Reduction in the Effect of Differing Wastewater Characteristics on Quantification of SARS-CoV-2 by Electronegative Membrane Adsorption Technique

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Introduction

Physical characteristics of raw influent are variable between geographic locations and over time, depending on the number of residents served, industries also using the sewershed, and storm water infiltration among other factors. As a result, an RNA extraction method for the detection of SARS-CoV-2 in raw influent may perform well in one location or time and poorly in another.

In 2020, a modified electronegative membrane adsorption technique to quantify SARS-CoV-2 in raw wastewater was developed using samples taken upstream of a WWTP headworks at 3 sewer-shed interceptor locations ("Interceptors") in Oakland, California. In January 2021, sampling location was changed to Influent Pump Station ("IPS") at the same facility. The procedure that was previously effective for recovering RNA from Interceptor samples yielded significantly different RNA recovery from IPS samples with corresponding lower SARS-CoV-2 quantification of the N1 region.

Materials and Methods

Wastewater concentration and RNA extraction method was developed by modification of Bivins *et al.* 2020:

- 100 mL sub-samples are spiked with 18,000 GC/mL BRSV (surrogate virus) then pasteurized
- Samples are centrifuged then filtered on 0.45 µm MCE filters
- RNA is extracted and purified directly from filters and captured solids using Zymo Environ Water kit, modified to work with 5 mL extraction tubes
- RNA is amplified and quantified using RT-ddPCR and Bio-Rad QuantaSoft software; quantification is in genetic copies per liter (GC/L) of sample

All samples are 24-hour composites taken from the following locations and dates:

1. Interceptors: Adeline; North; South
   - 12/9/20 to 12/16/20; 3/3/21 to 3/30/21
   - 1 sample/week at each of 3 locations (N = 19)
2. Influent Pump Station: at EBMUD WWTP headworks
   - 1/4/21 to 2/10/21
   - 2 samples/week from a single location (N = 11)

Following increase in TSS, sub-sample volume was decreased to 50 mL and/or particulate > 1 mm in size was excluded by passing sample through a sterile 1 mm stainless steel sieve prior to pasteurization.

Results

IPS samples from Jan. 2021 processed without modification to procedure yielded significantly lower surrogate recovery than samples from Interceptor in Dec. 2020 ($P = 0.0012$; $t = 4.21$, $df = 12$). Interceptor samples from Nov. to Dec. 2020 had a total suspended solids (TSS) range of 270 to 700 mg/L. Throughout Jan. 2021 an increase in visible solids was noticed in IPS samples; this observation corresponded with a measured increase in TSS from a low of 330 mg/L starting Jan. 11 to a high of 5600 mg/L on Jan. 27. Interceptor samples from Mar. 2021 had a TSS range of 210 to 1400 mg/L (Fig. 1).

In IPS samples, SARS-CoV-2 N1 quantification was improved by reducing sub-sample volume from 100 mL to 50 mL in conjunction with removing particulate > 1mm in size ($P = 0.0010$; $t = 8.52$, $df = 4$), with an average RPD of +54% between N1 GC/L values of 100 mL and 50 mL sample volumes. Following modifications to the procedure, the surrogate recovery in IPS and Interceptor samples were no longer significantly different ($P = 0.84$; $t = 0.204$, $df = 12$; Figure 2.)

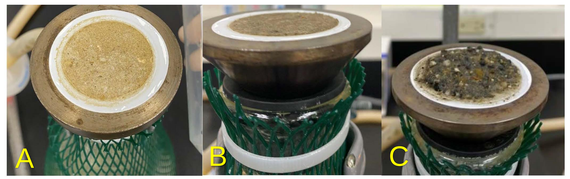


Figure 1. 0.45 µm MCE filters following filtration of 100 mL sample from the following locations: A. South Interceptor, TSS = 380 mg/L (3/17/21); B. North Interceptor, TSS = 1400 mg/L (3/10/21); IPS, TSS = 5600 mg/L (1/27/21).

IPS (n = 3) and Interceptor (n = 8) samples were prepared in duplicate with 50mL and 100mL Aliquots. In paired t-tests, reducing volume alone improved SARS-CoV-2 quantification in samples where TSS > 400 mg/L ($P = 0.0499$; $t = 2.78$, $df = 4$), but had no significant effect where TSS < 400 mg/L ($P = 0.17$; $t = 1.60$, $df = 5$). Four samples were prepared in duplicate with either removal of particulate > 1 mm or omitting this step; in paired t-test, the difference in SARS-CoV-2 N1 quantification between groups was not quite significant ($P = 0.088$; $t = 2.50$, $df = 3$).

Average surrogate % recovery improvement between base method and reduced sample volume was +12% (Fig. 3).

Figure 2. Mean Surrogate % Recovery by Location, Before and After Modifications to Procedure

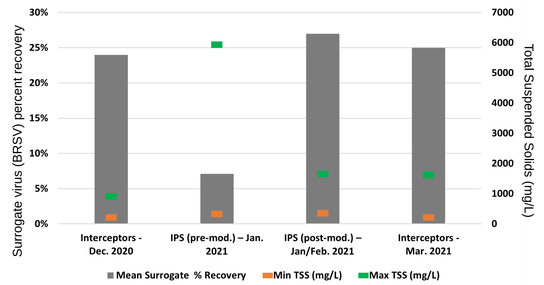
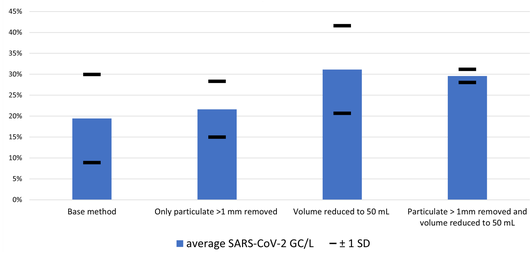


Figure 3. Average surrogate recovery from paired samples, comparing effects of removal of particulate and reduction of sample size



Conclusions

Differences in characteristics of wastewater between or within sampling locations over time can significantly impact RNA recovery using electronegative membrane adsorption techniques, which in turn impacts quantification of SARS-CoV-2 in wastewater.

In paired tests, reducing sample volume for samples where TSS > 400 mg/L was effective in increasing SARS-CoV-2 N1 quantification as well as surrogate recovery. Removal of particulate > 1 mm in size had a marginal effect on SARS-CoV-2 N1 quantification.

Laboratory familiarity with wastewater characteristics such as TSS at a sampling location can assist in determining when modifications to sample processing procedures should occur in order to obtain comparable results between samples.

Acknowledgments

Clayton Thompson, EBMUD
Yuyun Shang, EBMUD
Alicia Chakrabarti, EBMUD
Irene Lui-Wong, EBMUD
Eileen White, EBMUD

Literature cited

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