

# Improving Recovery of Respiratory Viruses from Arizona Wastewater



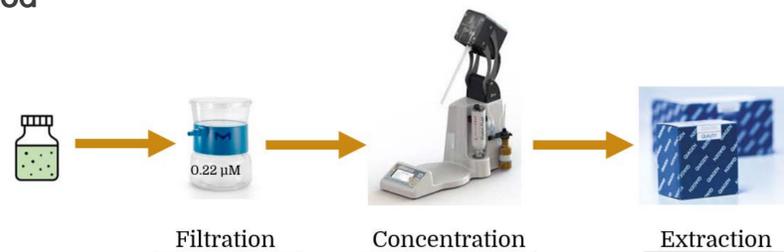
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## Abstract

In 2024, the Arizona State Public Health Laboratory (ASPHL) aimed to enhance the efficiency and quality of wastewater testing. Previously, the wastewater sample processing involved manual concentration using the CP Select and nucleic acid extraction with the QIAGEN AllPrep Powerviral DNA/RNA kit. Bovine Coronavirus vaccine (BCoV) was used as an internal control to assess the quality of wastewater extractions, requiring a minimum recovery of 2% for valid reporting. However, under the previous method, 11.97% of all wastewater samples failed to meet this threshold, likely due to the presence of inhibitors affecting nucleic acid recovery.

To address this issue, alternative concentration, extraction, and inhibitor removal methods were evaluated. The optimized protocol, which demonstrated the highest viral particle recovery, incorporated a 0.45  $\mu\text{m}$  pre-filtration, Ceres Nanotrapp Microbiome Particles A & B, MagMAX Wastewater Ultra Nucleic Acid Isolation Kit, and the Zymo OneStep PCR Inhibitor Removal Kit.

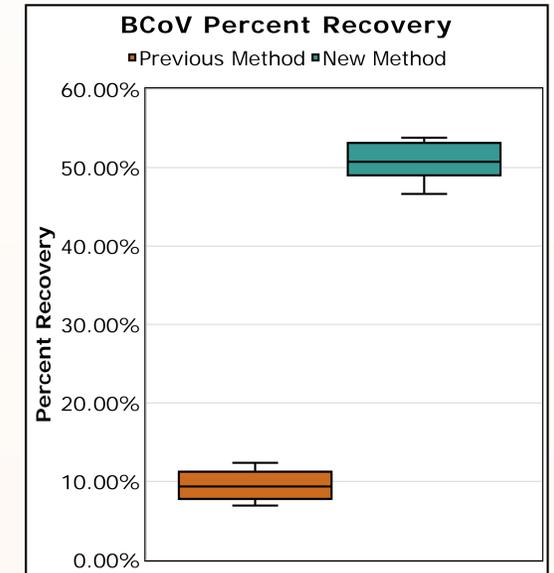
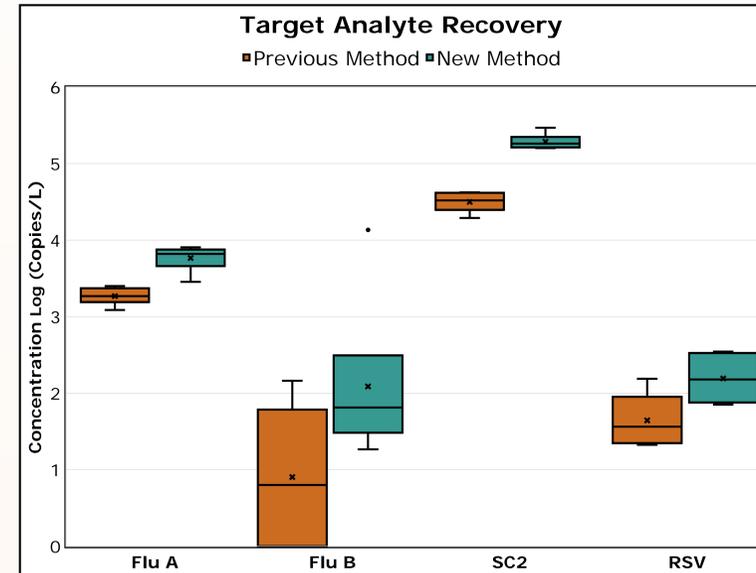
## Previous Method



## New Method



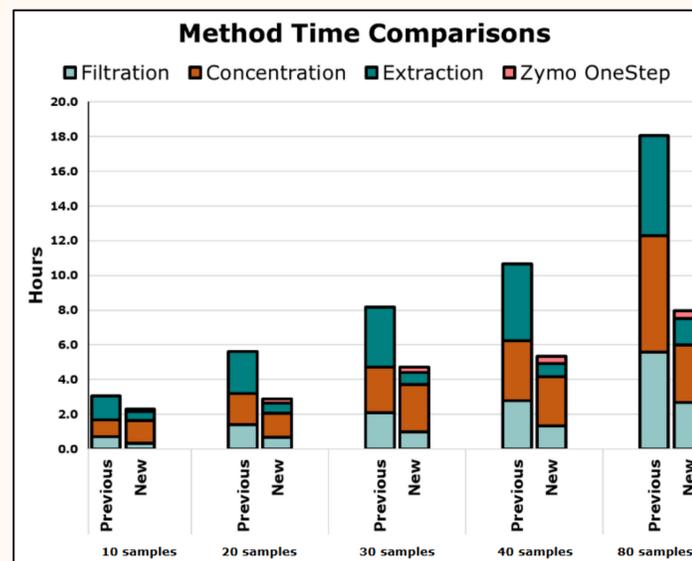
## Results



Inhibitor Mitigation Steps Reduce Average Percent Inhibition

No Inhibitor Mitigation	Filtration (0.45 $\mu\text{m}$ )	Zymo One-Step	Filtration & Zymo One-Step
98.80%	53.98%	80.20%	32.48%

## Improved Efficiency



## Conclusions

Implementation of the new processing method resulted in a **121.7%** increase in average viral recovery across all targets when compared to the previous method.

The new method also allowed for automation, increasing the efficiency of the work day.

These findings suggest that the new processing method significantly enhances the accuracy, reliability, and efficiency of viral detection in Arizona wastewater.