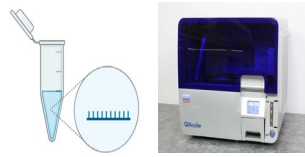
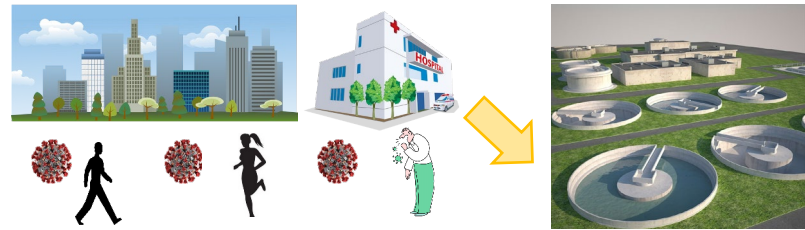


6- Detection and quantification of SARS-CoV-2 RNA with RT-ddPCR

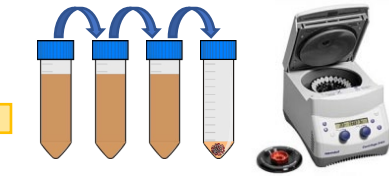


5- Viral RNA extraction

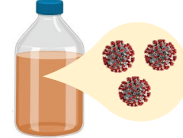


1- COVID-19 prevalence

2- Wastewater Treatment Plant



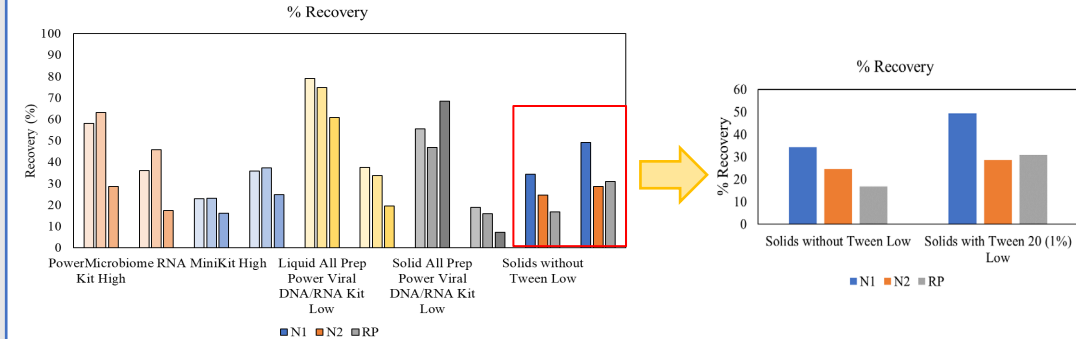
4- Virus concentration and precipitation with PEG/NaCl (liquid), direct extraction from solid



3- SARS-CoV-2 in Wastewater

Wastewater-Based Epidemiology

Analytical Performance & Results



Method standardization included the assessment of

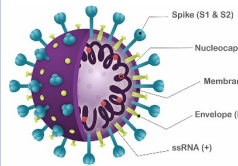
Liquids:

- RNA recovery (~48%)
- Limit of detection (182 cp/100 mL)

Solids:

- RNA recovery (~38%)
- Limit of detection (913 cp/g)

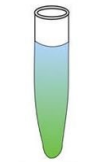
Why ddPCR?



Taken from Andrade Santos et al., (2020)

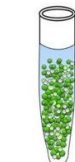
- SARS-CoV-2, the virus that causes COVID-19 is a RNA virus (ssRNA)
- Nucleocapsid (N) protein is responsible for defense and replication
- We use “primers” recommended by the CDC that target N1 and N2 regions of the nucleocapsid (N) gene, and quantify how many copies are present

RT-qPCR



One fluorescence measurement

RT-ddPCR



Thousands of fluorescence measurement

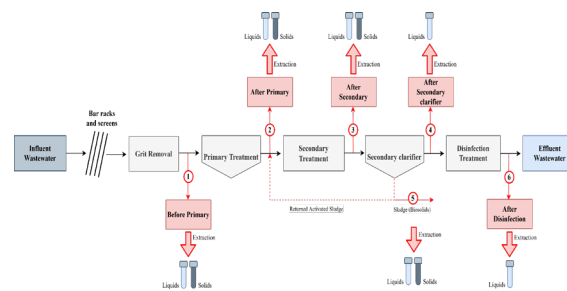
ddPCR advantages:

- Absolute quantification
- Higher precision
- Higher reproducibility
- Higher sensitivity

ddPCR disadvantages:

- Cost
- Time
- Specialized expertise to set up the reaction and analyze results

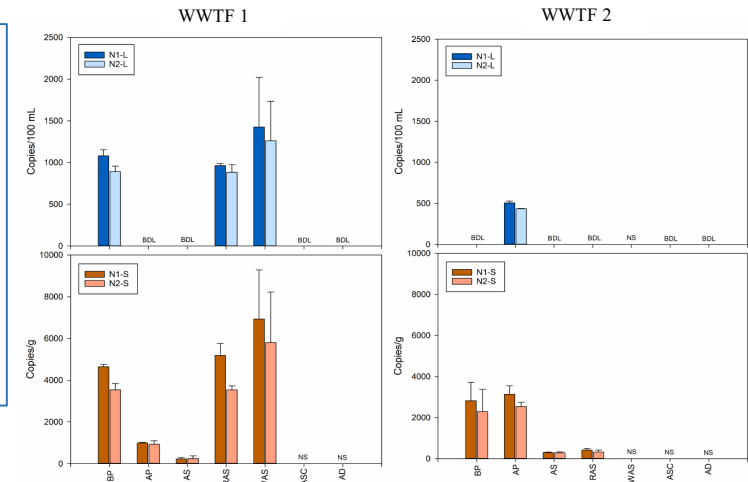
Process-based Study



- The efficacy of treatment processes and disinfection systems in viral RNA removal needs to be assessed
- Confirmation that the virus is removed prior to surface discharge and knowledge on its fate within the treatment train (e.g., sludge, air, liquids) is critical for proper public health management.

Preliminary results revealed:

- Inflow, primary treatment, RAS, and WAS contained SARS-CoV-2 biomarkers
- No SARS-CoV-2 biomarkers were present after secondary treatment phases, or after disinfection (effluent).
- The solid phase shows a 400x fold higher biomarker equivalent concentration than the liquid phase



Future Work Questions

6 out of 7 WWTFs have been sampled; 4 of 7 have been analyzed. Collection and analysis will wrap up February 2021.

Special thanks to everyone in Mouser Lab, WWTFs, including superintendents and operators, and New Hampshire Sea Grant Development Fund for making this work possible.