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Comparative performance of RTqPCR vs RTddPCR for the detection of SARS-CoV-2 in wastewater collected from a range of sites and scales across the sewer network of Calgary, Alberta

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Background

- Digital droplet PCR(ddPCR) partitioning of entire PCR solution into a large number of partitions (droplets) is thought to enhance the performance.
- We sought to compare wastewater (WW) SARS-CoV-2 RNA detection across a range of sites and scales using RTqPCR and RTddPCR.
- We compared performance of the assays at both high and low levels of community viral transmission to assess for benefits and drawbacks of each technique.

Methods

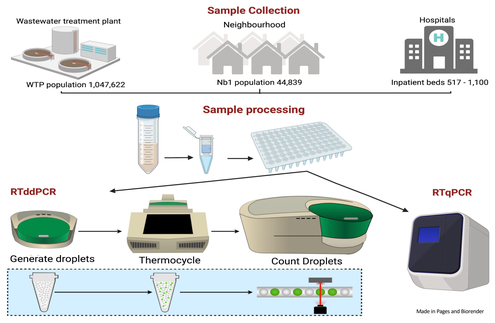

Figure 1. Sample collection, processing and molecular analysis. Comparing WW measured SARS-CoV-2 by different PCR platforms and targets with case occurrence.

- Composite 24h WW was collected from a WW treatment plant (WTP; n=18) population 1,047,622, a neighborhood (Nb1; n=12) population 44,839 and three hospitals; H-1, 517 inpatient beds, H-2, 615 inpatient beds, and H-3 (3-sites; A-C)(n=84) 1,100 inpatient beds.
- RNA was extracted using the 4S-silica column method. RTqPCR (QuantStudio5, ThermoFisher) and RTddPCR (C1000 Thermal Cycler and QX200 Droplet Reader, BioRad) quantified SARS-CoV-2 RNA nucleocapsid (N2) and envelope (E) genes in triplicate.
- Fisher's exact test was used to compare assay sensitivity.
- ROC curve was used to determine RTqPCR cut-offs. A cut-off < 2 droplet was used for RTddPCR.
- We compared WW detected signal with daily confirmed Covid-19 cases in the catchment area (defined by three-digit postal code of primary residence using 5-day rolling average) using Pearson correlation.

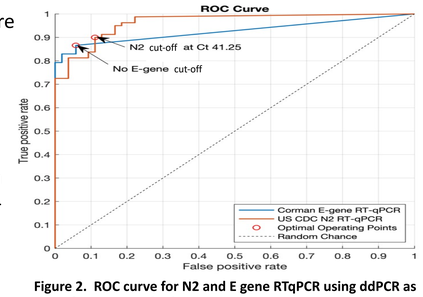

Figure 2. ROC curve for N2 and E gene RTqPCR using ddPCR as the reference standard.

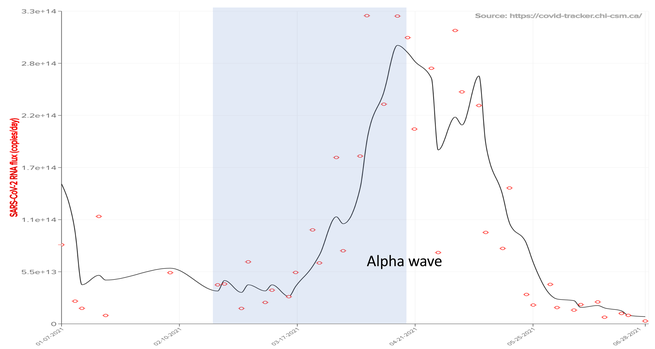

Figure 3. Sample collection time line and Calgary WWTP SARS-CoV-2 flux by RTqPCR.
Highlighted in blue is the period for sample collection before and during the Alpha (B117) wave in Calgary, Alberta, Canada. SARS-CoV-2 N2 RTqPCR weekly average of the mean flow rate adjusted copies/ml for three Calgary wastewater treatment plants.

Results

- 114 samples were tested (February 23, 2021 to April 22, 2021).
- SARS-CoV-2 N2 was identified in 77/114 (68%) by RTqPCR and 79/114 (69%) by RTddPCR (p=0.9).
- SARS-CoV-2 E was found in 72/114 (63%) by RTqPCR and 79/114 (69%) by RTddPCR, (p=0.4).
- Correlations between PCR platforms were strongest for N2 relative to E across all sites (see Table).
- N2 abundance correlated with clinically diagnosed cases for both PCR platforms greater at the level of the WTP (RTqPCR; r=0.8972, p<0.0001 and RTddPCR; 0.933, p<0.0001) relative to Nb1 (RTqPCR; r=0.6, p=0.04 and RTddPCR; r=0.9, p<0.001).
- E abundance correlated to a lesser degree with cases at WTP (RTqPCR; r=0.65, p=0.0035 and RTddPCR; 0.88, p=<0.001) relative to Nb1 (RTqPCR; r=0.19, p=0.55 and RTddPCR; r=0.84, p=0.0005).
- For hospital sites, correlation between WW and total hospitalized Covid-19 cases was not observed (data not shown).

Copies/ml	Target	WTP	Nb1	H-1	H-2	H-3A	H-3B	H-3C
RTddPCR vs RTqPCR	N2	r=0.88, P=<0.0001	r=0.77, P=0.003	r=0.72, P=0.001	r=0.99 P=<0.0001	r=0.99, P=<0.0001	r=0.99, P=<0.0001	r=0.54, P=0.03
	E	r=0.59, P=0.01	r=0.29, P=0.35	r=0.73, P=0.001	r=0.90, P=<0.0001	r=0.99, P=<0.0001	r=0.96, P=<0.0001	r=0.40, P=0.13

Table 1. Correlation RTddPCR vs RTqPCR.
Pearson correlation for N2 RTqPCR vs RTddPCR and E gene RTqPCR vs RTddPCR from different site locations.

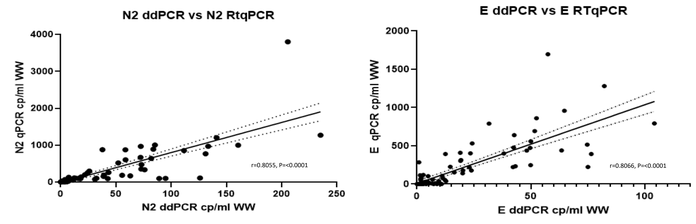

Figure 4. Correlation RTqPCR vs RTddPCR.
Pearson correlation of all samples for N2 RTqPCR vs RTddPCR and E gene RTqPCR vs RTddPCR.

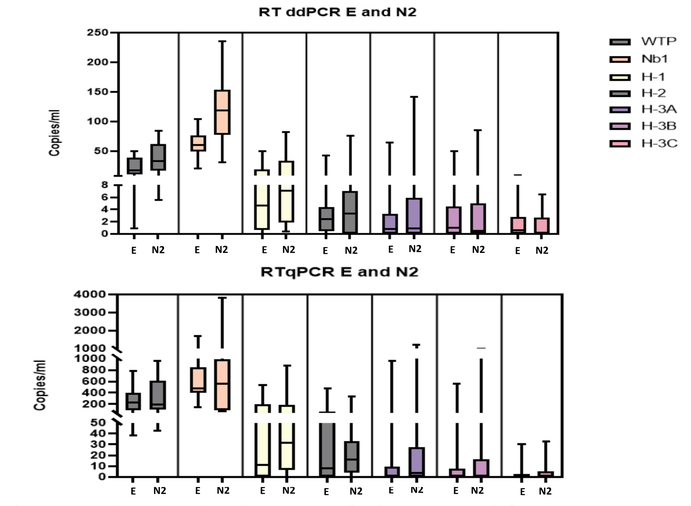

Figure 5. Comparing SARS-CoV-2 E and N2 gene target abundance across PCR platforms.

Conclusion

- Across a range of scales, SARS-CoV-2 N2 performance was similar between PCR platforms.
- RTddPCR correlated better with cases than RTqPCR for E gene.
- Correlation between WW and clinical cases was stronger for larger population sewersheds.

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