COVID-19 Surveillance Project

Presentation to the SCCWRP Commission
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Background

• Wastewater epidemiology is a great concept
  • Allows for tracking population levels of the SARS-CoV-2 virus
  • None of the drawbacks of individual testing

• California is a national leader in promoting the concept
  • More than 35 wastewater treatment plants collecting samples
  • More than a dozen research programs
  • CDC has chosen California as a pilot location

• State Board is partnering with California 5 utilities and CDPH to pilot use
  • One of only 8 states
  • SCCWRP member agencies account for 80% of this effort
Statement of the Problem

• Emphasis has been on speed
  • As a result, many different methods to measure the virus are in use

• Differences in methods leads to lack of compatibility of results across labs, plants, and regions

• These differences also lead to disparities in measurement sensitivity
  • Of particular concern when moving into small sewersheds to track outbreaks
Goals of this Project

• Collect and process samples to assist member agencies and the Public Health community

• Investigate sources of variability in method sensitivity
Coronavirus in Sewage at Inlets to Seven POTW’s
Approach to Goal One

• Collaboration with Stanford
  • Nationwide study (largest of its kind)
  • 25 plants throughout the state
  • SCCWRP responsible for the entirety of southern California

• Collect and measure SARS-CoV-2 virus in over 300 primary influent and settled solids samples (April – November)
  • Hyperion
  • San Jose Creek, JWPCP, Valencia
  • Point Loma, North City South Bay
  • OCSD
Approach to Goal Two

• Identify and investigate sources of variability in method sensitivity

• Use controlled experiments determine effect of individual factors on results
Potential Sources of Variability

Sample Type
Influent or Settled Solids

Sampling Strategy
Grab or Composite

Sampling Frequency
Daily? Weekly?

Virus Inactivation
Heat
Chemical

Fresh Processing, Storage, or Preservation
Refrigerate
Freeze
Chemically preserve

Virus Capture
Direct processing, Filter, precipitate, or concentrate

Virus Quantification
RT-qPCR or RT-ddPCR

Purification of Viral RNA
Commercial kit, published protocol, In-house protocol

Target Selection
Which gene target?
Sampling Frequency

• How often do we need to sample to adequately track virus levels in the population?

• We have four months of daily data that allows us to examine this question
How frequently should you sample?

- No significant relationship between Covid case counts and weekly wastewater samples
- Significant relationship with 3X or daily samples
Frozen Sample Preservation

• Many of you collected and froze samples for later processing

• Some more fragile viruses are highly susceptible to degradation when frozen and thawed in a water matrix
  • Important to know how freezing effects the SARS-CoV-2 virus levels

• We conducted controlled experiments with corona virus spiked into influent samples
Effect of Freezing
Virus Deactivation

• Clinical labs that work with SARS-CoV-2 are required to have a Biosafety Level 3 (BSL3) rating
  • Most labs processing wastewater for SARS-CoV-2 would require expensive modifications to meet this criterion

• Heat treatment (Pasteurization) prior to handling allows non-BSL3 rated labs to process wastewater samples
  • Common step in many methods

• We conducted controlled experiments to determine effect of heat treatment on measurement sensitivity
Virus Deactivation

![Graph showing virus deactivation](image)
Gene Targets

• Multiple primers sets are available (WHO, CDC, Commercial)

• When the pandemic hit, labs had to make a choice as to which of the available targets to measure
Targets for SARS-CoV-2 RT-PCR Assays

Comparison of N1 vs N2 Genes
Work in Progress

• Comparison between grab and composite samples

• Comparison between paired influent and settled solids samples

• Comparison of virus detection methods
Summary

• Methods matter
  • Several steps can change outcome by more than 2 orders of magnitude
  • Don't change methods over the course of monitoring

• When virus concentrations are low, you may need to switch to a more sensitive methods
  • Source tracking in small sewersheds, dormitories, or nursing homes

• Have only shown data for a few sources of variability we identified
  • Stay tuned
Next Steps

• We have submitted an NIH proposal with UCI to virus strain typing
  • Allows us to use mutations in the virus to track its spread throughout the region

• Sampling for Stanford Project is complete
  • Continuing to sample at two facilities at the request of member agencies