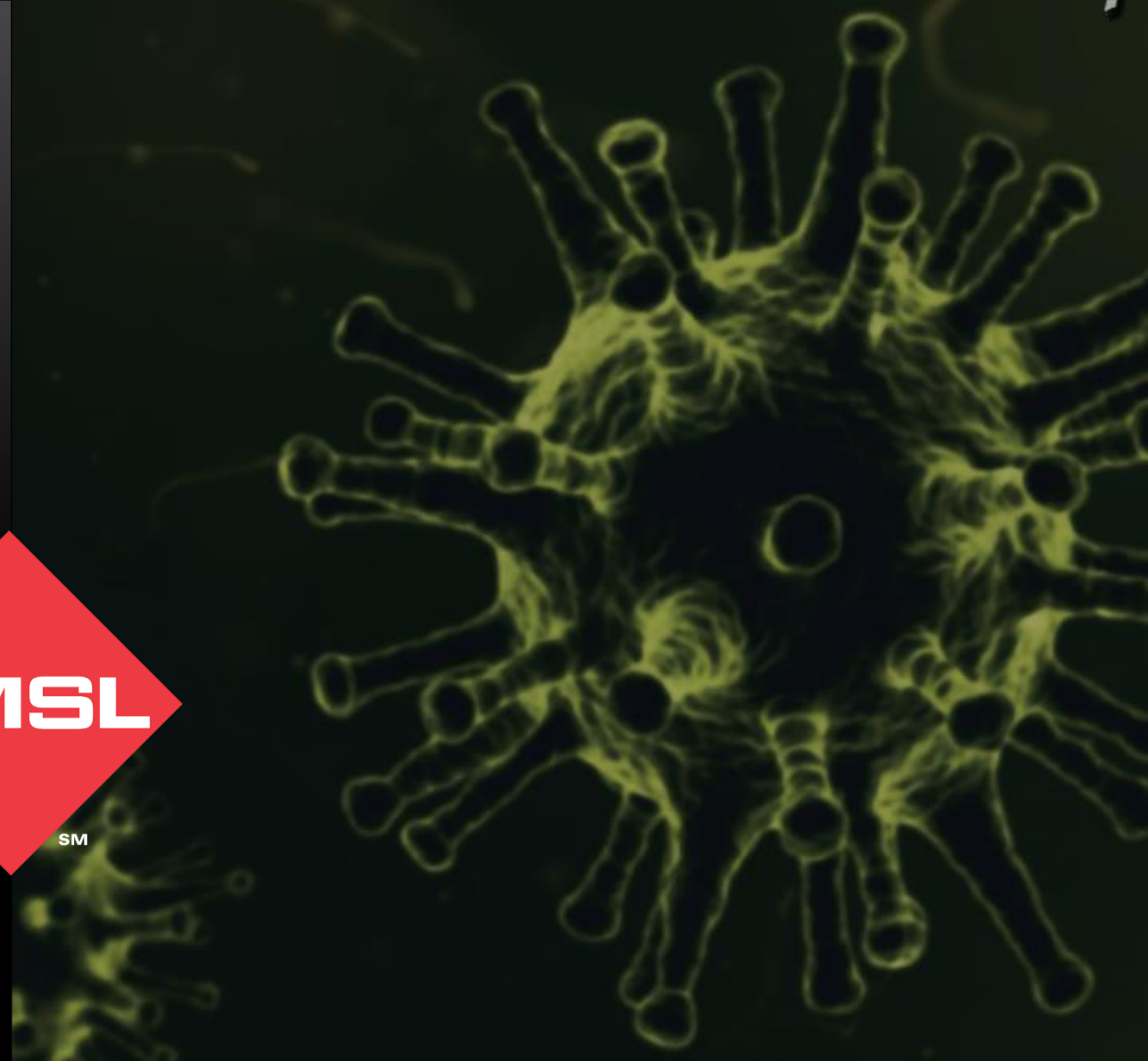
SARS-CoV-2 Environmental Sampling and qPCR Analysis



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Asbestos Division

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Microbiology Division

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EMSL Analytical, Inc. is one of the leading testing laboratories with 46 locations throughout the United States and Canada.

Transmission and Surface Contamination

- Main infection route is between people who are in close contact (within about 6 feet).
- Through respiratory droplets produced when an infected person coughs or sneezes.
- Currently, transmission from surfaces is not thought to be the main way the virus spreads, however the CDC's April 13th update remarked: *"COVID-19 is a new disease and we are still learning about how it spreads and the severity of illness it causes."*
- Possible by touching a surface or object that has the virus on it, and then touching the mouth, nose, or eyes.

Environmental Surfaces

- May remain infective on surfaces for hours to days
- Dependent on the surface material type (fabric, tile, steel, paper, etc.)
- Thorough cleaning and disinfection of frequently touched surfaces are recommended by the CDC and believed to be essential in preventing the spread of infection

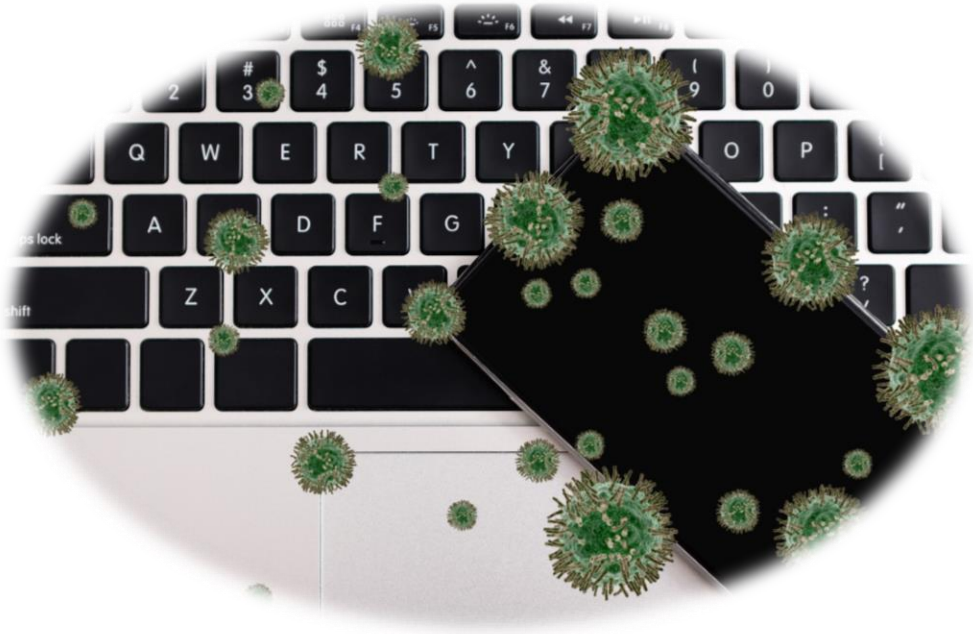
Stability of SARS-CoV-2 in Different Environmental Conditions

- Virus is highly stable at 4°C (14 days), but sensitive to heat
- Temperature increased to 70°C, the time for virus inactivation was reduced to 5 mins
- No infectious virus could be recovered from printing and tissue papers after a 3-hour incubation
- Day 2 treated wood and cloth
- Day 4 glass and banknote
- Day 7 stainless steel and plastic
- Surgical mask on day 7

https://doi.org/10.1016/S2666-5247(20)30003-3

Potential High Risk Areas to Sample

- Telephones, Keyboards
- Chairs, Tables, Light Switch
- Hand Soap Dispenser
- Garbage Bin
- Office Equipment, Panels
- Doorknobs
- Railings, banisters
- Faucet Handles
- Toilet Handles
- Air Returns, Ventilation Exits



Disinfectants Studied

Disinfectant (Working concentration)	Virus titre (Log $TCID_{50}$/mL)		
	5 mins	15 mins	30 mins
Household bleach (1:49)	U	U	U
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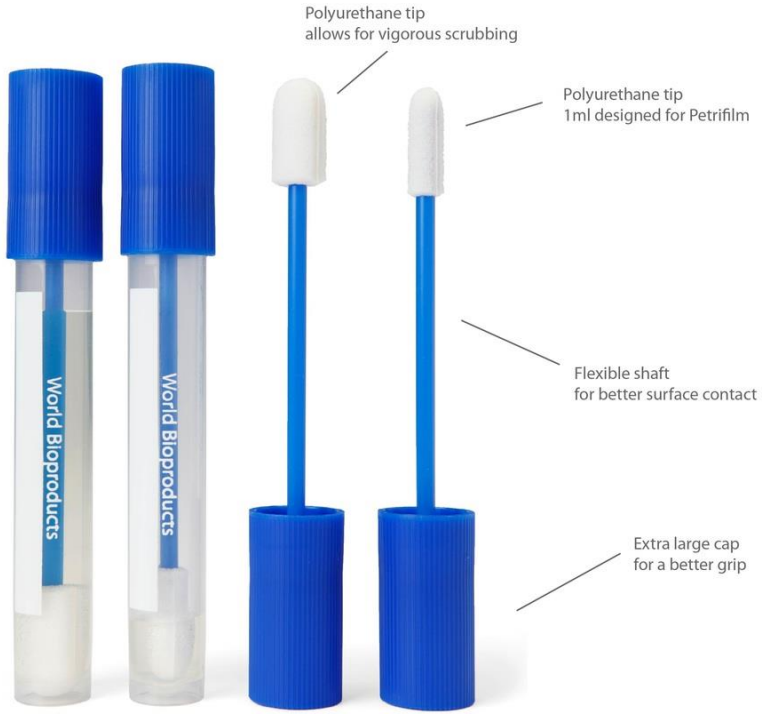
Hand soap solution (1:49)	3.6[#]	U	U
Ethanol (70%)	U	U	U
Povidone-iodine (7.5%)	U	U	U
Chloroxylenol (0.05%)	U	U	U
Chlorhexidine (0.05%)	U	U	U
Benzalkonium chloride (0.1%)	U	U	U

ATP Testing

- Adenosine Triphosphate – the energy molecule used by living cells
- Measured by luminometer (RLUs) onsite
- Commonly used in food processing
- Indicator of cleanliness (all cellular ATP)
  - food residues, blood spillages, fecal matter, dust, shed skin squama
- Not produced or stored by viruses
- Not a direct measure of viral presence
- Follow manufacture recommendation on sampling

Microbial Screening

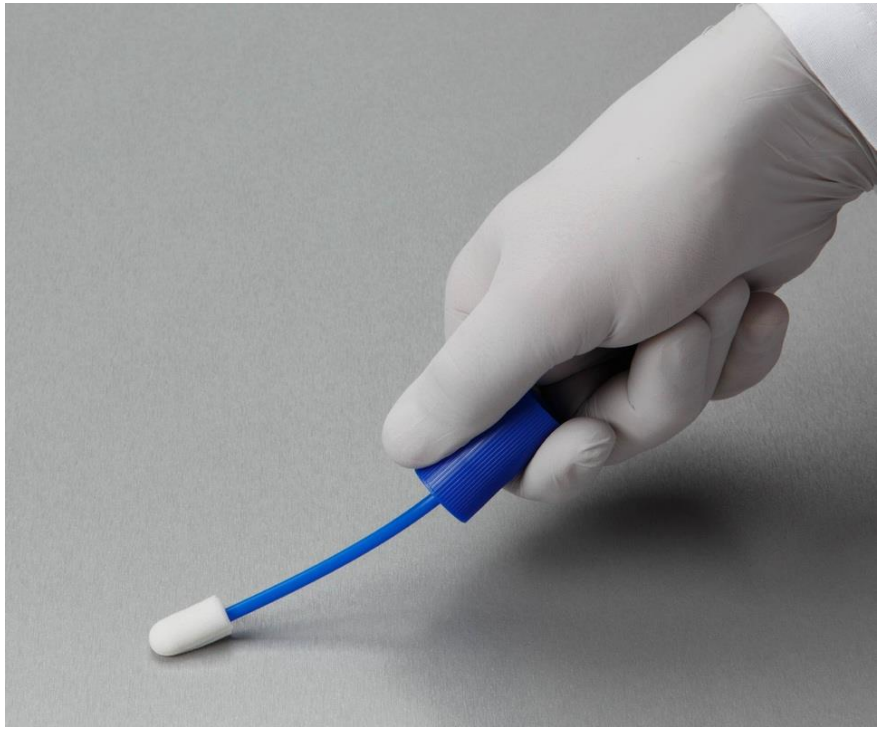
- Swab surfaces for bacteria/fungi as indicators of cleaning efficacy
- Assumes an EPA-registered virucide was included in the cleaning protocol and used according to labeling
- If results are free of bacteria and fungi then those surface have been properly cleaned



https://www.worldbioproducts.com/purblue.html

Microbial Screening

- Wait until disinfectant has dried
- Must use swab with neutralizing buffer
- Fungal plate counts, 5 day TAT
- Bacterial plate counts, 2 day TAT
- Ship swabs on ice packs



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SARS-CoV-2 PCR Testing

- Presence or absence of the viral genetic material on environmental surfaces
- RT-qPCR targets the SARS-CoV-2-nucleocapsid N gene
- Two indicators: 2019-nCoV_N1 & 2019-nCoV_N2
- Same method as specified by the CDC and authorized by the FDA for clinical COVID-19 diagnostics
- Human RNAse P gene is not analyzed
- Not for processing patient SARS-CoV-2 specimens

SARS-CoV-2 PCR Testing cont.

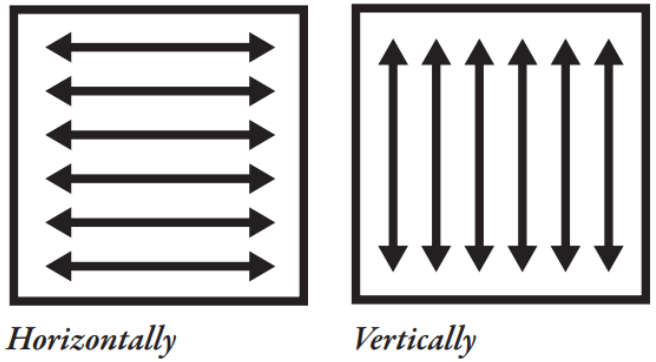
- Environmental samples frequently contain qPCR inhibiting substances found in dirt, soil, and dust
- Additional nucleic acid (NA) extraction method (clean-up) for environmental samples
- 'Detected' for either indicator can be interpreted with confidence that the area swabbed contained SARS-CoV-2 coronavirus particles that could be deemed as potentially infectious
- Test includes five controls: positive N1, positive N2, negative N1, negative N2, and qPCR inhibition control

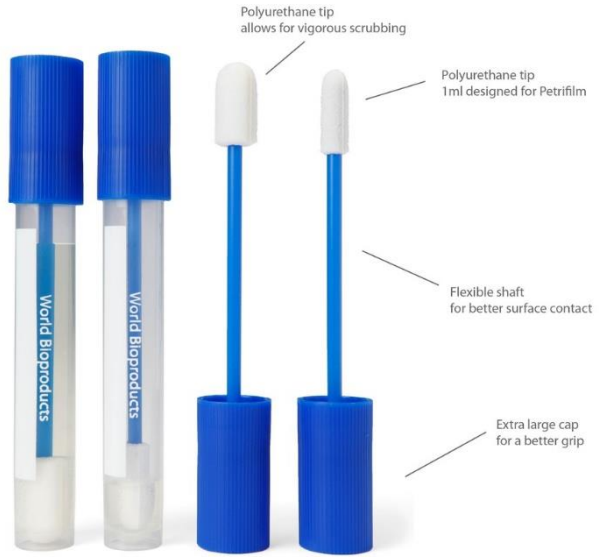
SARS-CoV-2 PCR Results

- Test is currently P/A results but may be quantitative in future
- Limit of detection (LOD) 1 RNA copy per microliter of transport medium
- The test is specific only to SARS-CoV-2
- There is no known cross-reaction to any other viruses
- Rapid TATs available: same day to 3 day typically offered by commercial labs

Swab Sampling Protocol

- Wear appropriate PPE during sampling
- Swab surface areas of interest (25 cm^2 is commonly used)
- Moderate pressure while moving in at least two different directions and rotating the swab
- Avoid letting the swab dry completely





Swab Sampling Protocol (cont.)

- Place back in labeled transport container
- Wipe with disinfectant
- Place all swabs in Ziplock bag, wipe bag
- Double bag and wipe outside of second bag
- Place COC in separate Ziplock bag

Air Sampling

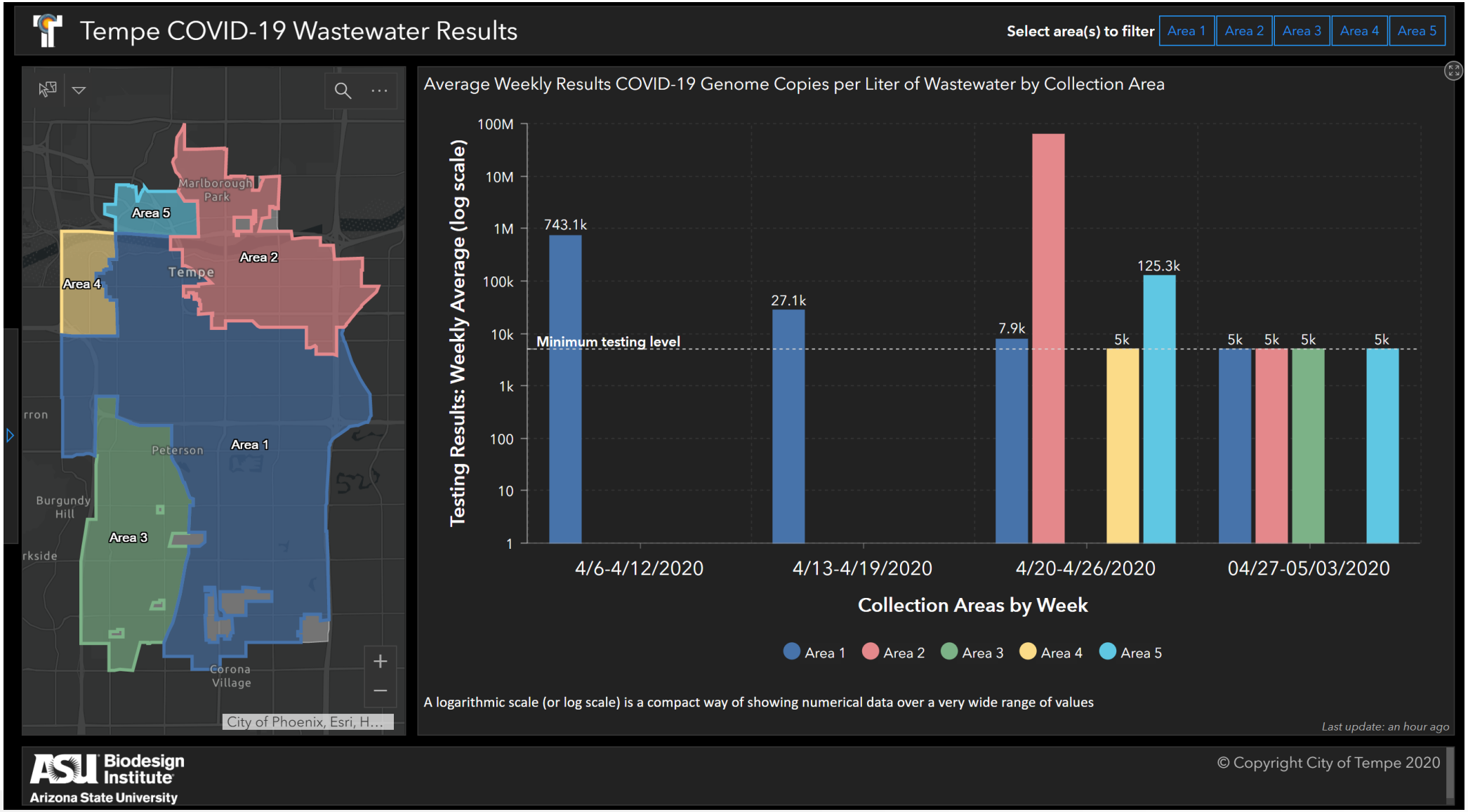
- Vira-Pore - 37mm 1 µm pore-size PTFE-laminated PTFE Zefon® "ZePore"™ filters
- Tested with 10 L/min flow rate for 10 mins
- Other samplers
  - BioSampler, Button Sampler (gelatin, short time)

SKC Specialists in Air Sampling Since 1962

ENVIRONMENTAL EXPRESS®

VIRA-PORE Viral Sampling Cassette

COVID-19 in Wastewater



Wastewater Sampling

- Collect wastewater as it enters treatment plant (untreated influent)
- Wear gloves and do not touch the bottle opening
- Sterile 120mL bottle (Only 105ml required for analysis)
- Wipe outside of the bottles, then double bag
- Ship same day to the lab in cooler with ice packs
- Samples may be stored up to 72 hours at 2-8^{o}C

Wastewater Samples

- IDEXX Water SARS-CoV-2 RT-PCR Test Kit (P/A)
  - Detects the same virus regions targeted during the analysis of environmental swabs or air samples
  - Sterile 120mL Bottle (Only 105ml required for analysis)
- New tests being developed for quantitative results

Sampling Strategy

- Detect the presence of COVID-19 within a sewershed
- Monitor trends in COVID-19 within a sewershed
- Targeted wastewater surveillance entails sampling wastewater from upstream in the wastewater network (e.g., lift stations, interceptors, manholes)
- SARS-CoV-2 RNA concentrations in wastewater cannot currently be used to determine the total number of infected persons in a community or the percent of the population that is infected.

Sampling Strategy

- A minimum of three samples is required to detect wastewater trends over time.
- Grab samples can be collected rapidly but are less representative
- Composite samples are collected by pooling multiple grab samples at a specified frequency over a set time period – typically 24 hours for wastewater surveillance.

From CDC - https://www.cdc.gov/coronavirus/2019-ncov/cases-updates/wastewater-surveillance/developing-a-wastewater-surveillance-sampling-strategy.html

Shipping Samples

- Pack well so samples are not damaged during shipping
- Samples must be shipped with an ice-pack
- Recommend shipping samples on the same day as collected
- Samples may be refrigerated up to 72 hours per WHO guidelines

Interpreting Results

- Results are reported in either 'Detected' or 'Non-Detected' for two indicators of the SARS-CoV-2
- Indicator one is 2019-nCoV_N1 and indicator two is 2019-nCoV_N2.
- Both indicators detect the nucleocapsid protein 'N' gene of the SARS-CoV-2 coronavirus.
- Either indicator can be interpreted that the area sampled did contain SARS-CoV-2 coronavirus particles that could be deemed as potentially infectious.

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