

## IVD\_08 - Evaluation of molecular assays for the detection of respiratory viruses with a view to structuring the national Wastewater-based epidemiology program

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**Introduction:** Along COVID-19 pandemic, several countries considered wastewater based epidemiology (WBE) as an additional surveillance approach. WBE works out as an early alert system, as viral concentration in wastewater precedes the increase in incidence in about 1-2 weeks. As a populational approach, it is not limited to clinical cases. Due to diversity in concentration methods that preclude detection, qRT-PCR targets, sample complexity and resulting bias in comparative outcomes, standardization is still a global challenge.

**Objectives:** Aligned with the Health Emergency Department, this study aims the development and standardization of laboratory assay for viral detection to support the establishment of a WBE program for outbreaks and health emergencies.

**Methodology:** Two main protocols were compared: (P1) viral concentration with electronegative membrane filtration (100mL) followed by direct manual extraction and (P2) concentration using magnetic nanobeads (10mL) followed by automated extraction. Virus like particle (VLP) containing SARS-CoV-2 (SC2), Influenza A and B (FLUA; FLUB), Syncytial Respiratory Virus (RSV) were inoculated into WW samples and negative control (0.1 to 0.5uL/mL). SC-2 quantification was determined based on a standard curve. Moreover, sets of primers/probes (Bio-manguinhos) for detection and quantification of respiratory viruses by qRT-PCR were optimized for WW samples.

**Results:** No inhibition of the primers/probes set was observed in the 8 samples tested with VPL, even in low concentrations. In polluted river samples (N=4), SC-2 concentration in P2 (10.8gc/uL ± 5.8) was comparable to P1 (4.4gc/uL ± 4.3) despite using a 10x smaller volume. In sewage samples (N=13) there was no significant difference between P1 (5.08gc/uL ± 6.7) and P2 (2.5 ± 2.4). Although, we identified the presence of FLU-A (3) and RSV (3). The P2 was the easiest handled and fastest one; For 20 samples using P2, 2hrs were necessary in comparison with 6.5h for P1.

**Conclusion:** The P2 protocol, when associated with Bio-manguinhos optimized RT-PCR assay, represents a more useful tool for WBE, reducing the volume of samples and processing time without affecting the sensitivity. This preliminary data shows our ability to optimize the assay with a view to scaling up of a national network.

**Keywords:** Wastewater; Surveillance