SARS-CoV-2 wastewater monitoring: Environmental surveillance to assess community-level incidences of COVID-19

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SARS-CoV-2 in Sewage

• Virus is shed in feces by individuals with symptomatic and asymptomatic infection – but not all shed

• Variable SARS-CoV-2 load in feces: $10^3$-$10^7$ RNA copies/gram\(^1\)

• Approximately 75-80% US is served by municipal sewage systems\(^2\)

• Low risk of wastewater as vehicle for transmission
  • Limited reports of infectious virus in feces\(^5,6\); none from sewage
  • No additional risk to wastewater workers\(^7\)

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Sample Processing and Analysis

24-hr composite sample, 225 ml

PBS OC43

Centrifuge 3000 x g, 15 min

Membrane filtration, 0.45µm

Ultrafiltration, 30 kDa MWCO

0.2 ml “Direct” sample

Pellet

Filter

UF Retentate
Sample Processing and Analysis

24-hr composite sample, 225 ml

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OC43

0.2 ml “Direct” sample

Pellet

Filtrate

UF Retentate

Nucleic Acid Extraction (RNeasy Power Water Kit)

RT-ddPCR: SARS-CoV-2 (N1, N2), OC43, PMMoV, Inhibition control; ddPCR: crAssphage

Turnaround time = 3 days
Questions

• Where is virus found in the sample? (Which fractions is it associated with?)

• What are the limits of detection for our samples and methods?

• What is our recovery efficiency? (What is the best way to assess this?)

• What is the effect of extended sample storage time?

• What is the effect of freezing the sample?
Where is virus found within the sample?

- 90% of measurable virus is found in the pellet and filter fractions.
What is the limit of detection?

• LOD is based on:
  • Volume of sample processed
  • Concentration factor
  • Volume of processed sample that is analyzed in PCR
  • Analytical sensitivity of instrument (i.e. minimum detectable concentration)

• Theoretical LOD represents ideal conditions; practical limits likely higher due to losses during processing

Mean, 95% CI

655 mol/L
What is our recovery efficiency?

We can understand this by measuring concentrations before and after processing of...

- **Endogenous virus** –
  - crAssphage: 84%
  - PMMoV (Pepper Mild Mottle Virus): 27%

- **Spiked virus** –
  - OC43 (Human Beta-Coronavirus): 6%

- Minimal PCR inhibition (<20%)
What is the effect of sample storage time?

- 24-hour composite shipped overnight, held at 4°C for up to 4 days
- No significant difference in SARS-CoV-2 RNA up to 4 days
What is the effect of freezing the sample?

- Up to five-fold reduction when samples were frozen at -70°C and then thawed for processing.
Ohio Wastewater Monitoring Network

- Currently 46 sites, more will be added
- Sample weekly, but increasing to biweekly
- ORD-Cincinnati = 10 sites
How will the data be used?

- Increased messaging to public on best practices to reduce transmission
- Mobilize additional individual testing
- Alert health care providers
- Closely monitor and evaluate data
- Provide recommendations to local leaders to take direct actions
Temporal trends in different sewersheds
Temporal trends in different sewersheds

<table>
<thead>
<tr>
<th>Sewer Creek</th>
<th>% Industrial</th>
<th>% Combined</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mill Creek (118 MGD)</td>
<td>5.0</td>
<td>40</td>
<td>0.5:1</td>
</tr>
<tr>
<td>Little Miami (37 MGD)</td>
<td>4.2</td>
<td>30</td>
<td>0.4:1</td>
</tr>
<tr>
<td>Muddy Creek (14 MGD)</td>
<td>&lt;0.05</td>
<td>30</td>
<td>0.5:1</td>
</tr>
<tr>
<td>Taylor Creek (3 MGD)</td>
<td>0</td>
<td>0</td>
<td>1.8:1</td>
</tr>
</tbody>
</table>

Mill Creek = pool of 488,000 people
Relating wastewater data to new cases

Hamilton County individual test data

Mill Creek wastewater data (serves ~80% of Hamilton County)

• Working with county to obtain sewershed-scale individual test data
Sewersheds and sub-sewersheds

- Lick Run as a possible “sentinel site” – more vulnerable population
- Working with county to obtain higher-resolution case data
Summary

• We have a low level of detection, but it coincides with observed increases of new cases

• We are using this data to develop models that account for factors influencing virus detection
  • Dilution of wastewater, Instrument limitations, Processing losses

• We will continue to measure SARS-CoV-2 in wastewater at specified sites and expand the Ohio network, focusing on trends or significant changes in the number of viral RNA detected

• This data will inform public health applications through the Ohio Monitoring Network
Thank you!