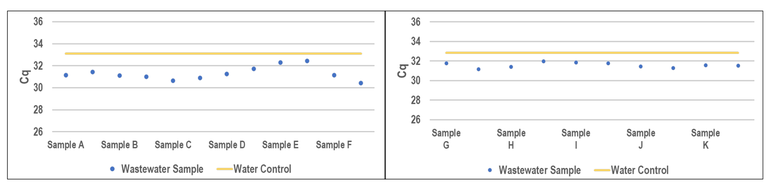


Figure 4. Nucleic acid concentration measurements (ng/µL) of dsDNA and RNA extracted from hospital and WWTP experimental samples. Results showed that overall, BB improved concentration of both dsDNA and RNA, but for bacterial target (i.e., dsDNA) BB was necessary for inclusion.



Results – qPCR Inhibition from Wastewater

Figure 5. Inhibitors that co-purify with wastewater DNA are effectively eliminated using the ZymoBIOMICS DNA Miniprep Kit. Eluting with 95 µL of Nuclease-free water plus additional 5 µL HIV DNA illustrate the effectiveness of qPCR amplification against a water control of the same volume. All samples succumbed to BB prior to DNA extraction.



Qualitative Results – Membrane Size Selection

Table 1. Qualitative assessment of membrane experimentation, for ability to remove a 400 µL supernatant from centrifuged, post-BB product (i.e., lacking bead/filter particles). Differing membrane sizes and volume of DNA/RNA shield can impact the ability to remove supernatant post-centrifugation.

Filter 'Size' Bead-Bashed	Volume of DNA/RNA shield (µL)	Was a "good-quality" 400 µL supernatant removable?	Qualitative Observations
Half-filter	**1,000**	**Yes**	**Very easy to perform, most of the filter pelleted, many filter pieces were small → medium sized (easily avoidable, great membrane destruction)**
Half-filter	800	Yes	Easy to perform, more difficult than 1 mL, more pieces than 1 mL bashing tube are smaller overall (good membrane destruction)
Half-filter	600	No	Not recommended, huge mess in cap, cannot avoid filter particles or beads upon removal of supernatant
Quarter-filter	1,000	Yes	400 µL easily removed, tight pellet, filter membrane appears almost powder-like, not messy upon tube lid removal
Quarter-filter	800	Yes	Easy to perform, less liquid left-behind than 1 mL so closer to beads, but able to remove full volume
Quarter-filter	600	No	Not able to remove a full 400 µL supernatant aliquot, poor quality many beads/filter pieces
Whole-filter	1,000	Yes	Difficult to avoid large filter particles, messy upon opening tube lid, not all filter pieces pelleted, few large pieces remain in lid
Whole-filter	800	No	Huge mess upon opening the tube, no space with whole filter so could not remove full 400 µL volume out without also collecting beads
Whole-filter	600	No	Huge mess upon opening the tube, could not collect even 200 uL with whole filter/pieces present, poor quality lots of beads

Conclusions

- Workflows to extract/isolate DNA-containing biological targets can be established from current SARS-CoV-2 protocols.
- BB has a significant impact on % recovered for bacterial species measured by qPCR, as well as downstream concentration (ng/ µL), but has limited impact on viral % recovery measured by qPCR.
- BB is necessary for optimizing DNA isolation workflows, but dependent on the biological target of focus (i.e., yeast, bacteria).
- Membrane-based filtration should be carefully optimized as differing membrane types + DNA/RNA shield yield different results for downstream isolation effectiveness, and laboratory performability.

Future Directions

- The application of technology such as III-HRC spin columns (Zymo Research) could benefit future dPCR assay optimization for effective inhibitor removal, prior to assay analysis.
- The inclusion, or exclusion, of BB within wastewater-based methodology could significantly impact the percent recovery of controls, and improve correlations between 'unknown' targets, such as SARS-CoV-2 or endogenous DNA-containing species within wastewater.
- The inclusion of BB for viral RNA could profoundly impact the ability to utilize extracted wastewater RNA for NGS applications.

References

- Babler, K. M.; Amirali, A.; Sharkey, M. E.; Williams, S. L.; Boone, M. M.; Cosculluela, G. A.; Currall, B. B.; Grills, G. S.; Laine, J.; Mason, C. E.; Reding, B. D.; Schurer, S. C.; Stevenson, M.; Vidovic, D.; Solo-Gabriele, H. M., Comparison of Electronegative Filtration to Magnetic Bead-Based Concentration and V2G-qPCR to RT-qPCR for Quantifying Viral SARS-CoV-2 RNA from Wastewater. *ACS ES&T Water* **2022**.
- Sharkey, M. E.; Kumar, N.; Mantero, A. M. A.; Babler, K. M.; Boone, M. M.; Cardentey, Y.; Cortizas, E. M.; Grills, G. S.; Herrin, J.; Kemper, J. M.; Kenney, R.; Kobetz, E.; Laine, J.; Lamar, W. E.; Mader, C. C.; Mason, C. E.; Quintero, A. Z.; Reding, B. D.; Roca, M. A.; Ryon, K.; Solle, N. S.; Schurer, S. C.; Shukla, B.; Stevenson, M.; Stone, T.; Tallon, J. J., Jr.; Venkatapuram, S. S.; Vidovic, D.; Williams, S. L.; Young, B.; Solo-Gabriele, H. M., Lessons learned from SARS-CoV-2 measurements in wastewater. *Sci Total Environ* **2021**, *798*, 149177.
- Solo-Gabriele, H. M.; Kumar, S.; Abelson, S.; Penso, J.; Contreras, J.; Babler, K. M.; Sharkey, M. E.; Mantero, A. M. A.; Lamar, W. E.; Tallon, J. J.; Kobetz, E.; Solle, N. S.; Shukla, B. S.; Kenney, R. J.; Mason, C. E.; Schurer, S. C.; Vidovic, D.; Williams, S. L.; Grills, G. S.; Jayaweera, D. T.; Mirsaeidi, M.; Kumar, N., Predicting COVID-19 cases using SARS-CoV-2 RNA in air, surface swab and wastewater samples. *Sci Total Environ* **2023**, *857*, 159188.
- Zhan, Q.; Babler, K. M.; Sharkey, M. E.; Amirali, A.; Beaver, C. C.; Boone, M. M.; Comerford, S.; Cooper, D.; Cortizas, E. M.; Currall, B. B.; Foox, J.; Grills, G. S.; Kobetz, E.; Kumar, N.; Laine, J.; Lamar, W. E.; Mantero, A. M. A.; Mason, C. E.; Reding, B. D.; Robertson, M.; Roca, M. A.; Ryon, K.; Schurer, S. C.; Shukla, B. S.; Solle, N. S.; Stevenson, M.; Tallon, J. J. J.; Thomas, C.; Thomas, T.; Vidovic, D.; Williams, S. L.; Yin, X.; Solo-Gabriele, H. M., Relationships between SARS-CoV-2 in Wastewater and COVID-19 Clinical Cases and Hospitalizations, with and without Normalization against Indicators of Human Waste. *ACS ES&T* **2022**, (Water).

Acknowledgements

This project is supported by the National Institutes of Health (NIH) Rapid Acceleration of Diagnostics - Radical (RADx-rad) grant 1U01DA053941; the NIH / NCI Cancer Center Support Grant 1P30CA240139-01 for the Sylvester Comprehensive Cancer Center; NIH grant P30AI073961 for the Miami Center for AIDS Research (CFAR); and NIH grant 1UL1TR000460 for the University of Miami Clinical and Translational Science Institute (CTSI). Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH. This project is also supported by University of Miami internal funding for wastewater surveillance at the university. We are grateful to the support from the University of Miami Facilities Administration and to the laboratory personnel from the Miami-Dade Water and Sewer Department who provided considerable logistical support for this project. We also thank Testing for America (501c3), OpenCovidScreen Foundation, the Bert L and N Kuggie Vallee Foundation, Igor Tulchinsky and the WorldQuant Foundation, Bill Ackman and Olivia Flatto and the Pershing Square Foundation, Ken Griffin and Citadel, the US National Institutes of Health (R01AI125416, R21AI129851, R01AI151059, U01DA053941), the Rockefeller Foundation, and the Alfred P. Sloan Foundation (G-2015-13964).