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Comparative evaluation of molecular methods for detection of respiratory viruses in wastewater to support the implementation of a national early alert system for epidemics and health emergencies

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Introduction

Wastewater-based epidemiology (WBE) has proven to be **a useful complementary approach for epidemiological surveillance**

WBE

Advantages
- Early warning system for incidence increase (1-2 weeks)
- Populational approach
- Detects asymptomatic cases

Challenges
- Matrix complexity
- Diversity in laboratory methods
- Bias in comparative results

Standardization of WBE is still a global challenge

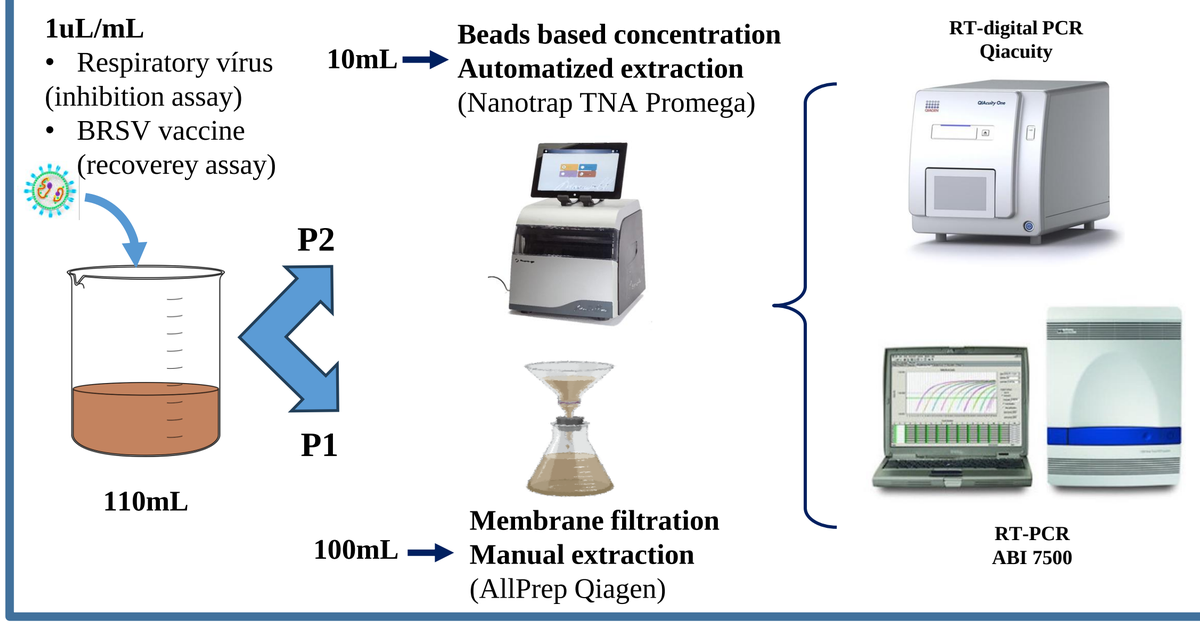
Objectives

Development and **standardization** of two end-2-end protocols for detection of **respiratory viruses** in wastewater samples

- Evaluate viral recovery
- Standardize a **multiplex** detection of viral RNA (SC-2, FLU A/B, RSV)
- Compare the efficiency of detection from **RT-qPCR** and **RT-dPCR**

Methods

- 20 Sewage samples were obtained from wastewater treatment plants (WWTP) from Rio de Janeiro (January to April 2024).
- A set of enzyme primers and probes (Biomanguinhos) were optimized at different concentrations for multiplex detection of SARS-CoV-2 (SC-2), Influenza A (FLU-A), Influenza B (FLU-B) and Respiratory Syncytial Virus (RSV).
- Two main protocols were tested (**P1 vs P2**)



Preliminary Results

- The performances of the multiplex primers and probes were previously evaluated by serial dilutions in several replicates. Concentrations were adjusted for best performance in complex samples with low RNA concentrations. No significant inhibitions were observed at a concentration of (0,1-0,5uM).
- Rt-dPCR - 18 paired (P1 vs P2) samples
- RT-PCR - 20 paired samples from January to April 2024.

Table 1. Positivity for SC-2, FLU-A, FLU-B and RSV RNA in wastewater samples from Rio de Janeiro using to distinct protocols, according to PCR methodology (RT-PCR and dPCR). Rio de Janeiro, Jan-Apr 2024)

Method/Vírus	P1 N (%)	P2 N (%)
dPCR		
SC-2	16 (88.9)	18 (100.0)
FLU-A	7 (38.9)	14 (77.8)
FLU-B	3 (16.7)	2 (11.1)
RSV	8 (44,4)	11 (61.1)
Total	18	18
RT-PCR		
SC-2	14 (70.0)	18 (90.0)
FLU-A	8 (40.0)	10 (50.0)
FLU-B	2 (9.0)	1 (3.0)
RSV	2 (10.0)	4 (20.0)
Total	20	20

Table 1 The data are presented by N (%) of positivity of the samples for (SC2) Sars-CoV-2 FLU-A (Influenza A), FLU-B (Influenza B) and RVS (Respiratory Syncytial Virus) by RT-PCR and RT-dPCR.

Figure 1. Comparison of P1 and P2 protocols for quantifying SC-2, FLU-A, FLU-B and RSV RNA by dPCR in wastewater samples from Rio de Janeiro

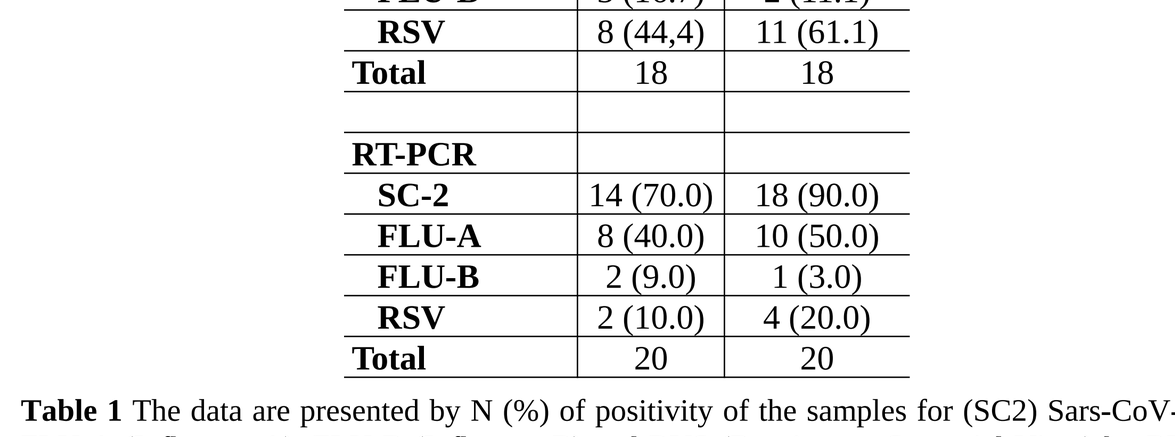


Figure 1. Bar graph with standard error representing the quantification in genomic copies per uL (gc/uL) detected through protocol 1 (red) and protocol 2 (green) through RT-dPCR. Analysis by Student's t-test or Wilcoxon test. **p* value <0.05; ***p* value <0.01; ****p<0,0001

Figure 2. Quantification of SARS-CoV-2, Influenza A and B and RSV RNA in wastewater samples from WWTP Alegria, Rio de Janeiro, according to epidemiological week, January to April 2024.

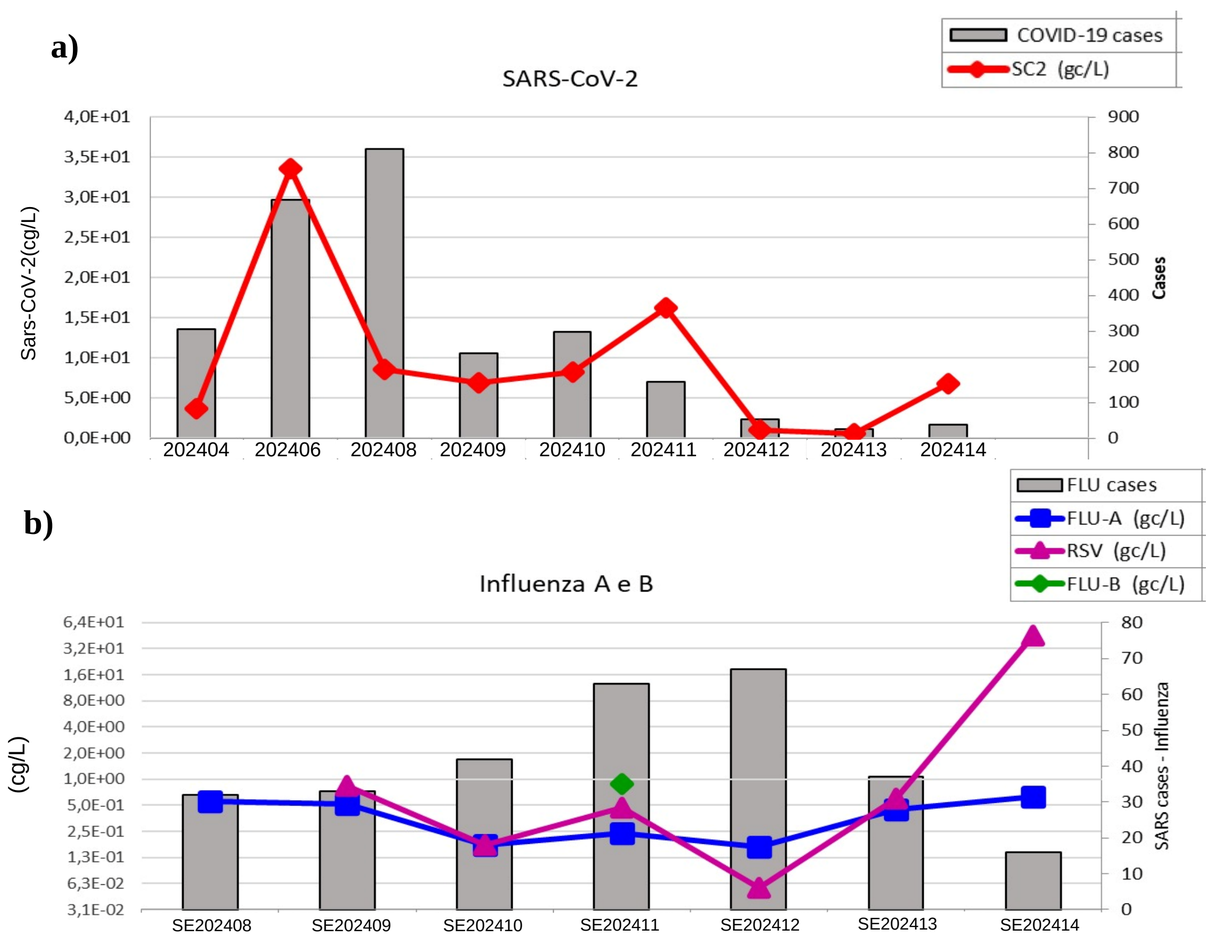


Figure 2. The columns represent the number of COVID-19 reported cases in the neighborhoods of WWTP Alegria, according to SMSRJ (a) and the number of acute respiratory infections according to Infogripe (http://info.gripe.fiocruz.br/) (b). The quantification of SARS-CoV-2, Influenza A, Influenza B and RSV RNA along time is shown in red, blue, green and pink lines, respectively.

Preliminary conclusions

- Despite the 10x smaller volume, **P2 showed better results and a higher detection rate** by using RT-PCR and dPCR. Besides a higher putative sensitivity, P2 may represent savings in processing time and storage space at a laboratory routine.
- **Molecular detection by dPCR seems to have higher sensitivity** than RT-PCR for Influenza A and RSV. Moreover, dPCR dispenses the need of a standard curve for viral RNA quantification. However, cost-benefit analyses comparing both methods should be implemented
- Quantification of viral RNA in wastewater was in line with the current epidemiological scenario in Rio de Janeiro.
- Cost-benefit of other protocols should be evaluated to support WBE as a surveillance strategy in Brazil.

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