EPA/ORD Method for SARS-CoV-2 Wastewater Monitoring

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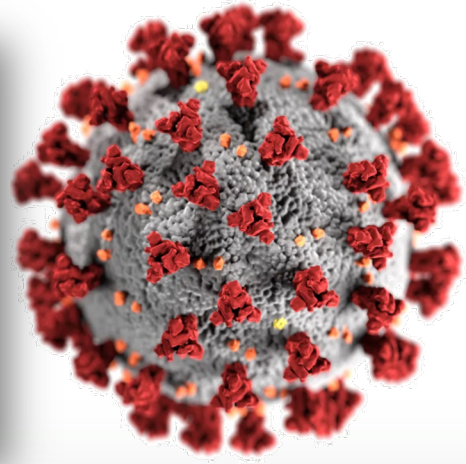
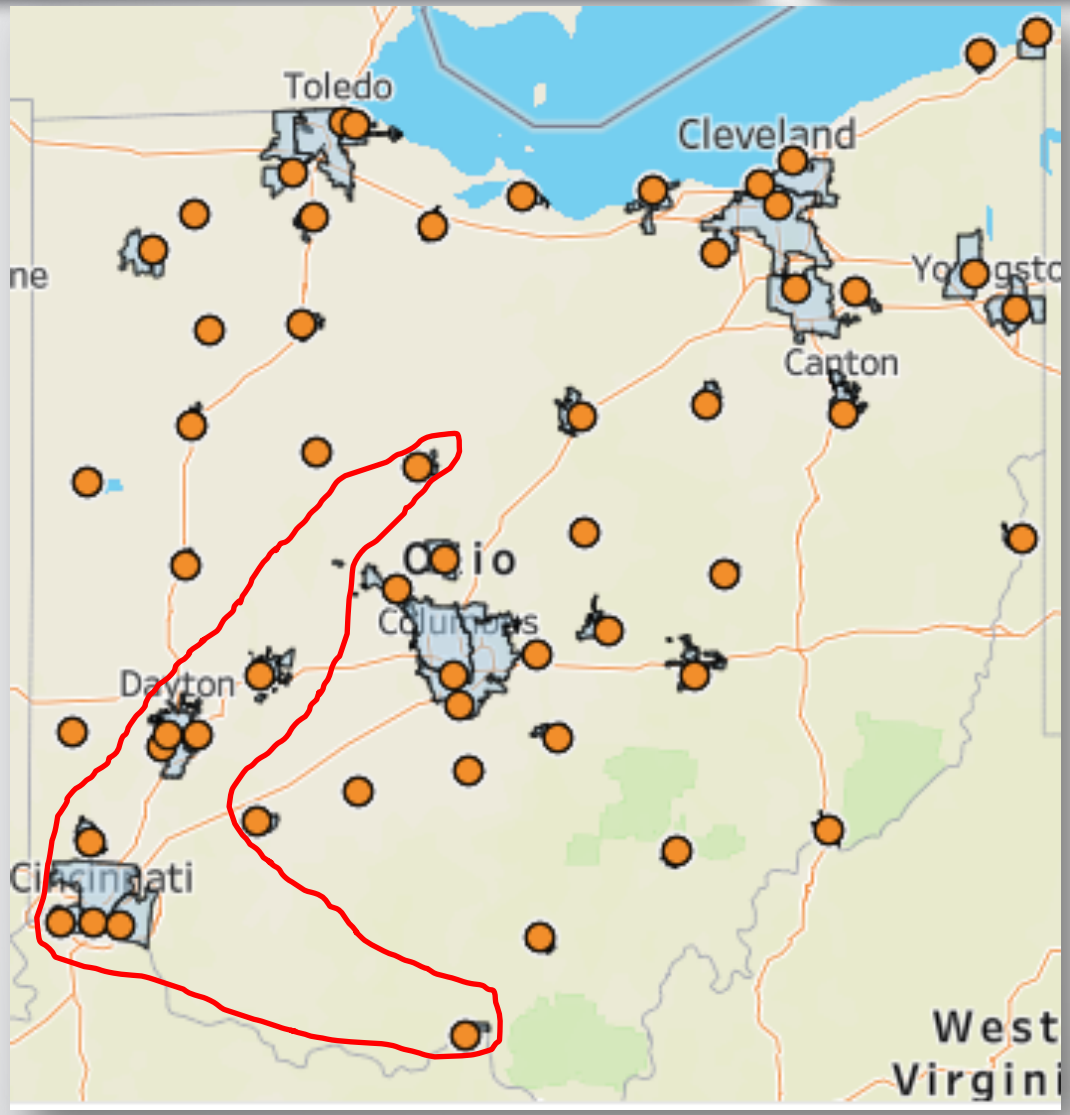
Office of Research and Development



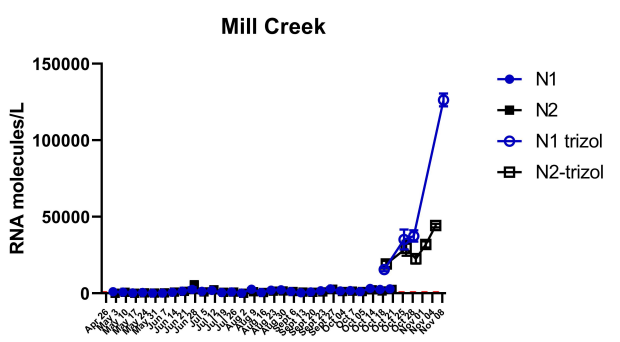
OH Coronavirus Wastewater Monitoring Network

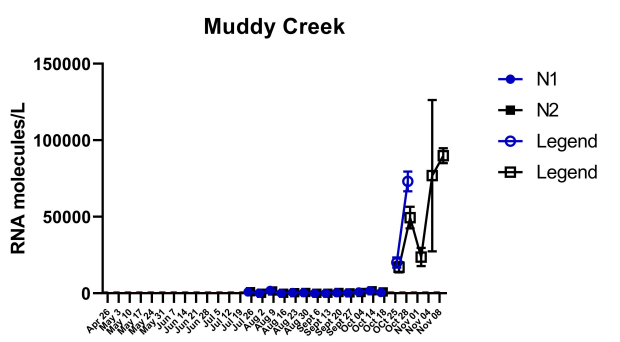


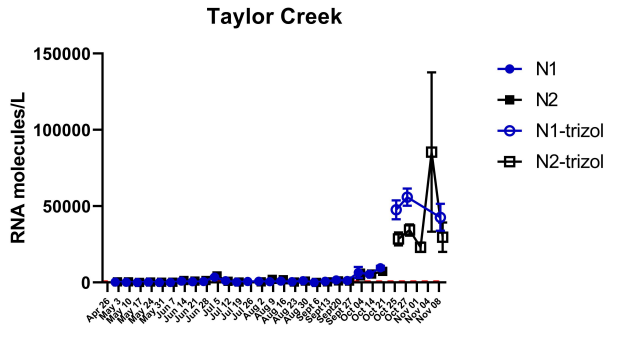
- **Initiated in May by Gov. DeWine**
- **Coordinated by Ohio Water Resources Center at OSU**
  - **Ohio Department of Health**
  - **Ohio EPA**
  - **56 wastewater utilities**
  - **University labs**
  - **Commercial labs**
  - **USEPA/ORD-Cincinnati**

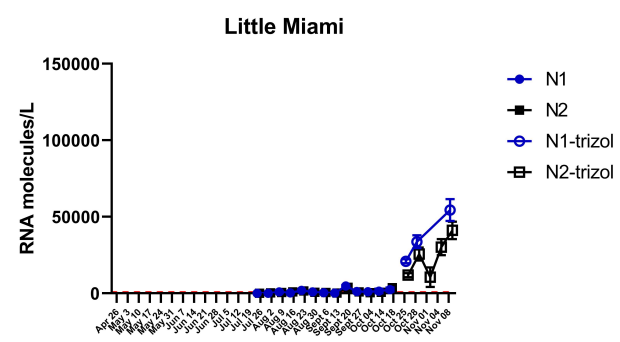
https://coronavirus.ohio.gov/wps/portal/gov/covid-19/dashboards/wastewater

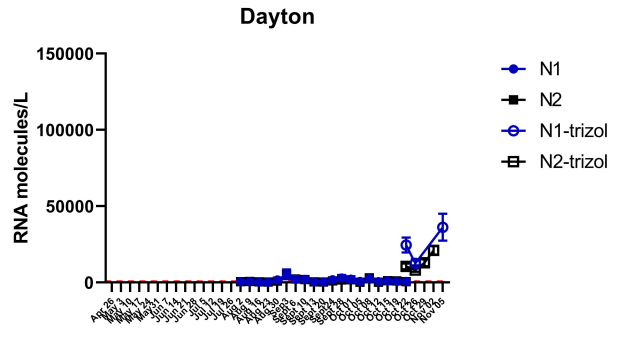
Temporal Trends of SARS-CoV-2 in Sewersheds

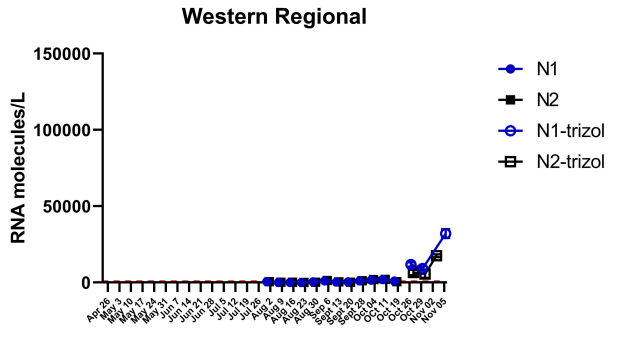


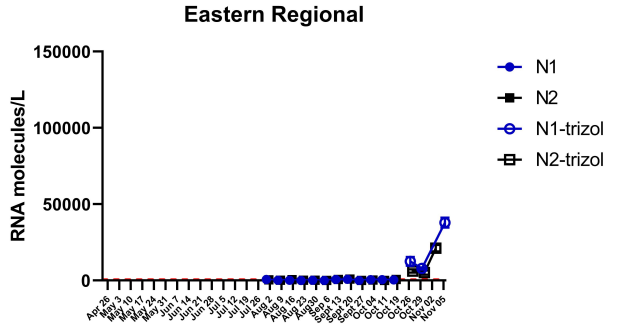


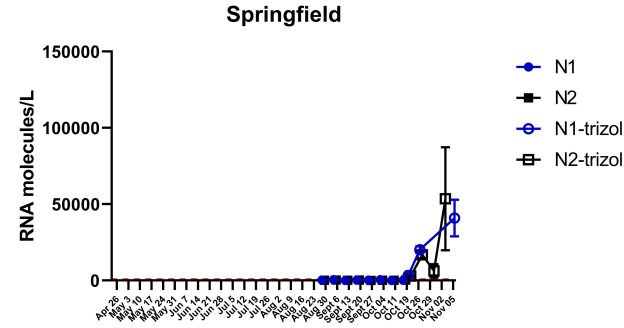


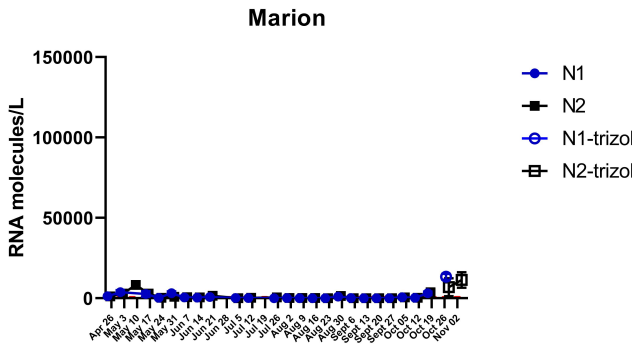


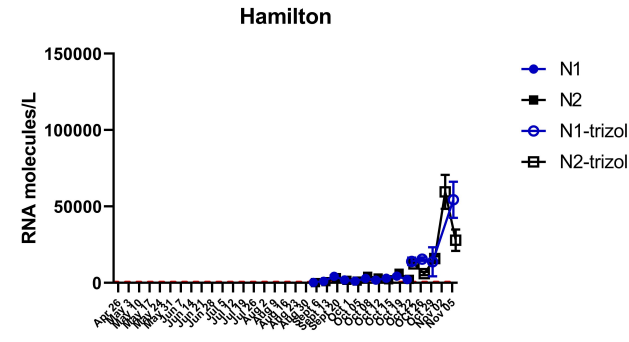










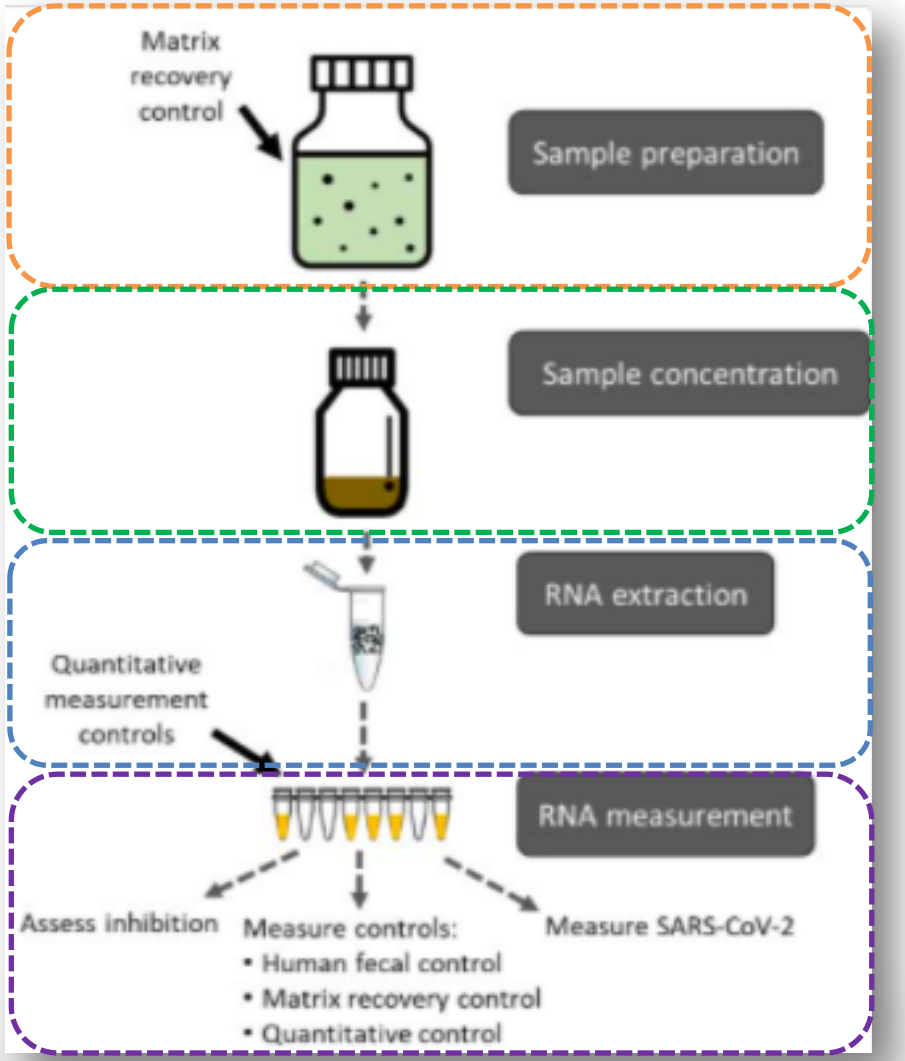


Method Considerations

Sample Type
Untreated wastewater
Primary sludge
Volume

Sample Preparation
Storage temperature
Homogenization
Additives
Matrix Spike
Clarification

Sample Concentration
Ultrafiltration
Electronegative membrane filtration
Polyethylene glycol (PEG) precipitation



Nucleic Acid Extraction
Silica columns
Magnetic beads
Precipitation

RNA/DNA Measurement
RT-qPCR
RT-ddPCR
Genetic targets

Other Considerations
Biosafety
Supply Chain issues
Practicality (time, equipment)
QA/QC

https://medrxiv.org/cgi/content/short/2020.11.02.20221622v1

https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance/testing-methods.html

https://cwn-rce.ca/covid-19-wastewater-coalition/phase-1-inter-laboratory-study/

Sample Processing and Analysis

PBS
OC43

24-hr composite sample, 225 ml

Centrifuge
3000 x g, 15 min

Super-natant

Membrane filtration, 0.45µm

Filtrate

Ultrafiltration, 30 kDa MWCO

0.2 ml Direct Extraction

Pellet

Filter

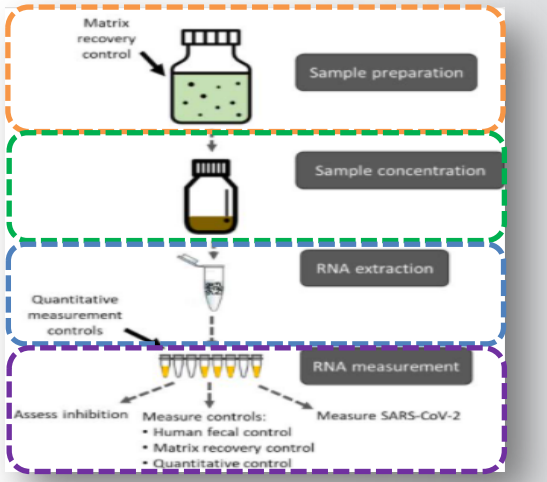
UF Retentate

Phi6

Nucleic Acid Extraction (RNeasy Power Water Kit – silica column)

RT-ddPCR:/ddPCR

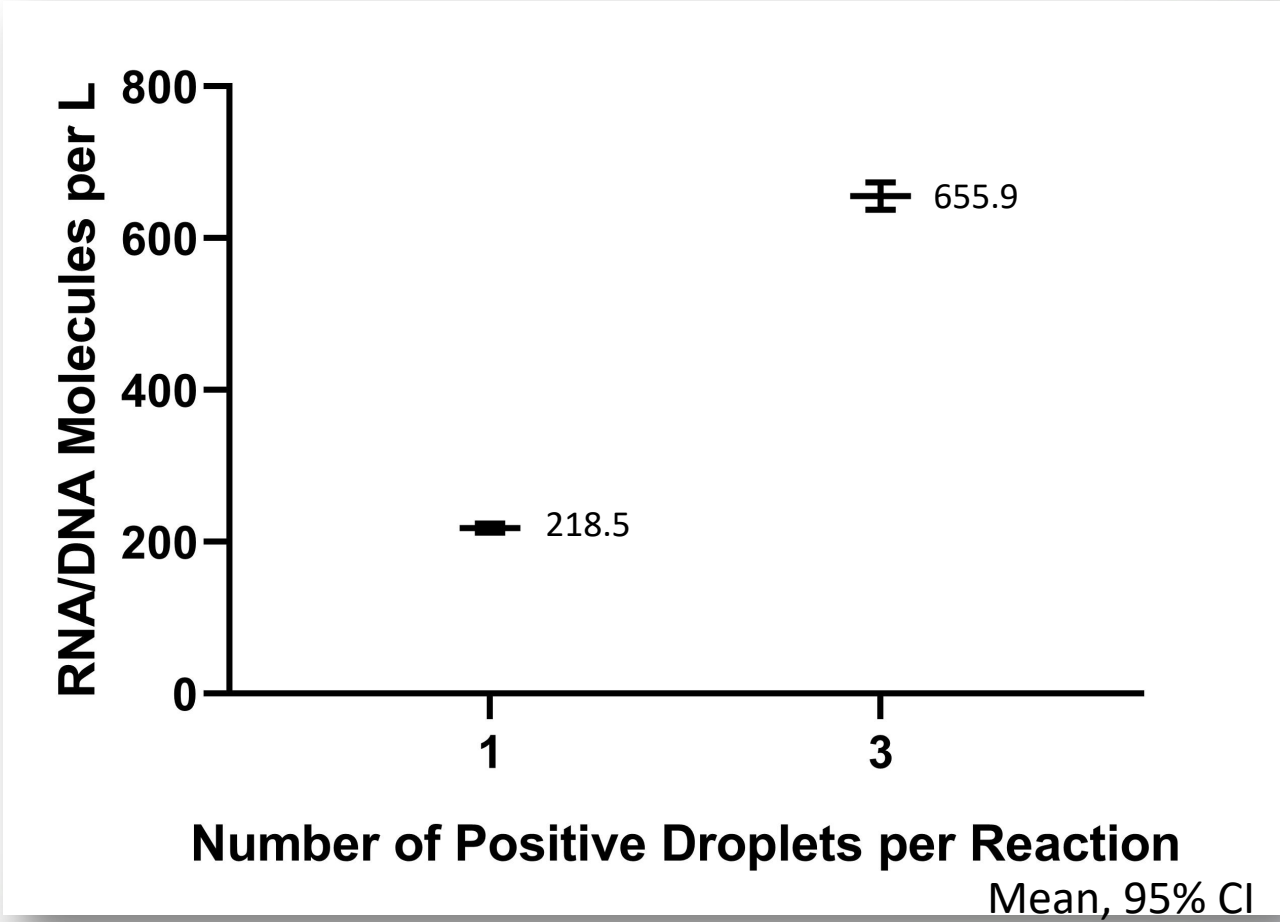
SARS-CoV-2 (N1, N2), RT-ddPCR QC, Inhibition control, Extraction Control, Matrix Recovery Control, Human fecal markers



Limits of Detection/Quantification

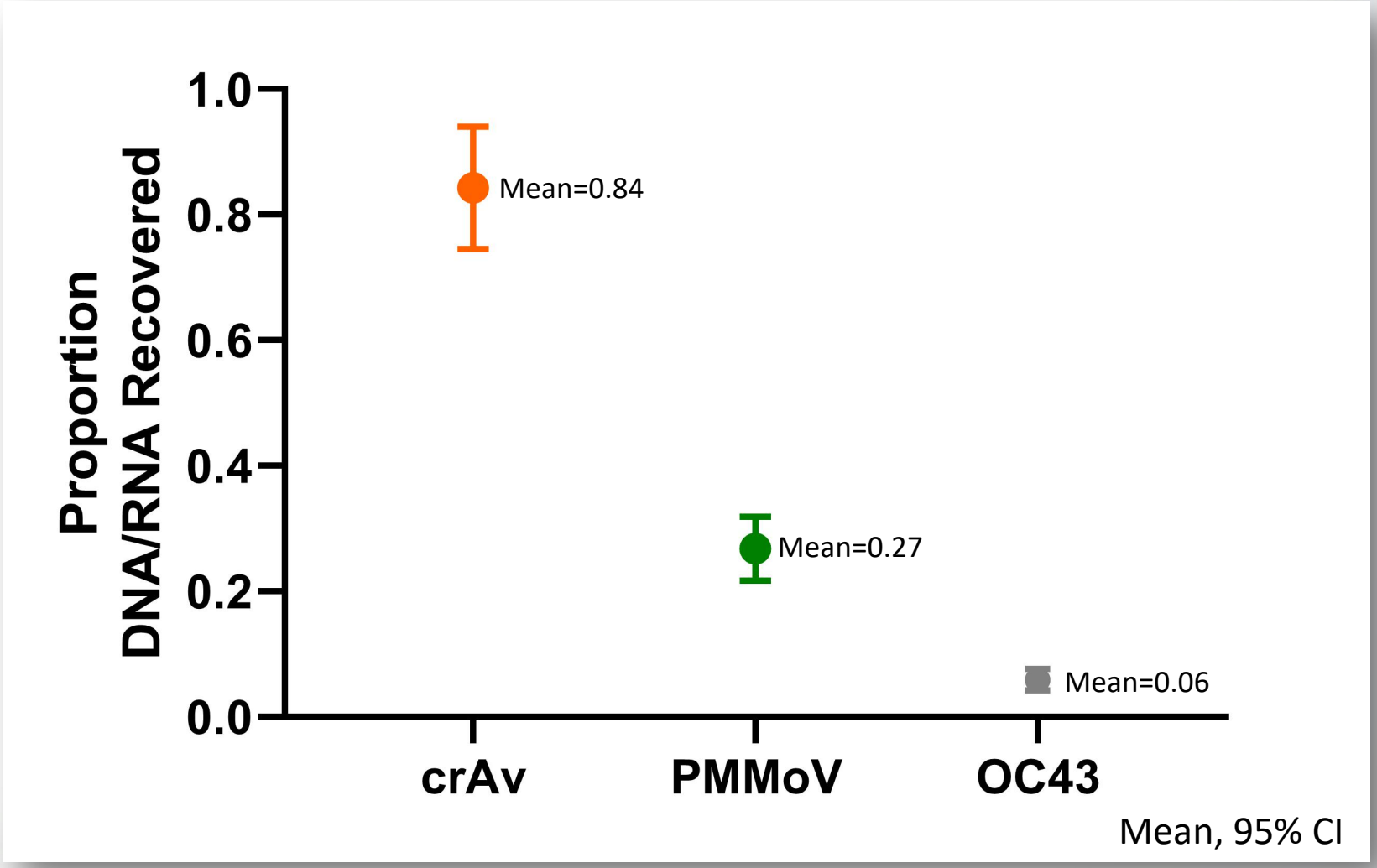
- Volume of sample processed
- Concentration factor
- Volume of processed sample analyzed
- Analytical sensitivity (i.e., minimum detectable concentration)

- Ideal conditions
- Practical limits likely higher due to losses during processing



Recovery Efficiency of Endogenous and Spiked Virus

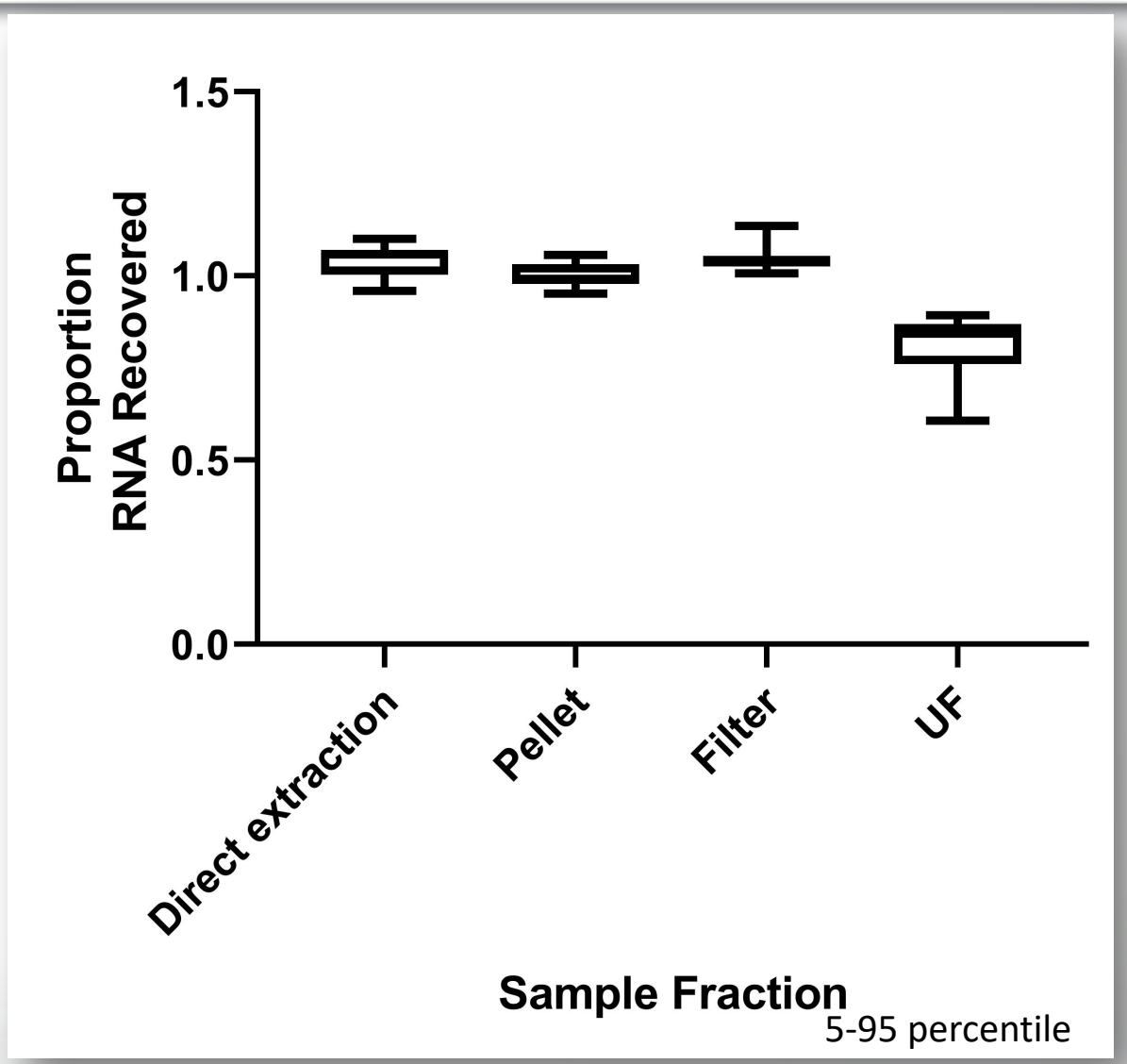
- **Endogenous virus**
  - crAssphage
  - Pepper Mild Mottle Virus
- **Spiked virus**
  - OC43
- **Measure concentrations before and after sample processing**



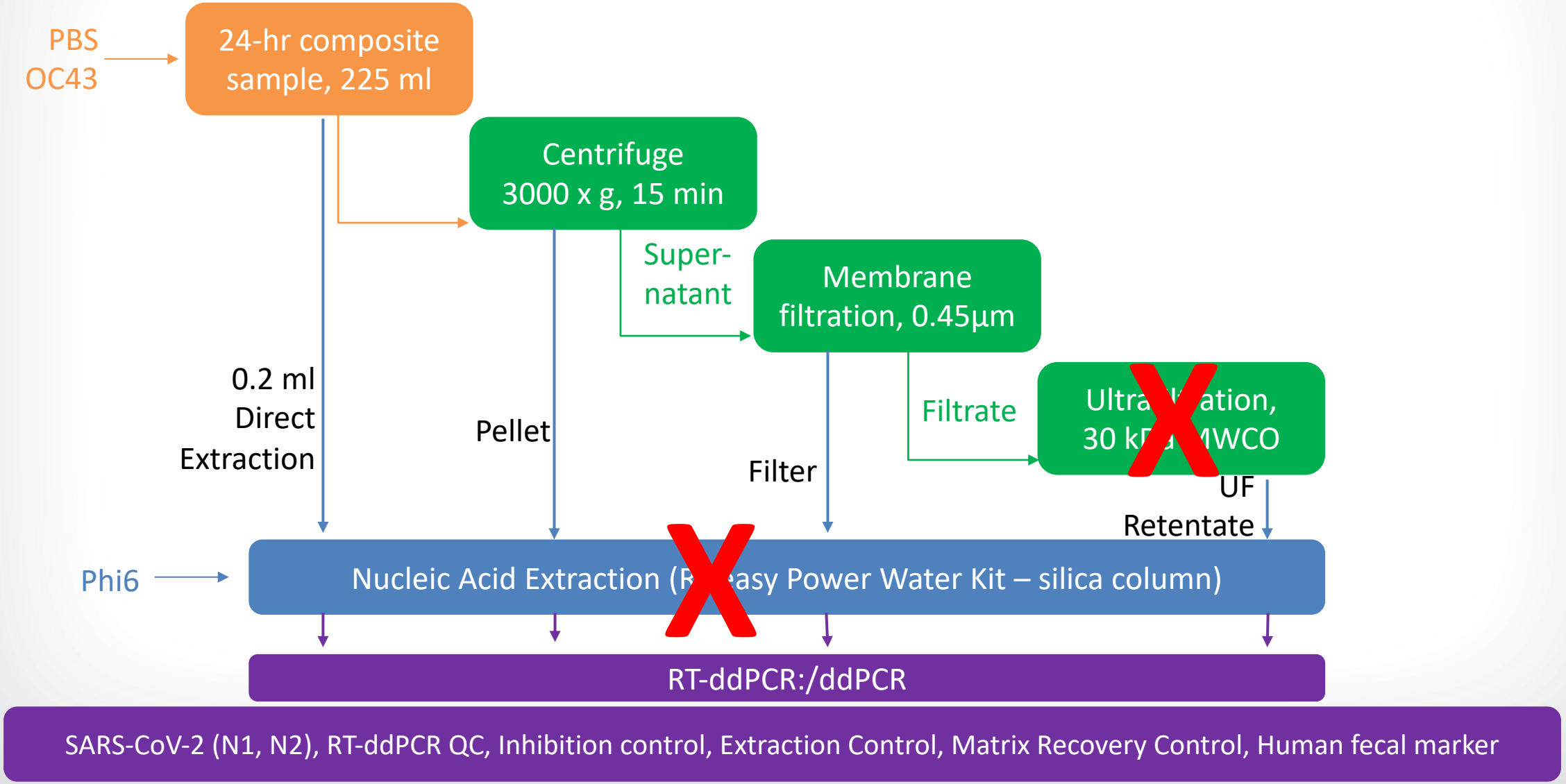
RT-ddPCR Inhibition

- Add RNA before RT-ddPCR
- Compare RNA concentration in sewage sample extracts and matrix-free controls

- Minimal RT-ddPCR inhibition observed

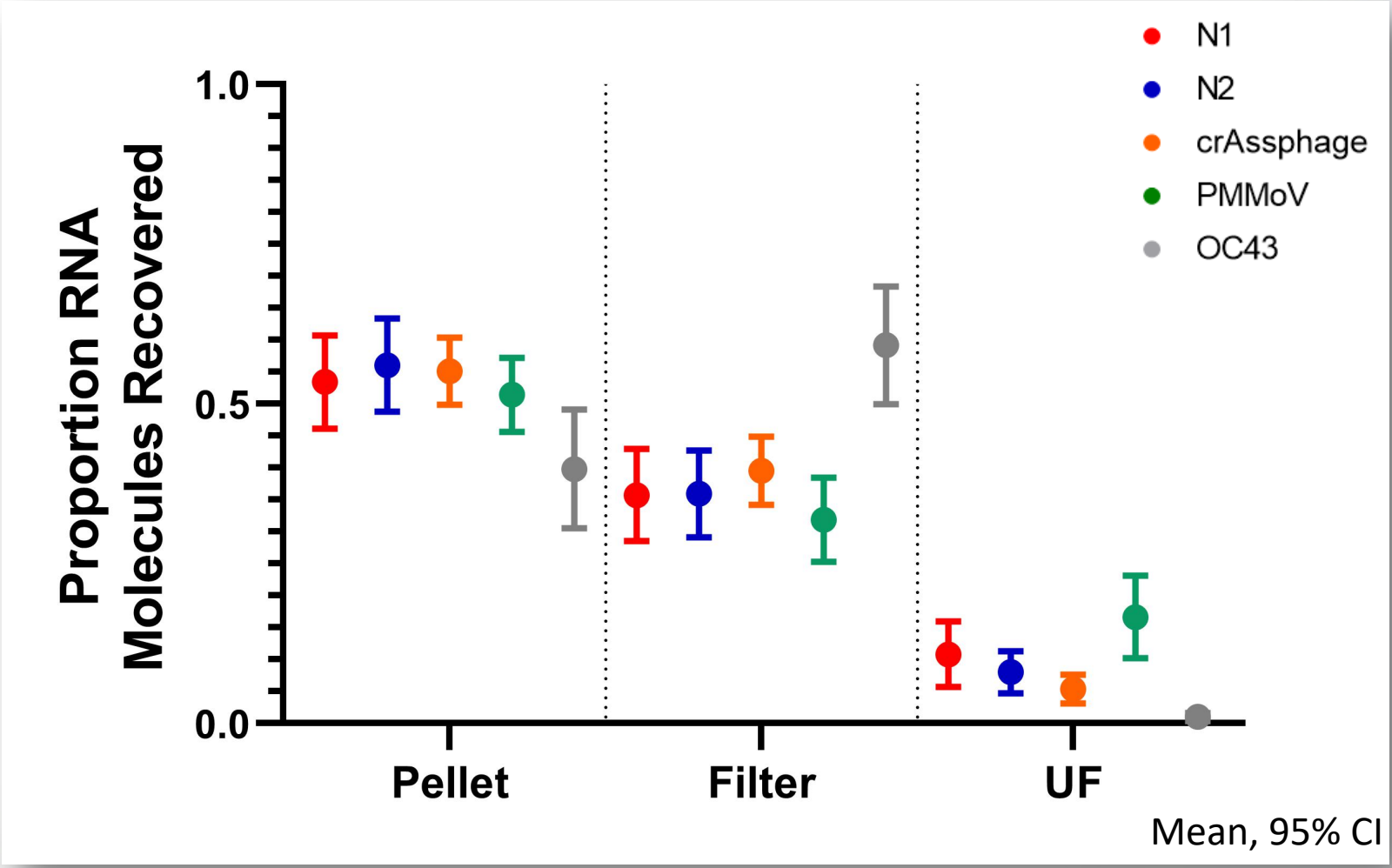


Supply Chain Issues

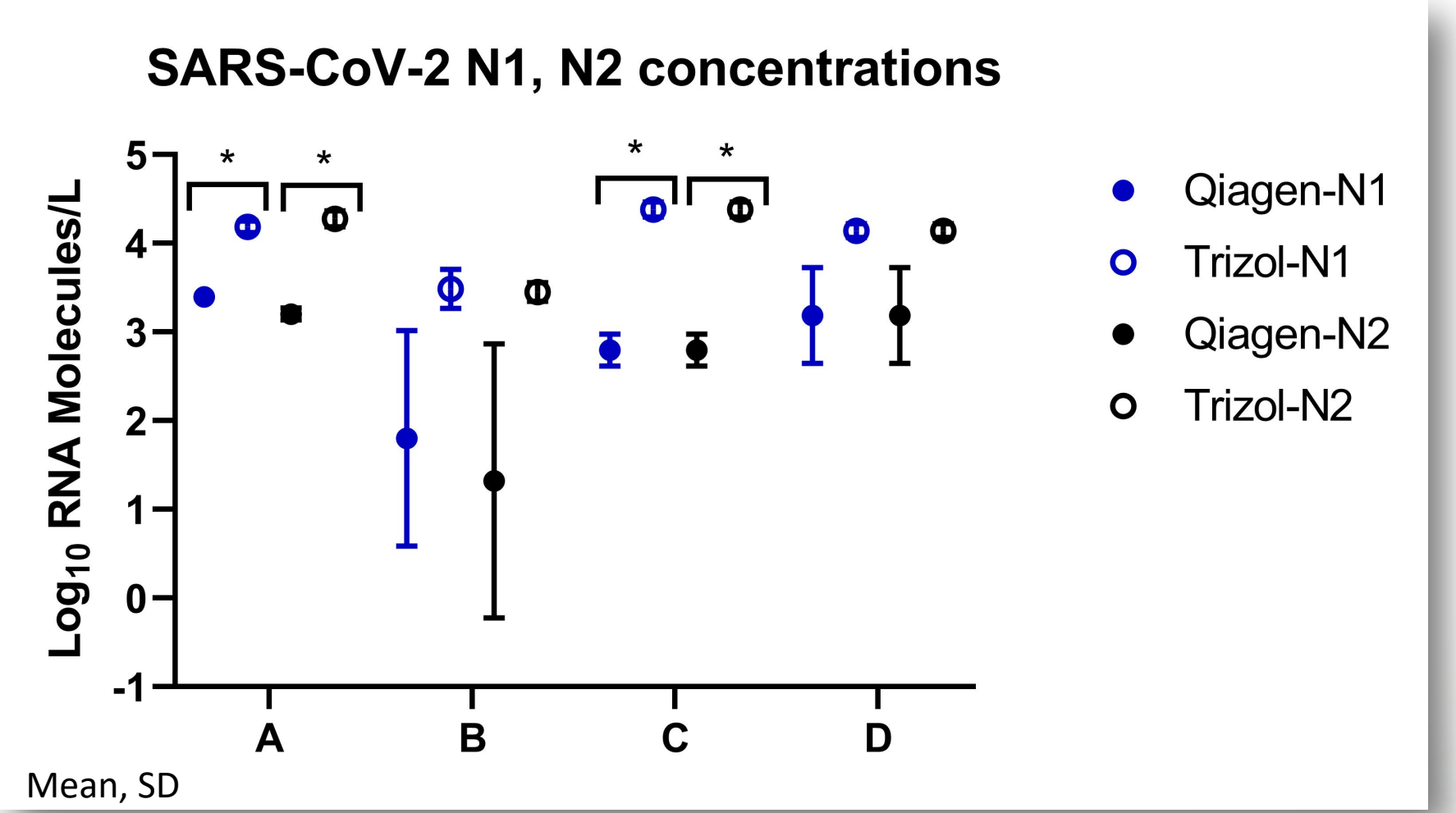


Partitioning of Virus in Sample Fractions

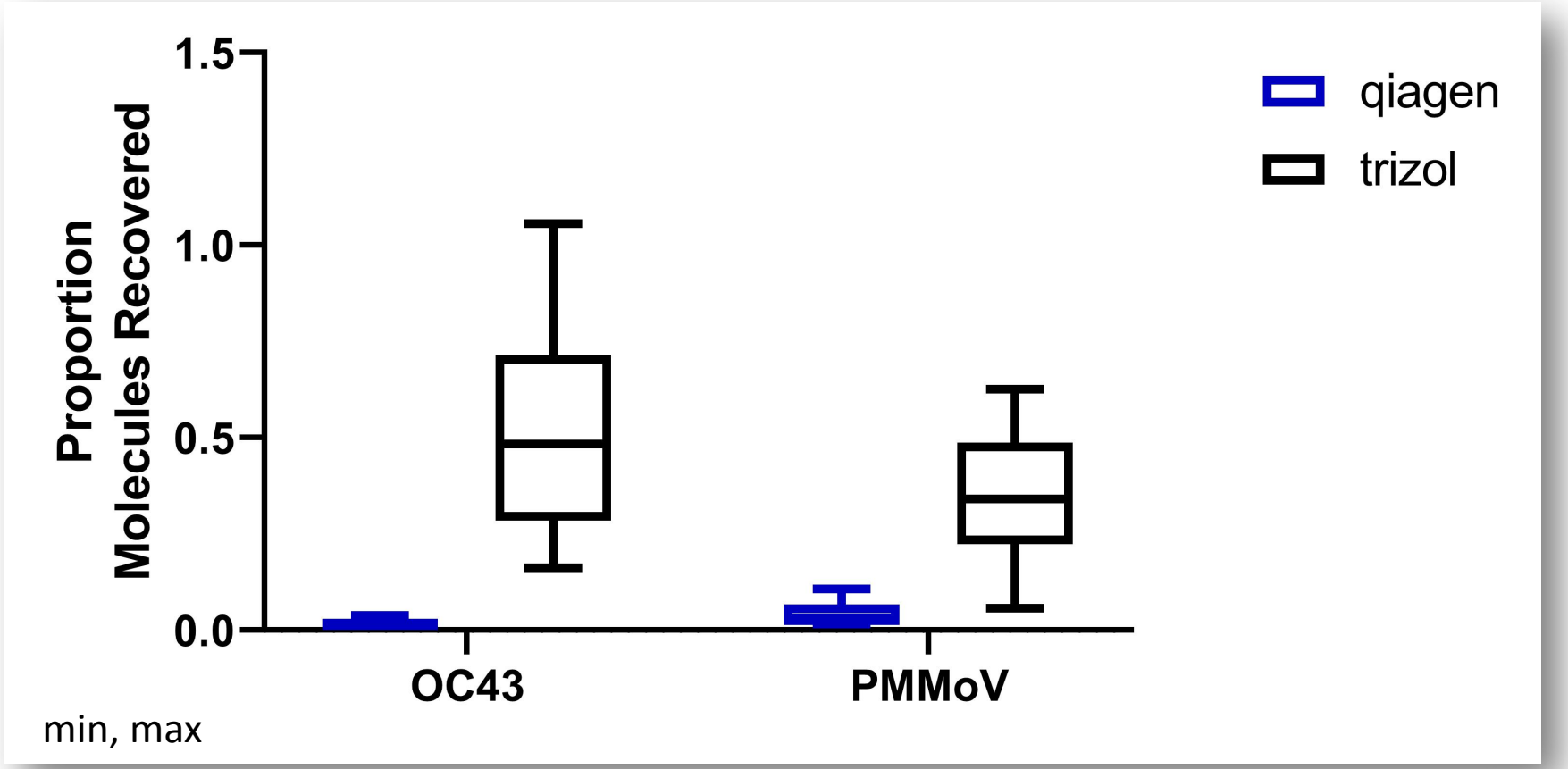
- **Where are viruses recovered within samples?**
- **Proportion of total virus measured in each sample fraction**
- **~ 90% measurable virus in pellet and filter fractions**



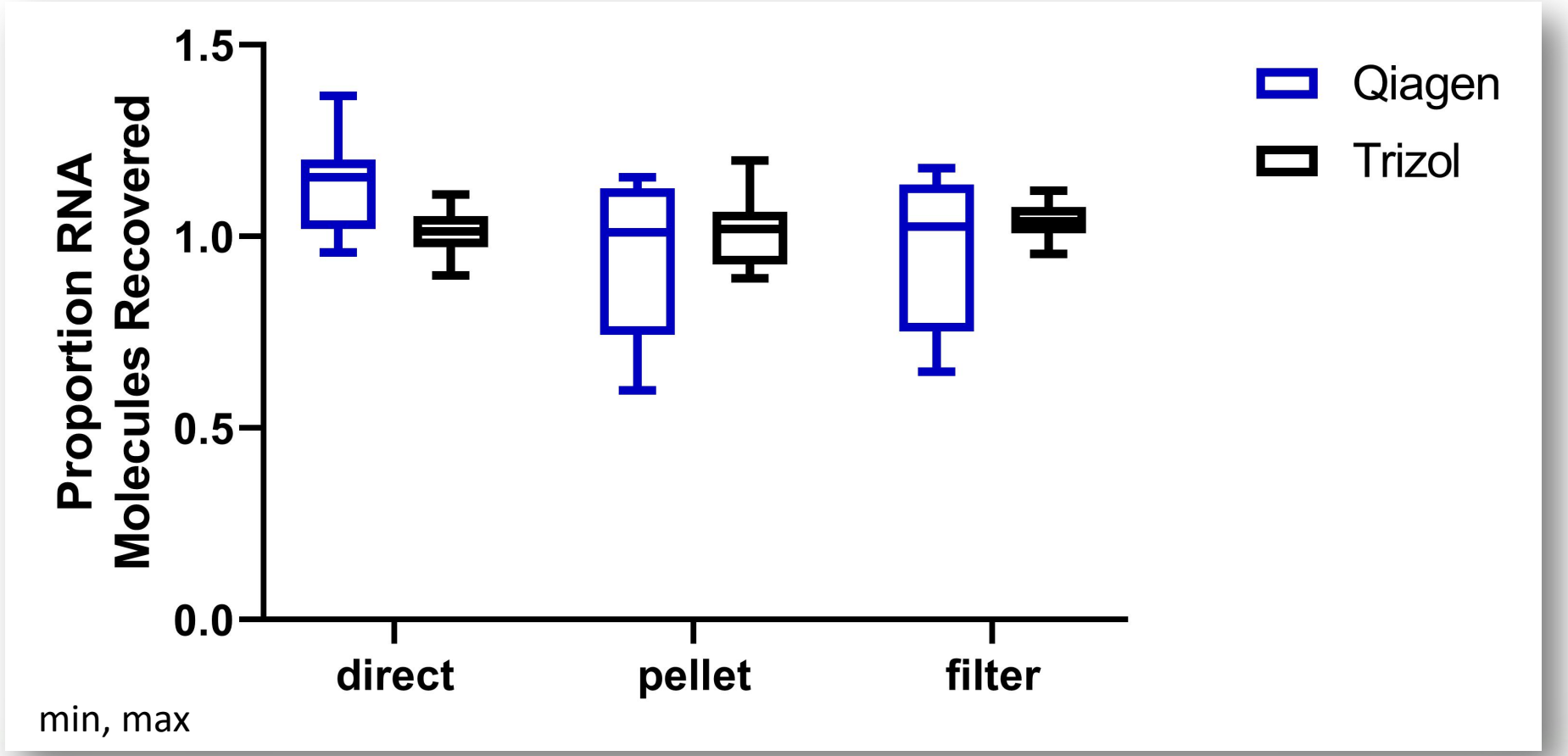
Trizol-chloroform and RNA precipitation



Recovery Efficiency of Endogenous and Spiked Virus



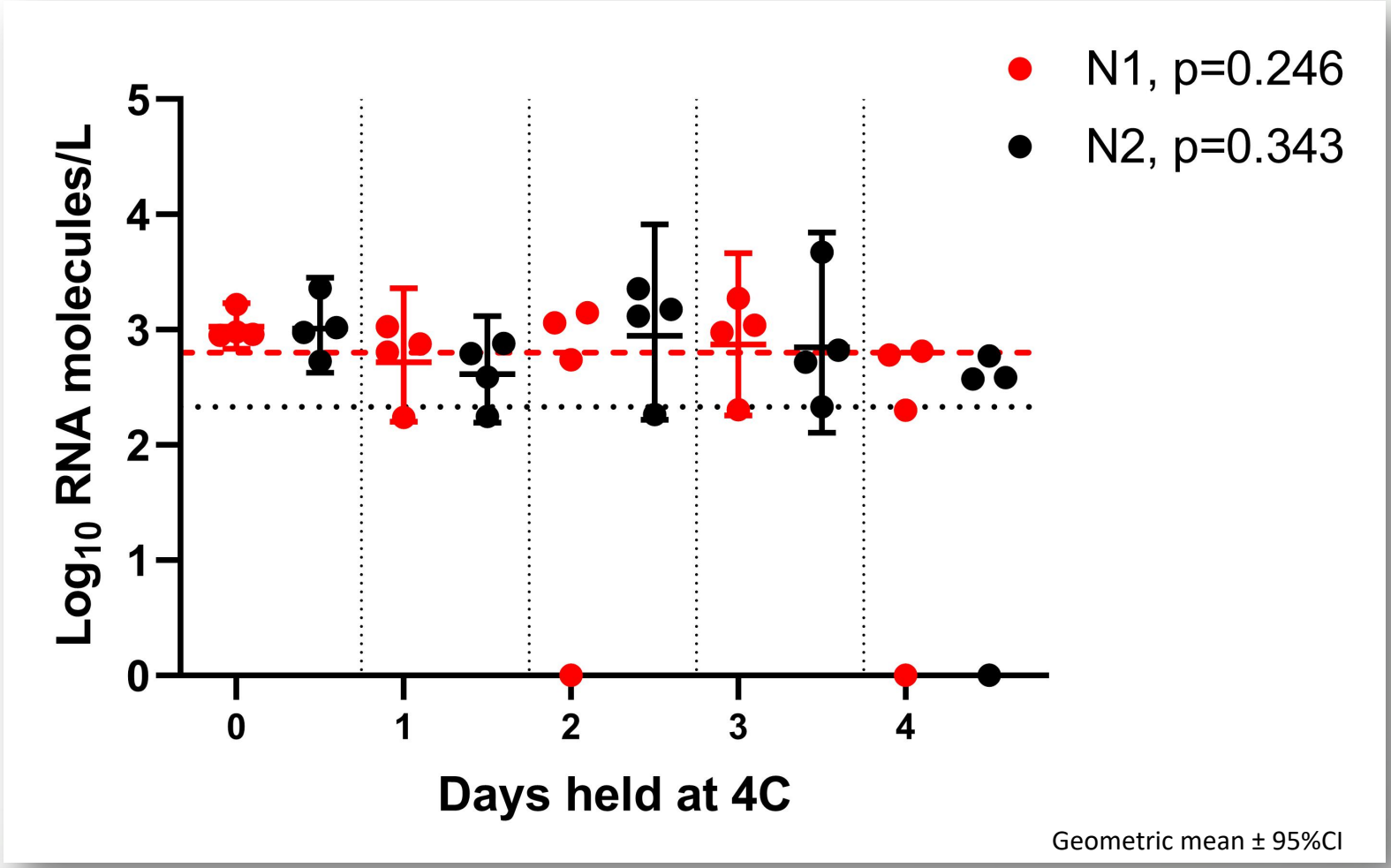
RT-ddPCR Inhibition



Sample Storage at 4°C

- 24- hour composite
- Shipped overnight
- How long can samples be stored before a significant decrease in viral RNA is observed?

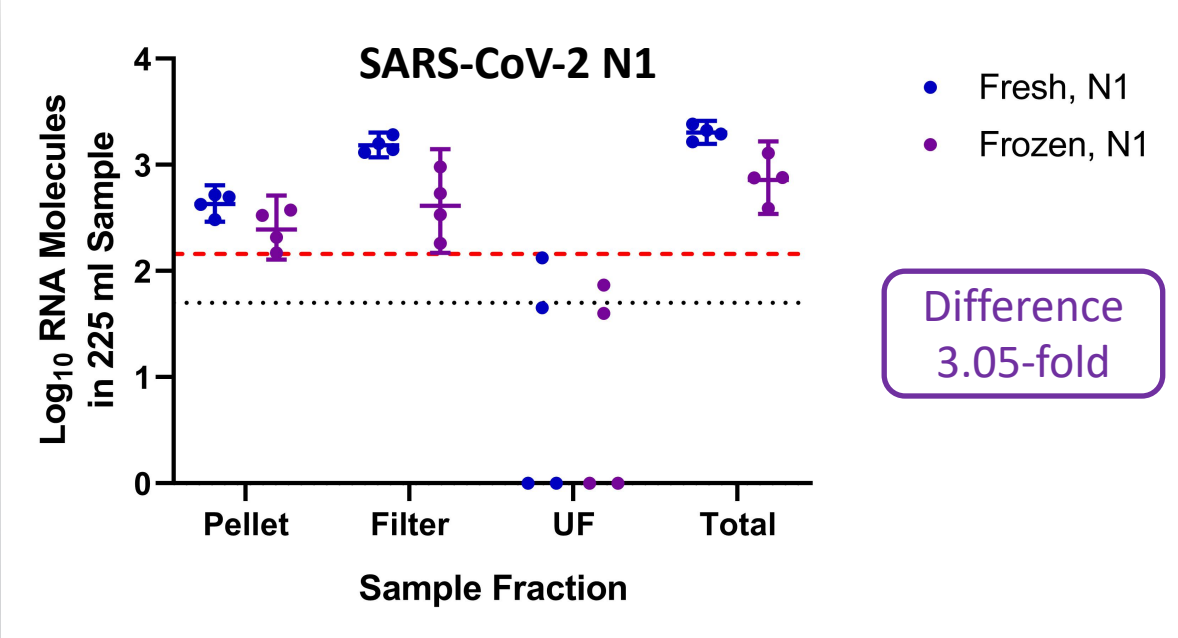
- No significant difference in SARS-CoV-2 RNA up to 4 days at 4°C

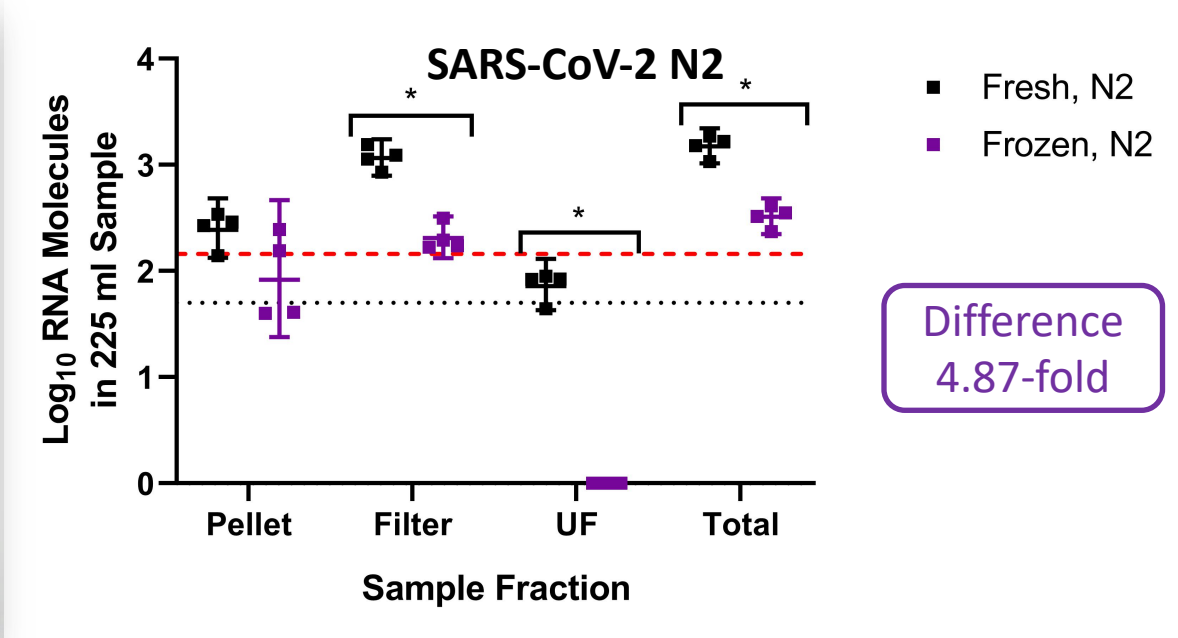


p-values for repeated measures ANOVA with time

Sample Storage at -70°C

- What is the effect of freeze-thaw cycle on detection of SARS-CoV-2 RNA?
- Sample collected and processed immediately
- Subsample frozen at -70°C, thawed at 37°C





Summary

- Wastewater is a complex and variable matrix
- No standard method for measuring SARS-CoV-2, but many options available
- Quality Control important for assessing method performance and matrix variability
- Supply chain can be disrupted
- Sample handling conditions – need more data
  - Temporary storage – no reduction at 4°C up to 4 days
  - Long-term storage – up to 5-fold reduction after freezing at -80 °C
- Continue to measure SARS-CoV-2 in wastewater at specified sites
- Focus on trends or significant changes in the concentration of viral RNA detected
- Continue to work with ODH to interpret trends for appropriate public health applications

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