Wastewater surveillance for COVID-19:
Variability in SARS-CoV-2 genome quantification affected by RNA extraction method and the time course of its stability

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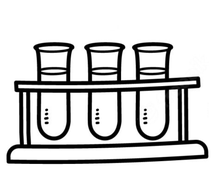
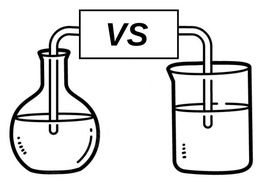
Background

Wastewater-based epidemiology is a powerful public health tool widely used to track the presence and the spread of SARS-CoV-2 within communities. However, wastewater is a complex matrix with fluctuating composition, where the sample homogeneity, the genome stability over time and the nucleic acid extraction method, may affect the analysis and hence increase measurment variability. For this reason, their study is fundamental for laboratory analysis in order to determine, for example, the maximum time delay between sampling and extraction, or the number of replicates for each extraction necessary for the study.

Materials & Methods



5 mL of wastewater naturally contaminated with SARS-CoV-2



Analysis using two distinct extraction principles

RT-PCR quantification (E and/or N1 targets)

Results

I – Comparison of different RNA extraction procedures : magnetic beads *vs.* silica columns

The NucliSens kit (BioMérieux) with magnetic beads without (DB70) and with a phenol-chloroform lysis step (DB70/PC) was tested for SARS-CoV-2 genome extraction; extracts were eluted in 70 µL of PCR-grade water. The Zymo Environ Water RNA kit (Zymo Research), including a concentration step by flocculation, was used for RNA extractions with silica columns in which the extracts were eluted in 100 µL (ZC) or reconcentrated in 15 µL (ZL). All RNA extracts were treated to remove inhibitors using the OneStep PCR Inhibitor Removal kit (Zymo Research).

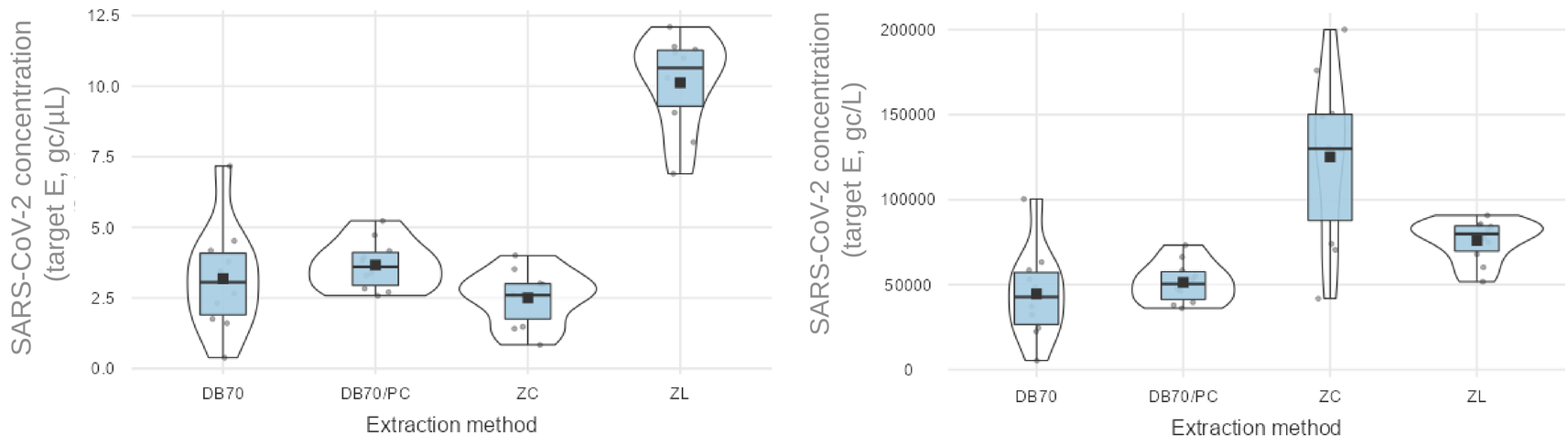


Figure 1 : Mean of SARS-CoV-2 genome concentrations and standard deviations according to each extraction method (n=10) presented in genome copies per µL of analyzed extract (A) or genome copies per liter of wastewater (B).

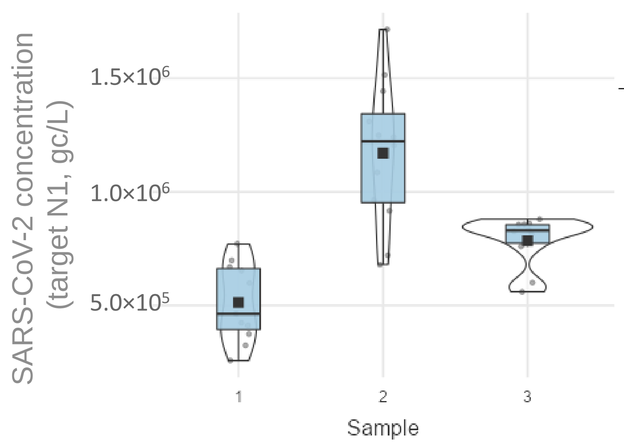
Table 1 : Coefficient of variation (CV) according to the extraction method

Extraction method	CV
DB70	60%
DB70/PC	24%
ZC	40%
ZL	16%

- ZL extracts showed the highest SARS-CoV-2 concentration in genome copies per µL (Figure 1A).
- ZC and ZL methods had the highest concentrations in gc/L of wastewater (Figure 1B).
- ZL method (column purification) showed a lower dispersion of measurements while the DB70 method (magnetic beads) had the highest levels of dispersion (Table 1).

II – Estimating the impact of sampling on quantification

Three wastewater samples of 200 mL were aliquoted from a bottle containing 2,5 L of wastewater after being shacked 5 times (manual agitation). Four repetitions were performed. All the 12 samples were extracted using the ZC method.



	Sample 1	Sample 2	Sample 3
Measures	11	12	10
Mean	5,13E+05	1,17E+06	7,85E+05
SD	1,72E+05	3,13E+05	1,13E+05
Min	2,57+E05	6,80+E05	5,60+E05
Max	7,70+E05	1,72+E06	8,80+E05
CV	**33%**	**27%**	**14%**

Figure 2 : Distribution of SARS-CoV-2 concentrations per sample

- Calculated variation can be considered as acceptable (Figure 2, CV 14-33%)
- There are small variations in Cq measurements per sample (Data not shown, CV < 2%)

III – Study of SARS-CoV-2 genome stability over time

A wastewater sample was incubated in the dark in a polyethylene vial at 5 ± 3°C for 39 days and SARS-CoV-2 RNA was extracted using the ZC method (n=3).

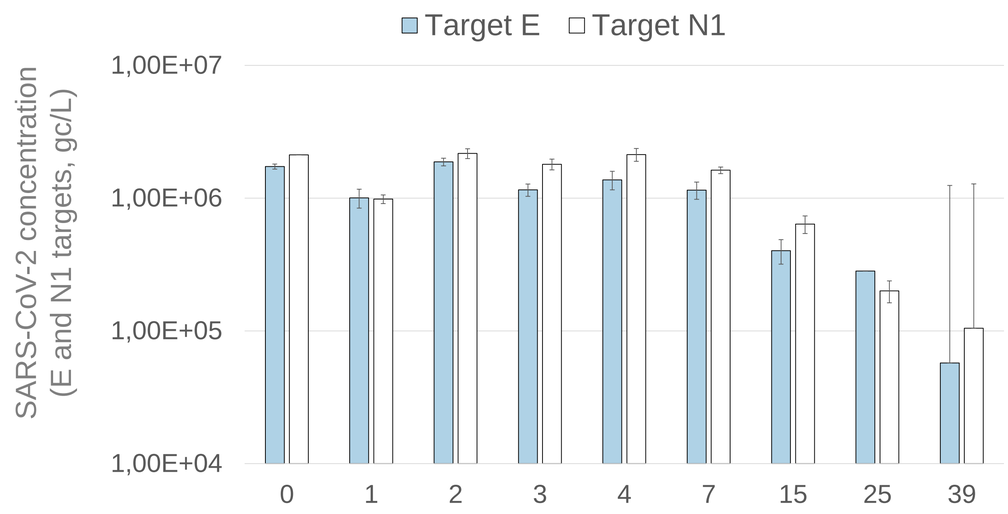


Figure 3 : Evolution of SARS-CoV-2 concentration over time, stored in the dark in a polyethylene vial at 5 ± 3°C

- SARS-CoV-2 RNA concentrations were stable up to day 7 for both E and N1 targeted genes.

Conclusions

- According to the analysed data, extractions using silica columns could reduce the dispersion of genome measurements. This finding needs to be verified and confirmed using other systems.
- While the ZL extraction method appears to be the most sensitive, the ZC method seems to be the method of choice in terms of execution speed, the concentration yield and the extraction repeatability.
- Our results demonstrate that manual agitation can provide sample homogeneity, ensuring the representativeness of the sub-samples to be analyzed.
- Storing wastewater sample at 5 ± 3°C ensures a stable amount of SARS-CoV-2 RNA copy number for up to one week after collection.
- Comparison with a second target seems to be necessary in order to confirm the obtained results.