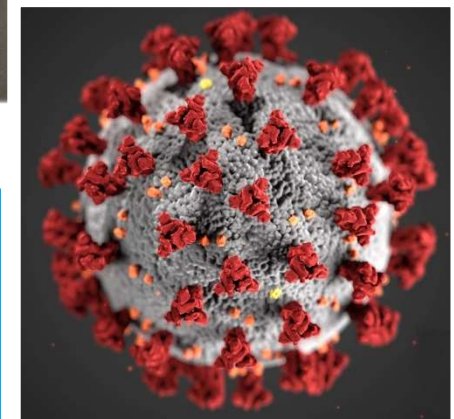


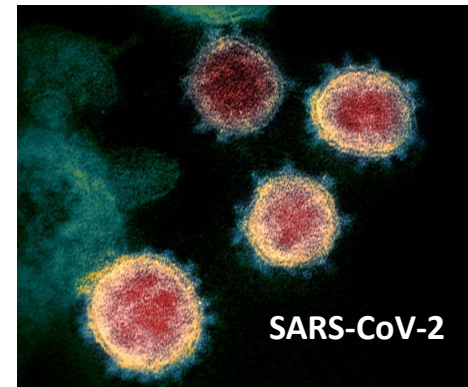
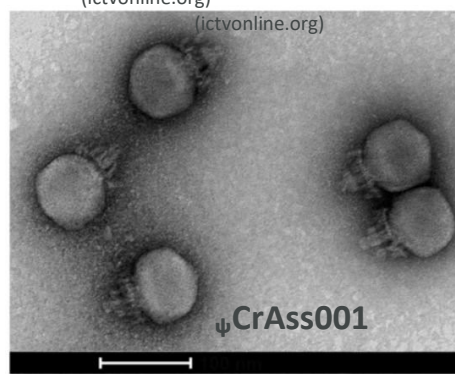
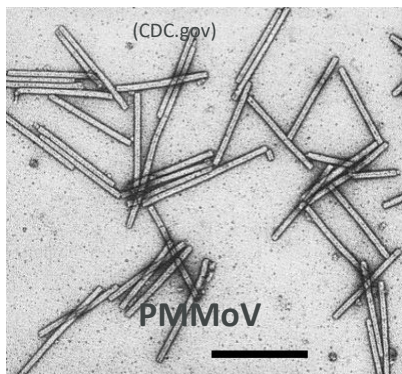
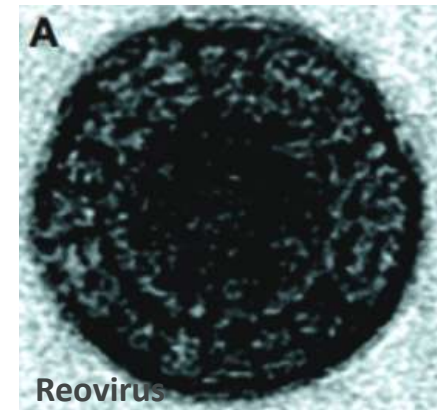
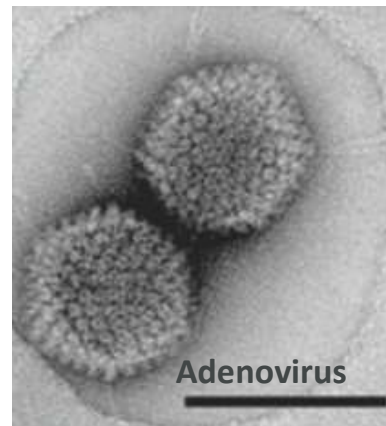
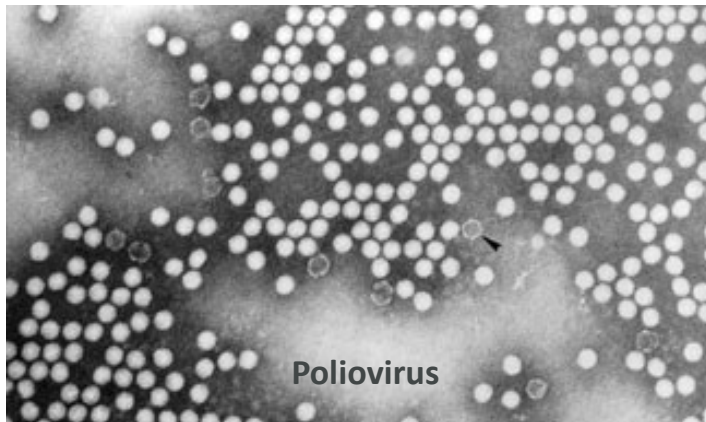


SARS-CoV-2 Variant Surveillance in Wastewater in Support of COVID-19 Mitigation Efforts

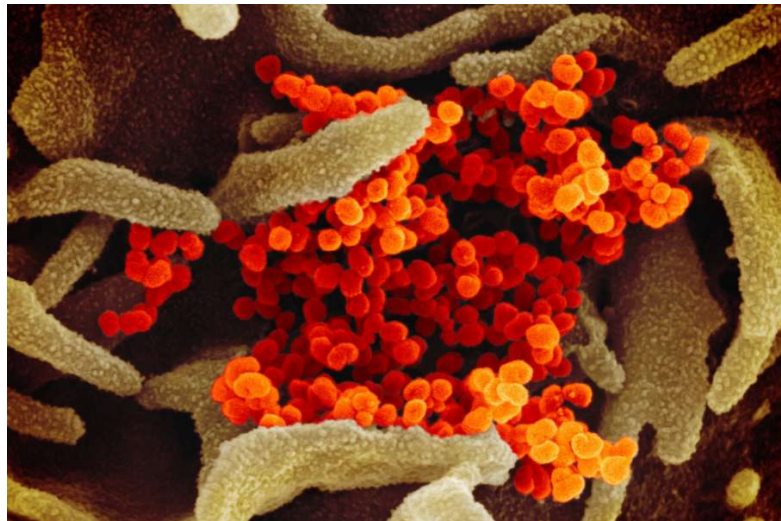
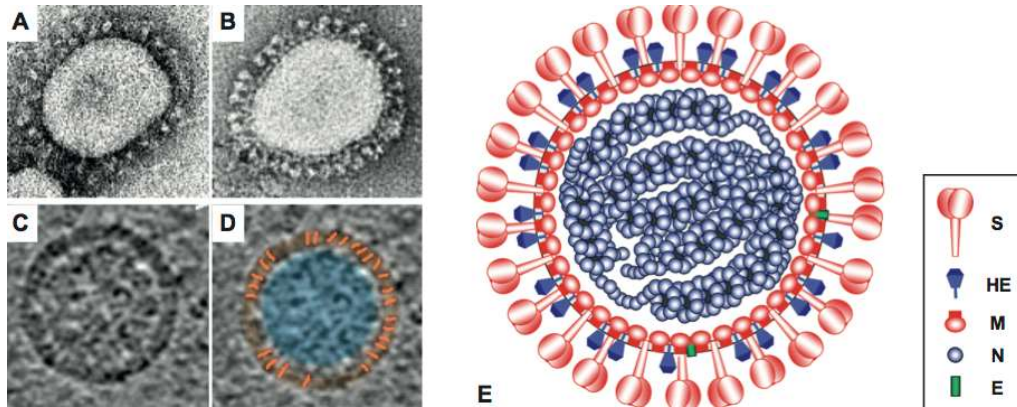
WALTER Q. BETANCOURT
THE UNIVERSITY OF ARIZONA, U.S.A



Transmission electron micrographs of viruses infecting humans, plants, and bacteria



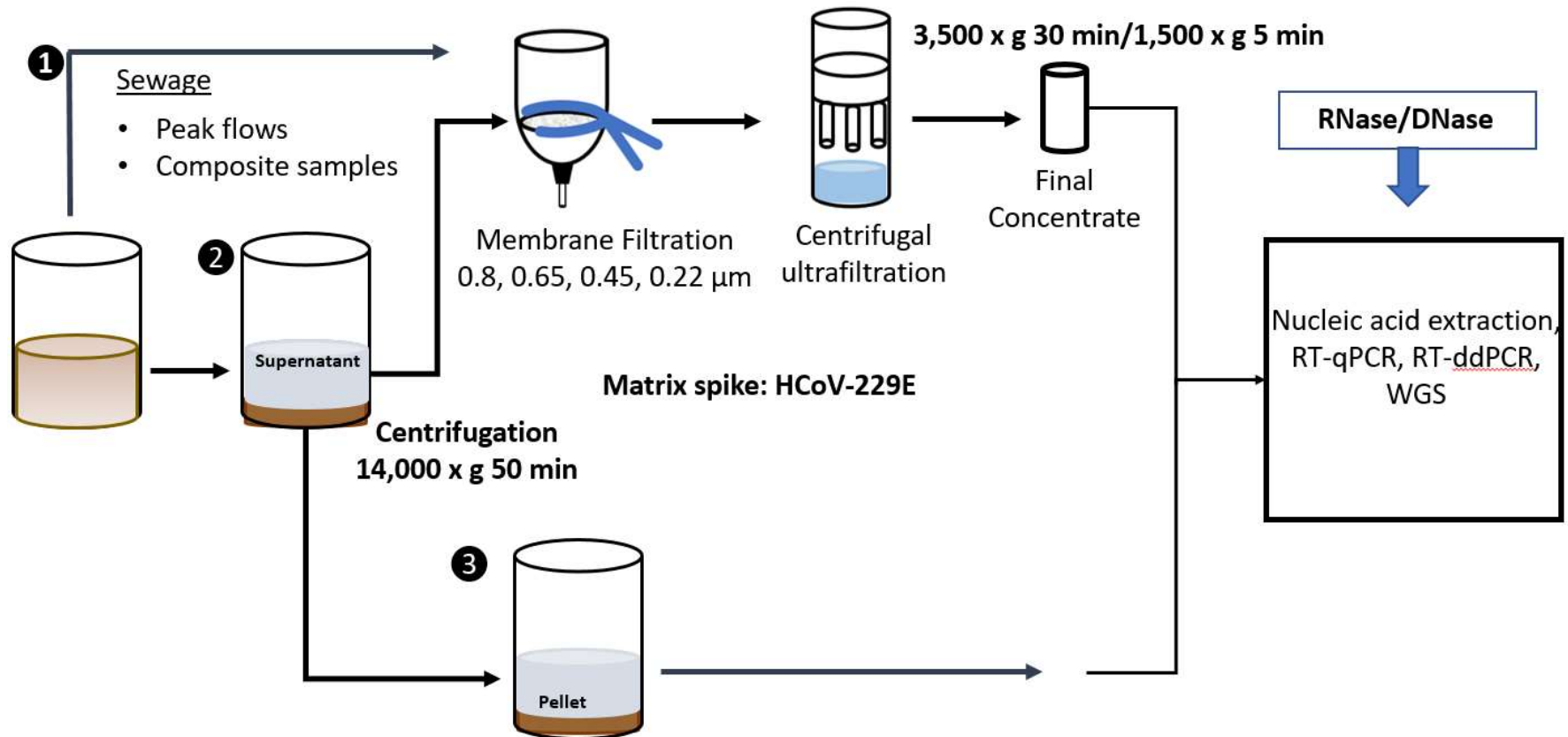
HCoV-19 / SARS-CoV-2 / 2019-nCoV



SARS-CoV-2 research at UArizona WEST Center: Implications for Water Reuse

- Monitoring SARS-CoV-2 viral RNA in municipal wastewater for COVID-19 infection surveillance at a population-wide level
- Evaluation of the removal of SAR-CoV-2 by full-scale wastewater treatment processes
- Monitoring sewer and wastewater operations' bioaerosols
- Surveillance of SARS-CoV-2 variants in UArizona dormitory sewage/Agua Nueva WRF
- Assessment of SARS-CoV-2 viability and persistence in sewage using in vitro cell culture and molecular methods

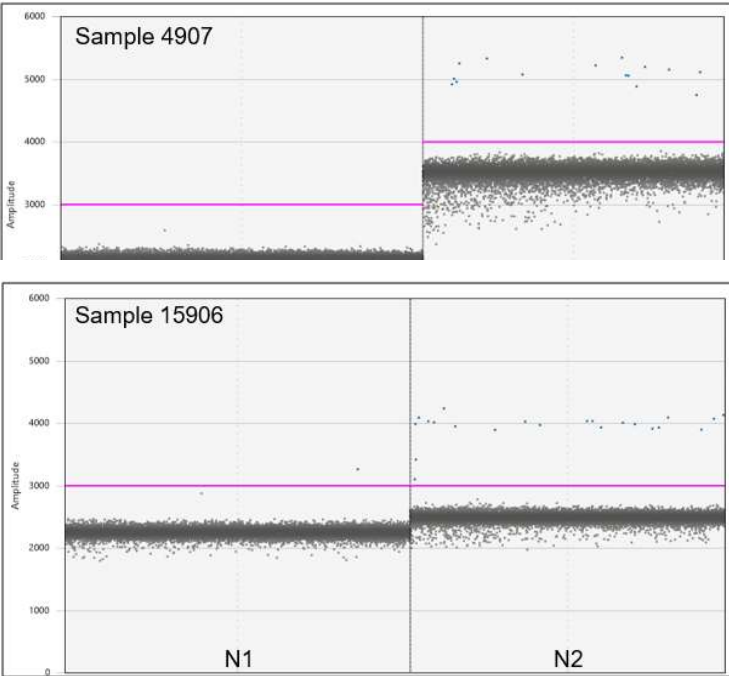
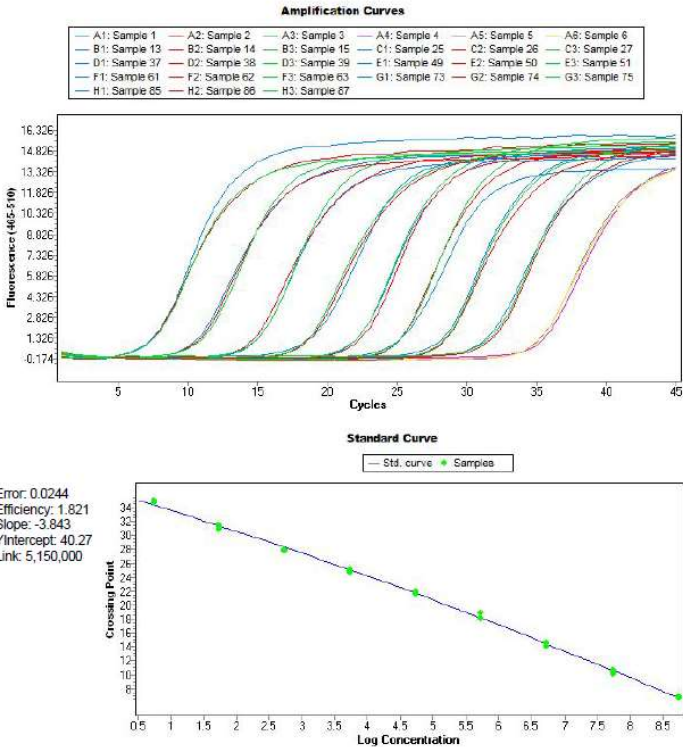
Methods for recovery and concentration of SARS-COV-2



Methods for detection of SARS-COV-2

RT-qPCR: relative quantification

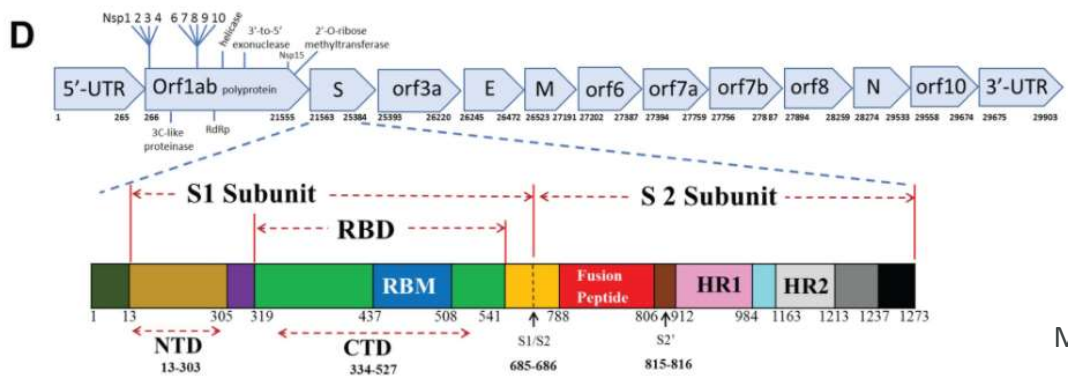
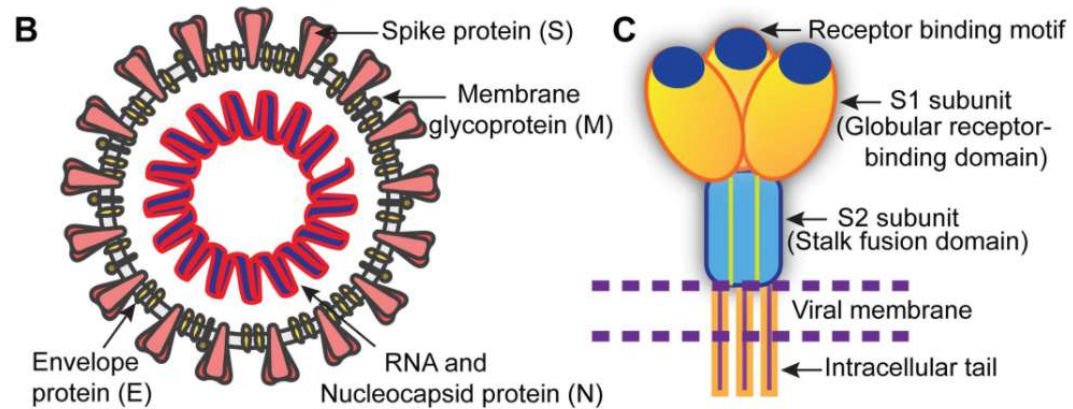
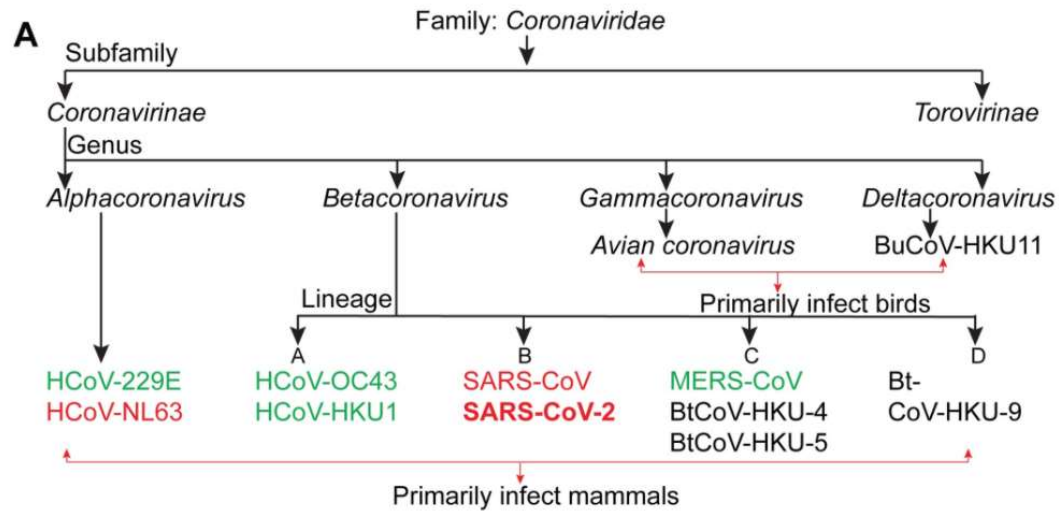
RT-ddPCR/dPCR: absolute quantification



Nucleocapsid gene: N1 and N2

Levels of SARS-CoV-2 RNA in untreated and treated wastewater

Sample type	Date	Location	SARS-CoV-2 RNA Concentration
Stormwater runoff (Hudson River)	April 2020	New York	10E+06 GC/L
Untreated wastewater	May – June 2020	Florida	10E+05 – 10E+06 GC/L
Untreated wastewater	May – August 2020	California	10E+03 – 10E+07 GC/L
Primary sludge	April 2020	Arizona	10E+08 GC/L
Primary sludge		Florida	10E+08 GC/L
Secondary effluent without chlorine disinfection	April 2020	New York	10E+03 GC/L
Secondary disinfected effluent	April 2020	Arizona	<ALoD
Tertiary effluent	April – June 2020	Arizona	<ALoD



Variants of Concern

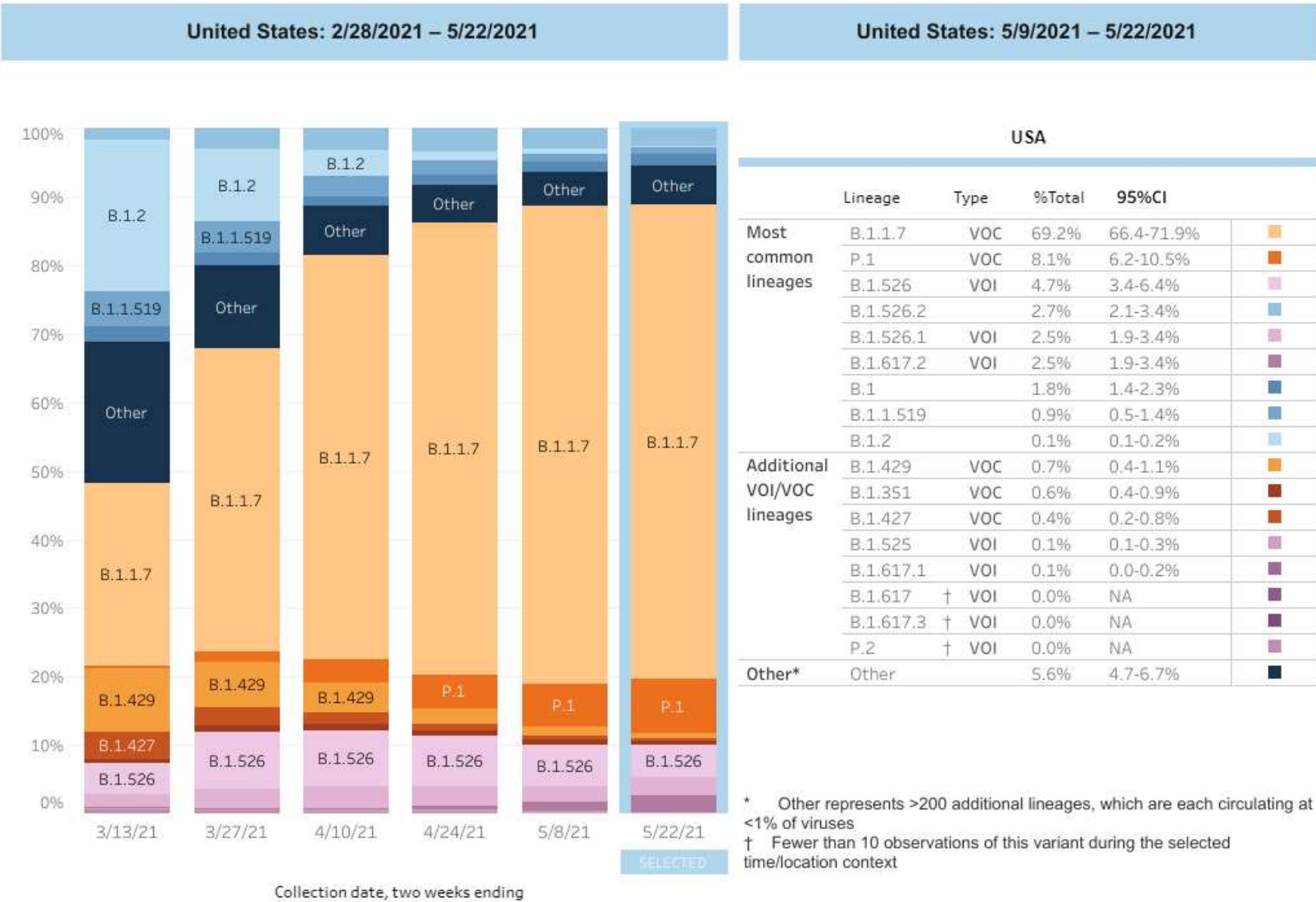
WHO Label	Pango Lineage	Earliest documented samples	Transmissibility	Immune Evasiveness	Vaccine Effectiveness
Alpha	B.1.1.7	United Kingdom	+++	--	√
Beta	B.1.351	South Africa	+	++++	√
Gamma	P.1	Brazil	++	++	√
Delta	B.1.617.2	India	++++	++	√



Potential consequences of emerging variants:

- Ability to spread more quickly in people
- Ability to cause either milder or more severe disease in people
- Ability to evade detection by specific viral diagnostic tests
- Decreases susceptibility to therapeutic agents such as monoclonal antibodies
- Ability to evade natural or vaccine-induced immunity

Estimated Proportions of SARS-CoV-2 Lineages in the United States



CDC's national genomic surveillance program

Estimated Proportions of SARS-CoV-2 Lineages in the United States

United States: 4/11/2021 – 7/17/2021

United States: 7/4/2021 – 7/17/2021 NOWCAST



USA			
Lineage	Type	%Total	95%PI
B.1.617.2	Delta	VOC	83.2% 79.2-86.8%
B.1.1.7	Alpha	VOC	8.3% 5.6-11.1%
P.1	Gamma	VOC	3.3% 1.5-5.1%
B.1.621			2.2% 0.8-3.8%
B.1.526	Iota	VOI	0.7% 0.0-1.5%
B.1.351	Beta	VOC	0.0% 0.0-0.3%
B.1.427	Epsilon	VOI	0.0% 0.0-0.3%
B.1.525	Eta	VOI	0.0% 0.0-0.3%
B.1.617.3		VOI	0.0% 0.0-0.3%
B.1.429	Epsilon	VOI	0.0% 0.0-0.3%
Other			2.2% 0.3-5.1%

* Other represents lineages each circulating at <1% of viruses over the last 12 weeks
 ** These data include Nowcast estimates, which are modeled projections that may differ from weighted estimates generated at later dates
 # Sublineages of P.1 and B.1.351 (P.1.1, P.1.2, B.1.351.2, B.1.351.3) are aggregated with the parent lineage and included in parent lineage's proportion. AY.1, AY.2, and AY.3 are aggregated with B.1.617.2.

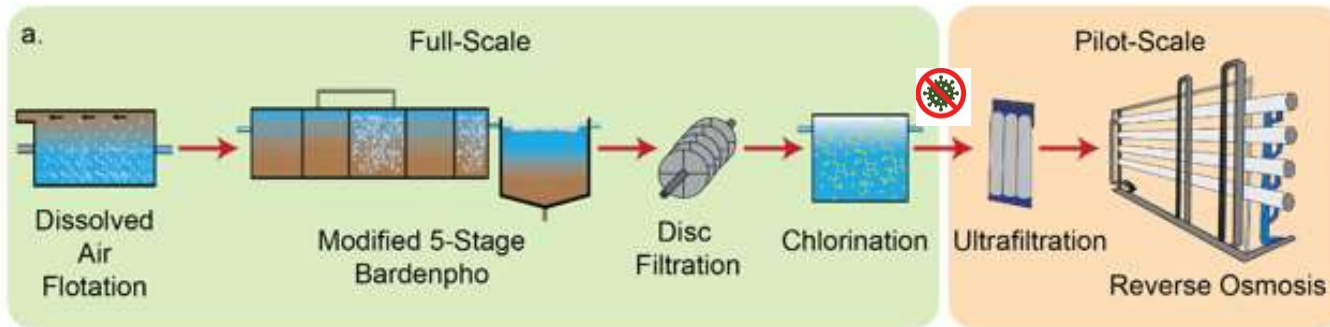
SARS-CoV-2 and Variants of
Concern in Wastewater:
Implications for Safe Water Reuse

SARS-CoV-2/COVID-19 virus and Variants of Concerns in Untreated Wastewater (Pima County, Arizona)

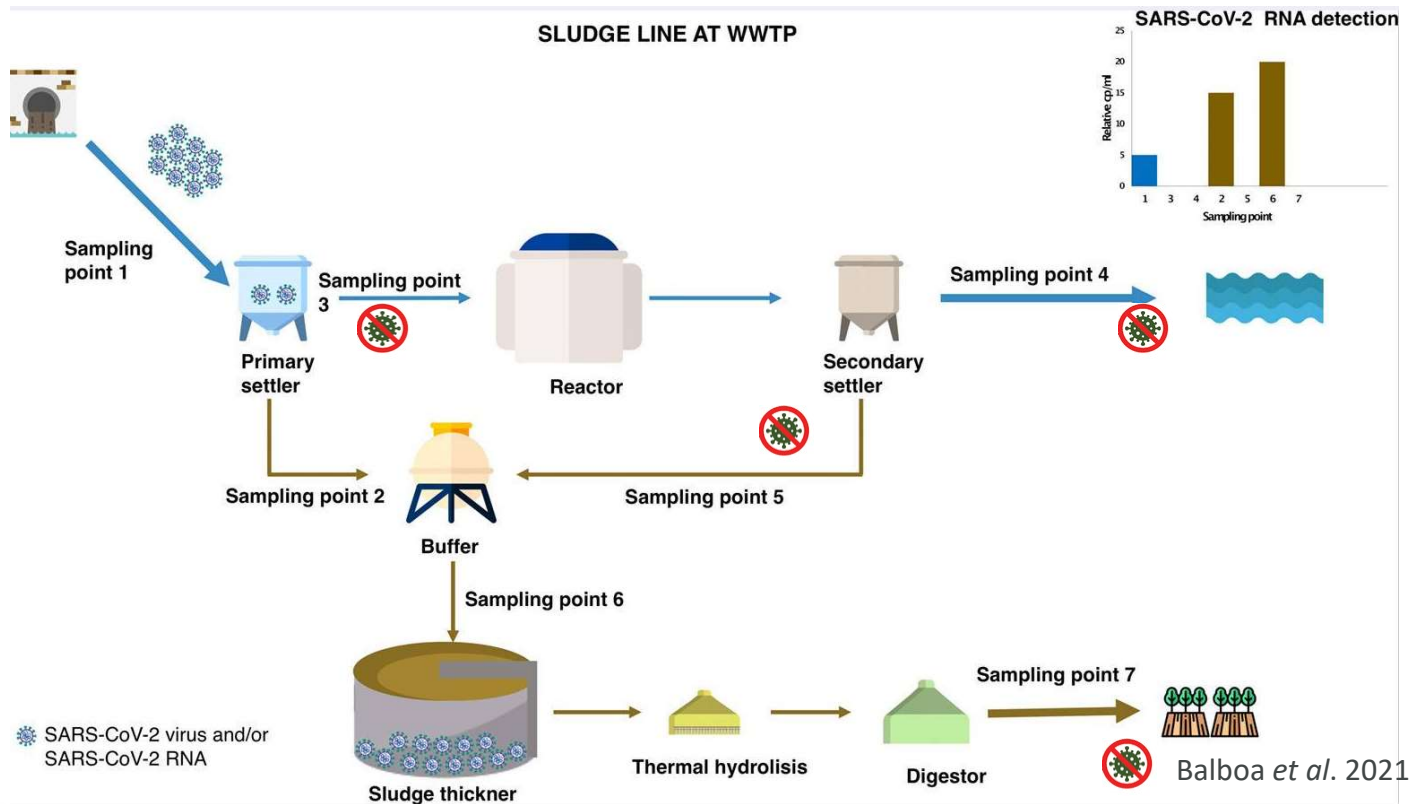
Sample type	Date	Levels of SARS-CoV-2	Presumptive variant
Untreated wastewater	6/16/21	10E+06 GC/L	Delta (B.1.617.2)*
Untreated wastewater	6/23/21	10E+06 GC/L	Delta (B.1.617.2)
Untreated wastewater	6/29/21	10E+06 GC/L	Delta (B.1.617.2)
Untreated wastewater	7/7/21	10E+06 GC/L	Delta (B.1.617.2)

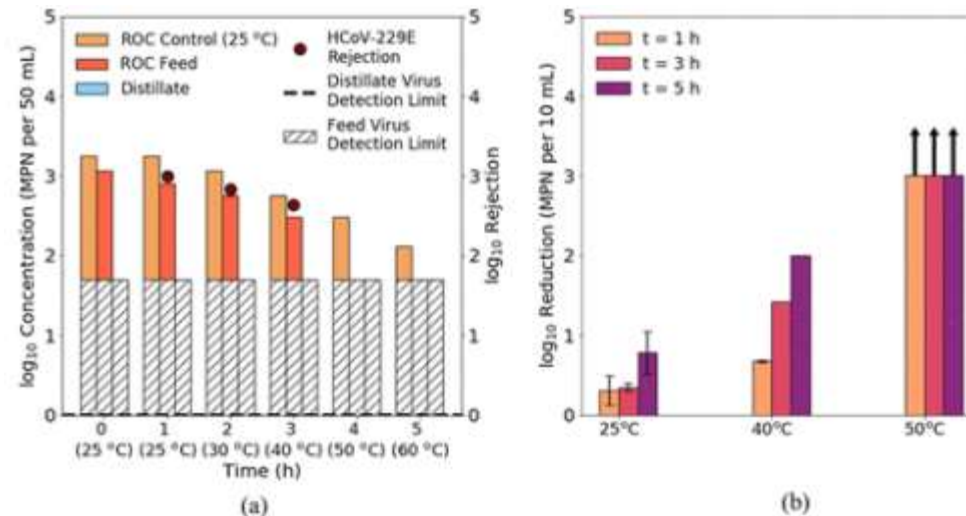
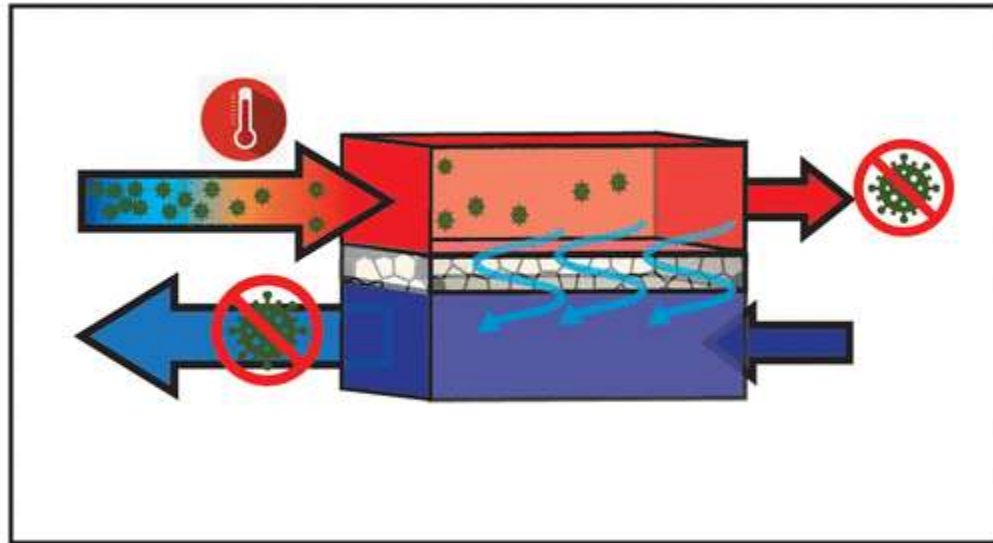
* T478K/L452R

SARS-CoV-2/COVID-19 virus in Wastewater Treatment Processes



WEST Center





Membrane distillation and Coronavirus removal (a) MD bench-scale test with RO concentrate as the feed solution, ultrapure water as distillate, and a control volume RO concentrate spiked with HCoV-229E at 25 °C. (b) RO concentrate spiked with HCoV-229E at different temperatures