Comparison of two rapid virus concentration methods for implementing SARS-CoV-2 Wastewater-Based Epidemiology

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An extended abstract

Introduction

The successful implementation of SARS-CoV-2 wastewater-based epidemiology (WBE) for outbreak control depends on precise and accurate viral copies quantification from wastewater, which has a lot of variables that can impact on the virus quantification. Virus concentration method is highly important especially in low COVID-19 prevalence area where viral titers in wastewater is not high enough to be detectable. So far, there are several common methods which have been used for virus concentration from wastewater, including PEG precipitation (La Rosa et al., 2020; Polo et al., 2020), centrifugal ultrafiltration (Nemudryi et al., 2020; Wu et al., 2020), and electronegative membrane filtration (EMF) (Haramoto et al., 2020; Barua et al., 2021). The drawback of the PEG precipitation method is long sample processing (4 to 24 h) and co-precipitation of PCR inhibiting materials (Kumblathan et al., 2021). Recently, a systematic comparison of enveloped virus recovery has been done by Ahmed et al., (2020a) where EMF showed maximum Murine Hepatitis Virus recovery (65.7%) compared to ultrafiltration (56%) and PEG precipitation method (44%). When these methods were applied for the SARS-CoV-2 quantification from aircraft wastewater samples, a similar higher trend of SARS-CoV-2 detection result was observed with this method compared to others (Ahmed et al., 2020b). About 13% SARS-CoV-2 WBE published articles so far used this method successfully for virus detection and quantification (Buonerba et al., 2021).

Ultrafiltration works based on size exclusion using centrifugal filters with different molecular cutoff ranging 10 to 100 kDa (Kitajima et al., 2020). This method widely used for concentrating SARS-CoV-2 virus from wastewater that has been reported in about 43% recently published WBE articles (Buonerba et al., 2021). A new ultrafiltration-based virus concentration method named Innovaprep concentrating pipettor (CP) is gaining attention because of its fast and high throughput sampling processing (Juel et al., 2021, Ahmed et al., 2021b). It is an automatic system that allow to concentrate bacteria or virus particles passing water or wastewater through either hollow or ultrafiltration based concentrating pipette tips. It can process large volume (up to 5 L) of sample depending on the turbidity of sample and concentrate to as small as 150 uL ((https://www.innovaprep.com). In this paper we will be evaluating the performance of Innovaprep CP select protocol (optimized) compared to the established electronegative membrane filtration method in terms of surrogate virus recovery rate, sensitivity of detecting SARS-CoV-2 from wastewater sample, and processing time.

Method

Wastewater samples were collected as part of the routine surveillance of UNC charlotte campus from November 2020 to January 2021. For the initial comparison, we selected 20 numbers of samples which were processed using the two methods side by side, followed by RNA extraction, and quantification following the same protocol. Wastewater samples were concentrated using the EMF or HA method following the protocol described in Gibas et al (2021). As part of the Innovaprep CP-Select™ protocol, we centrifuged the wastewater samples at 10000×g for 10 min to remove large particles. Then we added 10% Tween-20 to the supernatant at a ratio of 1:100 and filtered through the concentrator pipette tips (µm PS Hollow Fiber Filter) coupled with the CP-Select™ (Innovaprep). Viral particles trapped at the filter tips were recovered by eluting with 0.075% Tween-20/Tris elution fluid using Wet Foam Elution™ technology (Innovaprep) into a volume ranging from 250 - 500 uL. Then we added 1: 1 AVL lysis buffer (Qiagen) to this concentrated sample as an increased viral recovery was observed based on the preliminary result. 200 uL of concentrated samples were used for the RNA extraction using the QIAamp viral RNA mini kit (Qiagen). Reverse Transcriptase - real time PCR (RT-qPCR) was used to detect and quantify SARS-CoV-2 and Bovine coronavirus from extracted RNA. CDC recommended N1(Nucleocapsid) primers and probe set (Coreman et al., 2020) was used for SARS-CoV-2 quantification while a primers/probe set published by Decora et al.,(2008) was used for Bovine coronavirus quantification. The detailed method section can be found in Juel et al., (2021).

We also evaluated the performance comparison using the sample volume processed with the EMF and the CP Select protocol. We processed 40, 60, and 100 mL of wastewater side by side taking a separate set of samples (n = 10). Before concentrating these samples, we spiked Bovine Coronavirus (BCoV), a family of coronaviridae, in the wastewater at a ratio of 1: 10000 as an external process control. Both SARS-CoV-2 using N1 gene and BCoV were determined following the same protocol. We also investigated the impact of the centrifugation step on viral recovery. BCoV spiked wastewater samples were centrifuged at 10000×g for 10 mins as part of the CP Select protocol. Then the supernatant and pallets were processed for this purpose. For the quality purpose, we included filtration control, extraction control, negative control, and positive control throughout the study. The detailed method can be found in the published version at Juel et al., (2021).

Result

Results reported that both methods successfully detected and quantified SARS-CoV-2 viruses from wastewater samples, however, the CP Select method performed better than the EMF in terms of detecting SARS-CoV-2 positivity shown in **Figure 1.** Out of 20 wastewater samples, the EMF method quantified 6 samples that were above the limit of detection (LOD) while the CP Select protocol quantified 11 samples in that occasion which turned out 5 more samples as SARS-CoV-2 positive those were reported as negative using the routine EMF method. This result indicated that the optimized CP Select protocol is more sensitive for capturing viruses from low-tittered wastewater samples than the EMF method.

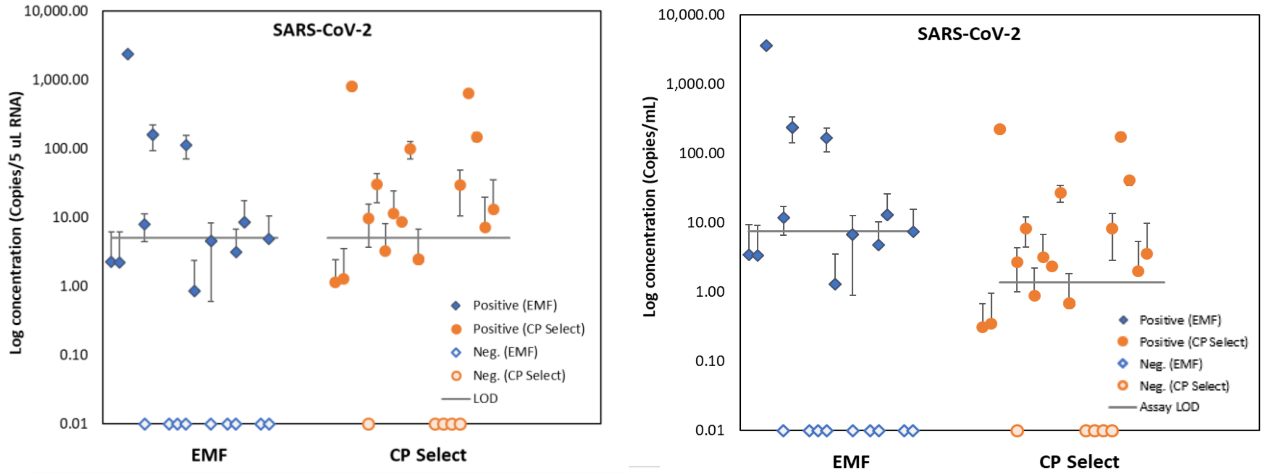

Figure 1: Quantification of SARS-CoV-2 from wastewater concentrated by EMF and Innovaprep CP Select protocol. Error bars indicate the standard deviation among replicates.

Performance comparison using the same sampling volume

Figure 1 illustrate the SARS-CoV-2 detection results for 40 and 60 sample volume processed with the two methods. An overall similar trend in SARS-CoV-2 detection result was also observed what we found previously in Figure 1. In both of the sampling size (40 and 60 mL), the number of EMF processed samples tested as SARS-CoV-2 positive those were also tested as positive concentrated with the CP Select protocol (Table 1 & 2). However, an additional number of samples were tested as positive that were non-detected with the EMF protocol (shown as highlighted color in Table 1 & 2). The result of the 100 ml input volume was not present as only 3 out of 10 samples were able to pass through the membrane filter. The larger input volume did not improve the sensitivity of the EMF method that may be because of the higher inhibitory substances deposited on the filter that can interfere during the qPCR amplification (Ahmed et al., 2020). This result is also supported by the BCoV recovery result for the EMF method. The lower parentage of the BCoV recovery was reported for the 60 mL sampling size compared to 40 mL sampling size (Figure 1). However, the CP Select protocol showed a higher mean BCoV recovery using 60 mL input volume compared to 40 input volume which is an agreement with the SARS-CoV-2 result (Table 1 and 2). When comparison was made between the two concentration methods based on the same sample volume, a statistically significant difference in BCoV recovery was observed for 60 mL sampling size ($p = 0.03$) though there is no significant difference found for the 40 mL sampling size ($p = 0.58$).

Table 1: SARS-CoV-2 detection from 40 mL wastewater sample concentrated by EMF and Innovaprep CP Select protocol.

Sample Id	**EMF (40 mL)**		**CP Select (40 mL)**	
	Mean Cq ± SD	**Result**	**Mean Cq ± SD**	**Result**
S1	-	Negative	-	Negative
S2	-	Negative	36.14*	Negative
S3	-	Negative	-	Negative

S4	31.14 ± 0.02	Positive	32.28 ± 0.56	Positive
S5	-	Negative	38.07 ± 1.45	Positive
S6	-	Negative	-	Negative
S7	35 ± 0.57	Positive	33.2 ± 0.32	Positive
S8	-	Negative	-	Negative
S9	36.32 ± 0.42	Posiitve	33.47 ± 0.50	Positive
S10	-	Negative	-	Negative

* indicates one replicate positive. SARS-CoV-2 positive was considered for at least two replicate signals.

Table 2: SARS-CoV-2 detection from 60 mL wastewater sample concentrated by EMF and Innovaprep CP Select protocol.

Sample Id	EMF (60 mL)		CP Select (60 mL)	
	Mean Cq ± SD	**Result**	**Mean Cq ± SD**	**Result**
S1	-	Negative	-	Negative
S2	-	Negative	37.12*	Negative
S3	-	Negative	-	Negative
S4	-	Negative	32.55 ± 0.11	Positive
S5	39.60 ± 2.85	Positive	35.08 ± 0.42	Positive
S6	-	Negative	-	Negative
S7	35 ± 0.57	Positive	32.00 ± 0.31	Positive
S8	-	Negative	37.24 ± 0.12	Positive
S9	31.53 ± 0.31	Positive	33.12 ± 0.42	Positive
S10	-	Negative	37.04*	Negative

* indicates one replicate positive. SARS-CoV-2 positive was considered for at least two replicate signals.

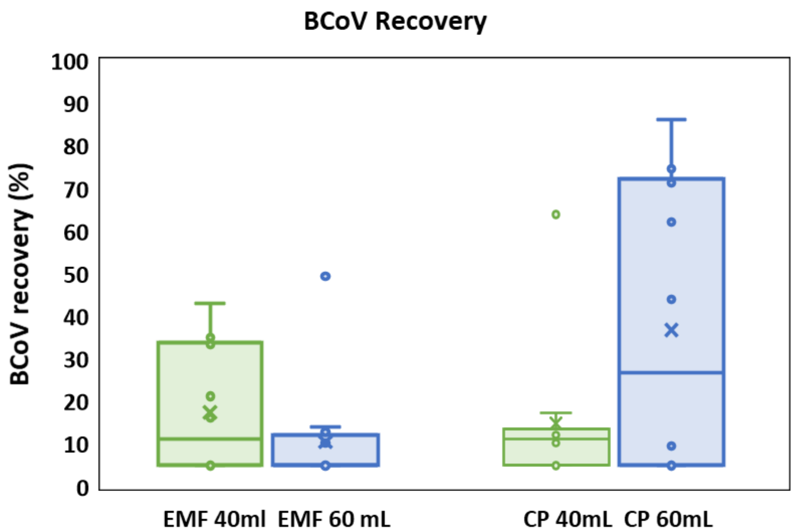

Figure 2: Effect of input filter volume on the performance of EMF and CP Select method in terms of BCoV recovery.

Virus partitioning and effect of sonication on the virus recovery

Result suggest that similar fraction of SARS-CoV-2 viruses were determined from the supernatant and the pallets ($p = 0.85$) though BCoV behaved differently than SARS-CoV-2. A higher fraction of BCoV was recovered from the supernatant than pallets ($p = 0.01$). This difference may be because of the different viral structure especially at the spike protein that may help attach to the solid surface. In an attempt to increased viral recovery, we applied ultrasonication (36khz) step for 1 min to wastewater samples just before the centrifugation step. This step increased the mean BCoV recovery by 19% compared to the samples that were not treated with the ultrasonication. SARS-CoV-2 result also improved for most of the samples.

Conclusion

The CP Select method is more sensitive than EMF method as about 25% wastewater samples concentrated with this method showed SARS-CoV-2 positive result that were non-detected when processed with the EMF method. The CP Select method is beneficial in situations where detection sensitivity and quick data reporting is important as it reduces around 30% processing time compared to the EMF method. Similar portion of SARS-CoV-2 viruses were found in the supernatant and pallets. The addition of sonication step increased the viral recovery from the wastewater.

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