COVID-19
Surveillance Update

Presentation to SCCWRP Commission
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Background

• COVID-19 pandemic has put wastewater based surveillance of viral pathogens at the forefront of public health

• California’s wastewater community continues to be active in wastewater based surveillance for SARS-CoV-2
  • More than 40 facilities are monitoring and have at least six months of data
  • Several facilities (including SCCWRP member POTWs) have more than a year of data
  • Participation in national studies

• WQMC Wastewater Based Epidemiology continuing to provide guidance on effective data use and interpretation
From Method Comparison to Effective Data Use

- Laboratory measurement method variability
- Measurement methods sensitivity
  - Guidance document with WBE Subcommittee
- SARS-CoV-2 variants in wastewater
Potential Sources of Variability

Stanford Collaboration

Sample Type
- Influent or Settled Solids

CSU Fullerton Collaboration

Sampling Strategy
- Grab or Composite

Sampling Frequency
- Daily? Weekly?

Virus Capture
- Direct processing, Filter concentrate

Virus Inactivation
- Heat
- Chemical

Fresh Processing, Storage, or Preservation
- Refrigerate
- Freeze
- Chemically preserve

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

Target Selection
- Which gene target?

Virus Quantification
- RT-qPCR or RT-ddPCR

Manuscript in revision
From Method Comparison to Effective Data Use

• Laboratory measurement method variability

• Measurement methods sensitivity
  • Guidance document with WBE Subcommittee

• SARS-CoV-2 variants in wastewater
Recommendations to Improve Sensitivity

• Remove heat inactivation step
  • 50% of treatment plants using heat inactivation

• Concentrate or measure a larger volume
  • ~30% of labs are not concentrating

• Measure SARS-CoV-2 using digital RT-PCR
  • 60% of labs are using RT-QPCR

• Use sludge samples
  • 25% of labs are collecting sludge
Sensitivity Case Study at JWPCP

• Opportunity to test sensitivity recommendations

• Split samples among four groups
  • Small volume qPCR (Zymo Research)
  • Small volume, concentrated qPCR (BioBot)
  • Large volume qPCR (UC Berkeley COVID-WEB)
  • Large volume digital PCR (SCCWRP)

• Preliminary results
• UC Berkeley and SCCWRP are highly correlated ($r^2=0.7$)

• 60% of BioBot samples below detection

• 50% of Zymo samples are below detection

• Zymo’s concentrations are much higher compared to others
Takeaways from the Sensitivity Comparison

• Larger volume concentration qPCR and digital PCR are sensitive enough at low concentrations

• Smaller volume qPCR methods too insensitive to be useful when prevalence is low

• WBE Subcommittee recommendations looking pretty good
From Method Comparison to Effective Data Use

• Laboratory measurement method variability

• Measurement methods sensitivity
  • Guidance document with WBE Subcommittee

• SARS-CoV-2 variants in wastewater
Focus on SARS-CoV-2 Variant Analysis

- Variant analysis is the next on the horizon for SARS-COV-2 analysis
  - High public health impact
  - Not a part of current SARS-CoV-2 monitoring

- Should variant analysis become a part of routine wastewater based surveillance?

- WBE Subcommittee has determined that variant analysis is not ready to be included in routine monitoring
  - Guidance document sent to CASA and Public health departments.
SARS-CoV-2 Variant Analysis in Sewage

• Wastewater is complex and multiple variants in sewage
  • Detection of a single mutation is not enough

• Approaches using both sequencing and targeted analysis are needed
  • Active area of research

• Interpretation of variant data in sewage will require collaboration across multiple agencies
Ongoing Variant Research at SCCWRP

• Sequencing SARS-CoV-2 genomes in sewage samples
  • Sewage RNA Virus manuscript in revision
  • Identifying SARS-CoV-2 mutations in sewage

• Quantifying Variants using digital PCR
  • Targeting prevalent variants
  • Using multiple gene targets to identify specific variants

• Collaborating with universities, POTWs, and public health agencies within California and across the US