Evaluation of PCR-enhancing approaches to reduce inhibition in wastewater samples and enhance viral load measurements

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Molecular-based assays are the most commonly used methods for the detection and quantification of viruses in wastewater. The variety of inhibitory substances present in the complex matrix of wastewater hinders downstream analysis and often leads to false negative results and underestimation of viral load [1]. The development of robust and inhibitor-tolerant detection methods is necessary in the context of wastewater-based epidemiology, a valuable tool that has gained further importance since the emergence of the Covid-19 pandemic. Various strategies are used to mitigate inhibition in the polymerase chain reaction (PCR) with the most prevalent of all: the dilution of the sample and the inhibitor removal kits [2]. In this study, we first indicated the presence of inhibitors in wastewater samples and the evaluation of eight different PCR enhancing strategies were further performed using reverse-transcription PCR (RT-qPCR) protocol. False negative results were eliminated through four approaches evaluated, a 10-fold dilution of the extracted sample, addition of T4 gene 32 protein (gp32), addition of Bovine Serum Albumin (BSA), and using an inhibitor removal kit. Among the methods that removed inhibition, the most significant for the removal of inhibition was the addition of gp32 (at a final concentration 0.2 μg/μl). This optimized protocol was further applied to wastewater samples tested for Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and a direct comparison study was further performed with reverse-transcription droplet digital PCR (RT-ddPCR). The detection frequency of both methods was 100% and the obtained viral concentrations were higher by RT-ddPCR; the optimized RT-qPCR assay showed a good correlation (Intraclass Correlation Coefficient: 0,713, p-value < 0,007) with RT-ddPCR. This is the first study to directly compare common strategies for eliminating inhibition in wastewater and demonstrates the importance of developing robust assays to accurately assess the recovery rates and viral loads of the targets tested, in a simple, cost-effective and high-throughput manner.

[1] W. Ahmed, S.L. Simpson, P.M. Bertsch et al, Sci. Total Environ. 805 (2022), 149877

[2] C. Schrader, A. Schielke, L. Ellerbroek, R. Johne, J. Appl. Microbiol. 113 (2012), 1014–1026