

OVERVIEW AND METHODS

- Recent studies indicate high concentrations of SARS-COV-2 in wastewater sludge (YALE SOURCE)
- Current concentration methods using wastewater require 30-50 mL wastewater per test with 0.01-0.04% solids.
- A comparison between current concentration methods, Imhoff cone settling and solid collection, and sludge centrifugation was carried out to determine the optimal concentration method to maximize Sars-Cov-2 sensitivity for PCR.
- Influent wastewater and primary sludge were collected from the Portland Columbia Boulevard WWTP on 7/21 and 7/29.
- Concentration Methods:
 - Wastewater: electronegative filtration
 - Imhoff cones: solids collected after settling for 2, 3, and 4 hours, with some batches being acidified or kept in ice.
 - Sludge: centrifuged in a standard centrifuge and a min-spin centrifuge
- All samples had PCR processing done at Oregon State University with N1, N2, and RP primers.

WASTEWATER SOLID CONCENTRATION METHODS TO MAXIMIZE SARS-COV-2 SENSITIVITY

A comparison of wastewater filtration, settled solids, and centrifuged sludge for N1 and N2 PCR sensitivity.

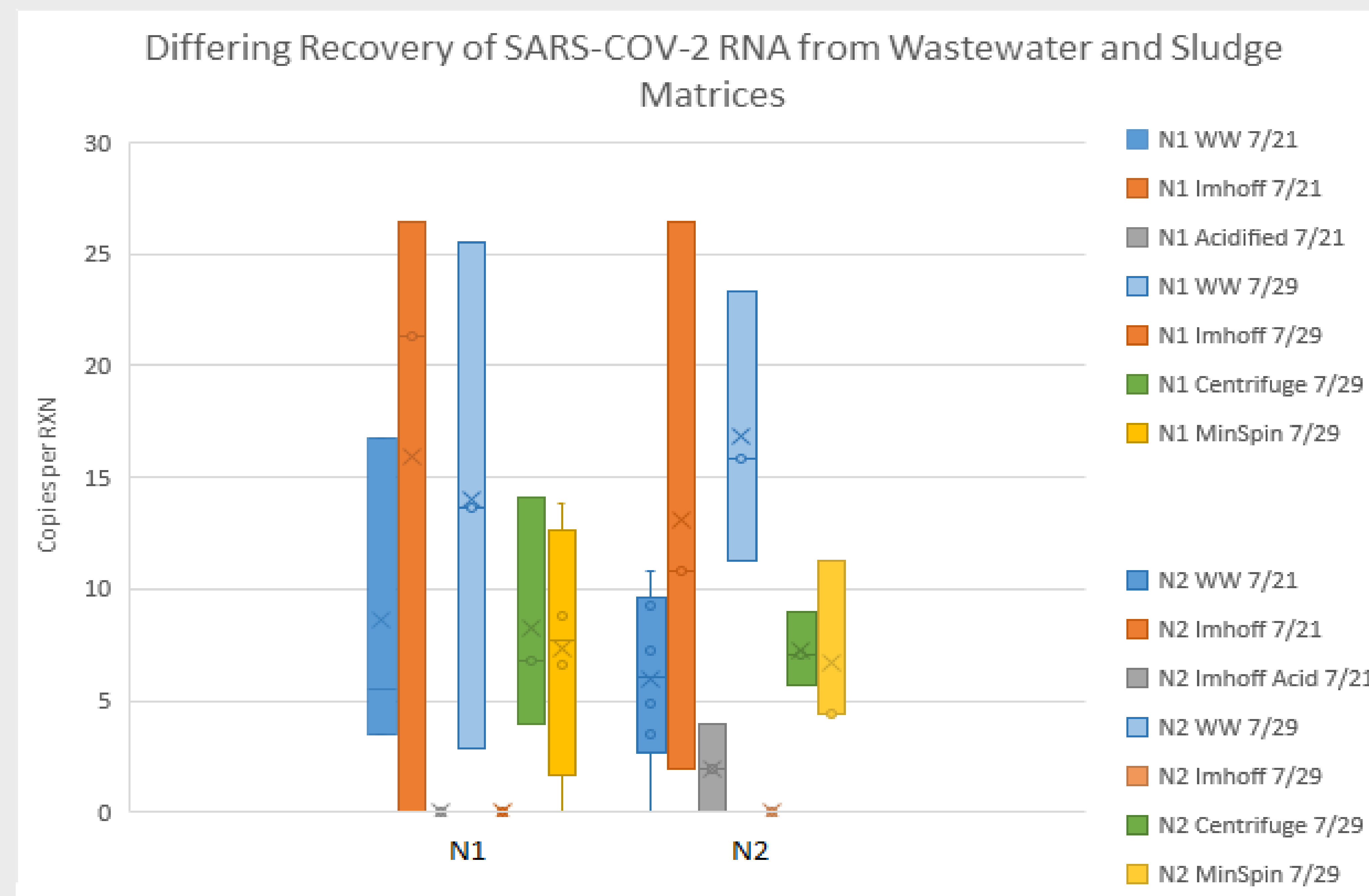


Figure 1. Comparison of concentration methods and their respective copies per RXN of N1 and N2 primers via dd-PCR. 7/21 data indicates wastewater filtration and Imhoff cones produce the highest sensitivity, but zero data for Imhoff cones on 7/29 is being investigated.

ABSTRACT

The use of sewage-based wastewater analysis to track or confirm the presence of a disease is becoming more important as we look for ways to protect communities from COVID-19. As more communities move forward with viral studies, maximizing SARS-COV-2 PCR sensitivity is vital. Filtration of wastewater via electronegative filters was compared with solids collected from Imhoff cones and primary sludge that underwent centrifugation.

Wastewater filtration and Imhoff cones produced the highest copies per RXN, with centrifugation being lower likely due to a significant decrease in volume (2 mL of sludge vs. 30+ mL wastewater). Imhoff cone results for 7/29 were inconclusive, and tests are being rerun to confirm 7/21 data indicating Imhoff cones give higher yield than standard wastewater filtration methods.

DATA

7/21 data indicates Imhoff cones give greater copies/RXN when compared to conventional filtration.

- 7/29 data indicates centrifuge is comparable to wastewater, but requires 2 mL of sludge compared with 30 mL wastewater.
- 7/29 data initially gave 0 results for Imhoff cones. Tests were rerun with additional 7/29 wastewater for varied settling times and temperatures, still resulting in 0 data.

- Longer settling time or increased temperatures may have contributed to 0 results.

RESULTS AND FUTURE WORK

- Current filtration methods or Imhoff settling seem to offer highest yield and ease of process.
- Continued testing to confirm Imhoff required.
- For future work with OHA and treatment plants across the state, electronegative filtration or Imhoff cones should be used. Electronegative filtration uses equipment plants would already have for TSS testing and takes a few minutes per sample. Imhoff cones could be implemented in cases where many samples are needed to be ran at one time.
- Decay rate of SARS-COV-2 RNA being analyzed using Imhoff cone time tests (2, 3, and 4 hours).

