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Performance Evaluation of RT-qPCR and RT-dPCR Platforms for the wastewater surveillance of SARS-CoV-2

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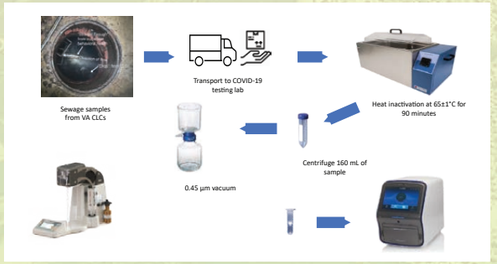
Introduction

The COVID-19 pandemic is an ongoing global health emergency. Wastewater-based epidemiology is a valuable tool for supplementing clinical testing in identifying infected individuals early thus containing disease transmission. To assess early detection of COVID-19, a building-level wastewater-based surveillance pilot project was implemented within VHA. Here, we report the results from 2 methods of polymerase chain reaction (PCR) testing of 1073 wastewater samples from VHA CLCs (i.e., nursing homes).

Methods



Daily (Monday-Friday) wastewater samples were collected (January 11, 2021, to July 2, 2021) at eight CLCs located across the US and shipped overnight for processing. The samples were heat inactivated by incubating samples in a 65±1°C heating circulating water bath for 90 minutes. The virus in the wastewater was concentrated using InnovaPrep concentrating pipette select, and RNA was isolated from the concentrate and subjected to reverse transcription quantitative PCR (RT-qPCR), RT-digital PCR, whole genome sequencing.

Acknowledgements

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Results

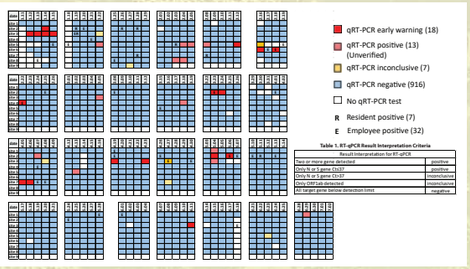

Table 1. RT-qPCR Result Interpretation Criteria

Result Interpretation for RT-qPCR	
Two or more gene detected	positive
Only N or S gene Ct≤37	positive
Only N or S gene Ct>37	inconclusive
Only ORF1ab detected	inconclusive
All target gene below detection limit	negative

Fig. 1. RT-qPCR detection of SARS-CoV-2 RNA from wastewater

MS2	N	ORF1ab	S	Result
27.65	32.949	32.62	33.078	Positive
26.866	32.656	32.966	33.124	Positive
29.171	33.112	33.614	33.947	Positive
27.764	34.241	35.05	34.106	Positive
31.073	35.555	37.266	35.553	Positive
30.75	35.717	37.827	36.064	Positive
27.959	35.083	34.908	36.146	Positive
29.562	31.11	31.959		Positive
27.639	33.867	32.751		Positive
26.548	35.11	33.637		Positive
26.405	35.471	36.139		Positive
29.618	36.309	37.622		Positive
30.86	36.513	37.714		Positive
28.003	34.94		34.849	Positive
30.74	38		36.208	Positive
29.093	38		37.712	Positive
29.607	34.901			Positive
29.551	34.95			Positive
27.369	35.072			Positive

MS2	N	ORF1ab	S	Result
27.712	35.28			Positive
29.13	35.636			Positive
29.182	35.891			Positive
31.671	36.13			Positive
30.246	36.15			Positive
31.697	36.293			Positive
28.199	36.523			Positive
29.101			34.122	Positive
31.458			36.514	Positive
31.639	37.946			Inconclusive
29.688	37.958			Inconclusive
27.464	38.396			Inconclusive
25.997		38		Inconclusive
29.704		37.477		Inconclusive
28.542		37.296		Inconclusive
31.05		37.019		Inconclusive
31.194		35.885		Inconclusive
27.15		35.217		Inconclusive

Table 2. Ct values of positive and inconclusive samples.

If SARS-CoV-2 was detected in the wastewater within the prior 10 days of a virus-positive occupant, the wastewater positivity was regarded as an early warning. Thirty-one positives and 7 inconclusive results were reported by RT-qPCR during the surveillance. Among the 31, 18 wastewater positives qualified as early warning and 13 positives were not verified by occupant positivity.

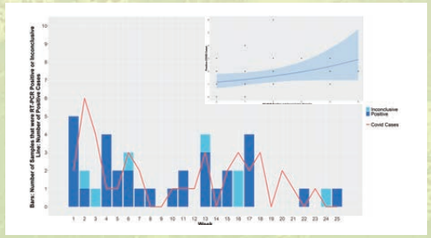

Fig. 2. An association between the SARS-CoV-2 detection in the wastewater and COVID19 positive cases in the occupants.

There is some evidence of an association between the SARS-CoV-2 detection in the wastewater and the COVID19 positive cases in the occupants. A Poisson regression model was used to determine the association between wastewater detection and COVID19 cases. The coefficient for the effect of 1 positive wastewater detection on COVID19 cases was 0.18 (-0.02, 0.38), on the natural log scale, indicating some evidence, though not at the 95% level.

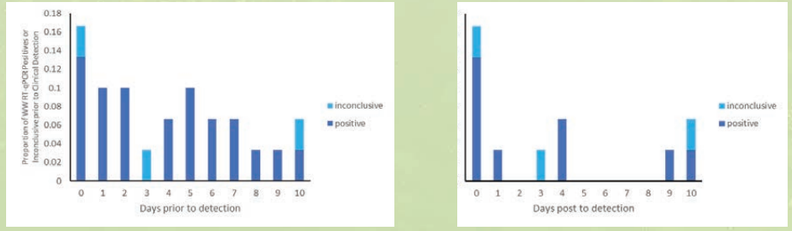

Fig. 3. Proportion of RT-qPCR positives before and after clinical detection

More SARS-CoV-2 detection in the wastewater occurred prior to occupants' cases than after the cases.

Digital PCR with a cutoff value of 0.25 copies/µL of RNA for defining positivity had 30 positives qualifying as early warnings, rest 113 positives were not verified by occupant positivity. Whole genome sequencing data are more closely correlated to the RT-qPCR data than RT-digital PCR data.

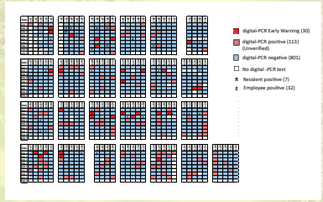

Fig. 4. RT-digital PCR detection of SARS-CoV-2 RNA from wastewater

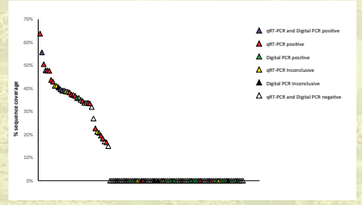

Fig. 5. Correlation of whole genome sequencing data and RT-qPCR and RT-digital PCR

Conclusions

The overall viral loads of the wastewater samples were very low corresponding to the dip in cases seen in the US during the study period. Although sensitivity of digital PCR appears higher than that of RT-qPCR, there were more occurrences of unverified early warning that could impact precision. More controlled studies are needed to determine sensitivity and precision as well as to standardize RT-digital PCR cutoffs to define for routine use.

References

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