EPA Tools & Resources Webinar: Detection of SARS CoV-2 in Wastewater to Inform Public Health

Jay Garland, PhD
US EPA Office of Research and Development

December 16, 2020
SARS-CoV-2 in Sewage

- Virus is shed in feces by individuals with symptomatic and asymptomatic infection
- 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\(^2\)
- SARS-CoV-2 has been detected in raw sewage
  - US, Europe, Australia, Africa, etc.
  - Up to $10^7$ RNA copies/L\(^3\)
- Low risk of wastewater as vehicle for transmission
  - Limited reports of infectious virus in feces\(^4,5\); none from sewage
  - No additional risk to wastewater workers\(^6\)
  - Treatment and disinfection are likely effective

Wastewater Surveillance

Illicit Drugs in Municipal Sewage
Proposed New Nonintrusive Tool to Heighten Public Awareness of Societal Use of Illicit-Abused Drugs and Their Influence on Public Health
Christian G. Daughton

Estimating Community Drug Abuse in Urban Areas
Ettore Zuccato, Chiara Chiabrand, Sara Castiglioni, Riccardo Gori
Department of Environmental Health Sciences, Istituto di Ricerche Farmacologiche Mario Negri, Milan, Italy

Israel’s Silent Polio Epidemic Breaks All the Rules
Leslie Roberts

REVIEW ARTICLE
Role of environmental poliovirus surveillance in polio eradication and beyond

Research
Environmental surveillance of poliovirus in Dakar, Senegal (2007-2013)
Abdou Kader Ndiaye, Pape Amadou Mbaye Diop, Ousmane Ndiaye

Retrospective Surveillance of Wastewater To Examine Seasonal Dynamics of Enterovirus Infections
Nichole E. Brinkman, G. Shay Fout, Scott P. Keely

Epidemiol. Infect. (2012), 140, 1-13. © Cambridge University Press and World Health Organization; WHO has granted permission to Cambridge University Press to publish the contribution written by the author(s) of the article. This article may not be reprinted or reused in any way in order to promote any commercial product or service. doi:10.1017/S095026881000316X

OPEN ACCESS
OA

NEWS & ANALYSIS INFECTION DISEASE

Retrospective Surveillance of Wastewater To Examine Seasonal Dynamics of Enterovirus Infections
Nichole E. Brinkman, G. Shay Fout, Scott P. Keely

American Society for Microbiology
mSphere®

Check for Updates
Wastewater-based SARS-CoV-2 Surveillance

- Complements existing COVID-19 surveillance systems
- Advantages
  - Non-invasive
  - Pool of individuals
  - Asymptomatic and symptomatic individuals
  - Inexpensive
  - Data for communities where individual testing data are underutilized or unavailable
  - Scalable
  - Unbiased
  - Can be a leading indicator of changes in community-level infection
The sewer as a mirror of society

Gertjan Medema
Outline for Presentation

• Analytical method development
• Understanding dilution and degradation in the sewer
• Relating the sewer signal to community case rates
• Building a statewide network of sampling
• Translating the information into public health decisions
**Method Considerations**

**Sample Type**
- Untreated wastewater
- Primary sludge

**Volume**

**Sample Preparation**
- Storage temperature
- Homogenization
- Additives
- Matrix Spike
- Clarification

**Sample Concentration**
- Ultrafiltration
- Electronegative membrane filtration
- Polyethylene glycol (PEG) precipitation

**Nucleic Acid Extraction**
- Silica columns
- Magnetic beads
- Precipitation

**RNA/DNA Measurement**
- RT-qPCR
- RT-ddPCR
- Genetic targets

**Other Considerations**
- Biosafety
- Supply Chain issues
- Practicality (time, equipment)
- QA/QC

---

Biosafety

• Wastewater risk is the same
• Increased risk with processes that could generate aerosols
  – Centrifugation
  – Membrane filtration
• CDC recommendations
  – Biosafety Level 2 laboratory
  – Biosafety Level 3 precautions
    • Respiratory protection
    • Designated donning/doffing area
• Borrowing lab space in AWBERC Biocontainment Suite
• Safety, Health and Environmental Management (SHEM)
• ORD’s BioRisk Management Advisory Committee

EPA Sample Processing and Analysis

PBS OC43

24-hr composite sample, 225 ml

Centrifuge 3000 x g, 15 min

0.2 ml Direct Extraction

Supernatant

Membrane filtration, 0.45µm

Pellet

Filtrate

Ultrafiltration, 30 kDa MWCO

Filtrate

Nucleic Acid Extraction (RNeasy Power Water Kit – silica column)

Retentate

Phi6

RT-ddPCR:/ddPCR

SARS-CoV-2 (N1, N2), RT-ddPCR QC, Inhibition control, Extraction Control, Matrix Recovery Control, Human fecal markers
Method Performance Metrics

- **Limit of Detection**
  - 655 RNA Molecules/L

- **Recovery Efficiency**
  - Endogenous virus
    - crAssphage 84%
    - PMMoV 27%
  - Matrix spike
    - Betacoronavirus OC43 (up to 50%)

- **RT-ddPCR Inhibition**
  - Minimal (< 20%)
Supply Chain Disruption #1

PBS OC43

24-hr composite sample, 225 ml

Centrifuge 3000 x g, 15 min

Membrane filtration, 0.45µm

PBS

0.2 ml Direct Extraction

Supernatant

Pellet

Filter

Filtrate

Ultrafiltration, 30 kDa MWCO

Retentate

Nucleic Acid Extraction (RNasey Power Water Kit – silica column)

Phi6

RT-ddPCR:

SARS-CoV-2 (N1, N2), RT-ddPCR QC, Inhibition control, Extraction Control, Matrix Recovery Control, Human fecal marker
Supply Chain Disruption #1

Ultrafiltration – Millipore Centricon Plus-70 centrifugal Units

~ 90% measurable virus in pellet and filter fractions
Supply Chain Disruption #2

PBS OC43 → 24-hr composite sample, 225 ml

Centrifuge 3000 x g, 15 min → Supernatant filtration, 0.45µm

Membrane filtration, 0.45µm → Filtrate

Ultrafiltration, 30 kDa MWCO → UF Retentate

0.2 ml Direct Extraction → Pellet

Nucleic Acid Extraction (RNeasy Power Water Kit – silica column)

RT-ddPCR:/ddPCR

SARS-CoV-2 (N1, N2), RT-ddPCR QC, Inhibition control, Extraction Control, Matrix Recovery Control, Human fecal marker
Supply Chain Disruption #2

Trizol-Chloroform Extraction
RNA precipitation

Phase separation  Isopropanol precipitation

- Aqueous phase
- Interphase
- Organic phase
- RNA pellet

Ana Braam

Lysis → Bind → Wash A → Wash B → Dry spin → Elute → Viral RNA
New extraction approach increased recovery efficiency 10-fold
The Metropolitan Sewer District of Cincinnati has various sewersheds, including Mill Creek, Taylor Creek, Little Miami, Muddy Creek, and Indian Creek. Each sewershed has data on MGD (Million Gallons per Day), % Industrial, % Combined, and Dilution.

<table>
<thead>
<tr>
<th>Sewershed</th>
<th>MGD</th>
<th>% Industrial</th>
<th>% Combined</th>
<th>Dilution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mill Creek</td>
<td>118</td>
<td>5.0</td>
<td>40</td>
<td>0.5:1</td>
</tr>
<tr>
<td>Taylor Creek</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>1.8:1</td>
</tr>
</tbody>
</table>
Sub-Sewershed Sampling: Cincinnati

Mill Creek

Lick Run
Combined Sewer Overflow

Remote Composite Sampler
~10L between 8-11 am
~500 ml every 15 min

Dry Weather Flow Within Structure

Access to Sewer

Sub-Sewershed Sampling – Lick Run
Accounting for Dilution Impacts

Mill Creek
118 MGD, 5% Industrial, 40% Combined

Taylor Creek
3 MGD, 0% Industrial, 0% Combined

<table>
<thead>
<tr>
<th></th>
<th>Correlation Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>0.43</td>
<td>0.0322</td>
</tr>
<tr>
<td>N1/crAv</td>
<td>0.59</td>
<td>0.00195</td>
</tr>
<tr>
<td>N2</td>
<td>0.483</td>
<td>0.0148</td>
</tr>
<tr>
<td>N2/crAv</td>
<td>0.563</td>
<td>0.000385</td>
</tr>
</tbody>
</table>

Flow volume
TSS
crAssphage
PMMov
HF183

<table>
<thead>
<tr>
<th></th>
<th>Correlation Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1</td>
<td>0.6</td>
<td>0.00201</td>
</tr>
<tr>
<td>N1/crAv</td>
<td>0.505</td>
<td>0.012</td>
</tr>
<tr>
<td>N2</td>
<td>0.521</td>
<td>0.00925</td>
</tr>
<tr>
<td>N2/crAv</td>
<td>0.529</td>
<td>0.00809</td>
</tr>
</tbody>
</table>
Different Views of Community Infection

Potential role of sentinel sites?

Red Line – County Infection Peak in early July
Temporal Trends of SARS-CoV-2 in Sewersheds

Range of minor peaks early, strong consistent increases across communities now.
What do these data mean?

• If you want to relate SARS-CoV-2 wastewater data to the number of infected individuals, you need to know:
  • Concentration of SARS-CoV-2 in wastewater
    – Measured concentration
    – Recovery Efficiency
    – Dilution
    – Decay
  • And how much SARS-CoV-2 shed in feces (uncertain)

• Or focus on relative changes at a given site
Governor DeWine initiates wastewater SARS-CoV-2 monitoring project May 2020

Ohio EPA - $2,000,000 for wastewater monitoring project via CARES funds ODH is project lead Ohio WRC project coordinator June 2020

Monitoring and Analyzing July 2020
- 7 large cities
- 15 locations sampled
- 3 laboratories – OSU, UT, US EPA

Adding Sites August – October 2020
- Medium and smaller cities
- 4 added laboratories – UA, KSU, Commercial lab, BGSU
- Sampling frequency twice a week
- Currently 52 sites

Workgroups created
Part of CDC national monitoring network
Working on analytical methods
Working on data analysis

Developing the Ohio Wastewater Monitoring Network
• Once a month
• SARS-CoV-2 positive sample send to all the labs
• Normal protocols performed
• Results analyzed
The focus is on **trends or significant changes** in the number of viral gene copies detected.

Currently action is taken when at least 3 samples show a sustained increase of at least 10-fold (1 log)

- Notify the local health district and utility
- Provide information on how to interpret the data and link to message toolkit
- Notify the state pandemic testing team for linkages to establish pop-up testing sites and the state contact tracing team to offer assistance

Ohio Public Health Applications

Development of toolkit for local health districts and utilities
• Additional messaging to public on best practices – social media, twitter

New focus on monitoring multiple sites on campus to support colleges/universities across state

Ohio is coordinating on data reporting approaches and with CDC on their National Wastewater Surveillance System
Monitoring SARS-CoV-2 virus in wastewater as an indicator of changes in community-level infection is a topic of interest to many different organizations, and EPA is committed to leveraging partnerships and collaborations to achieve results. Some examples:

- **CDC** – Weekly exchange with staff scientists to both provide status of EPA work and info on the National Wastewater Surveillance System

- **Ohio Wastewater Monitoring Network** – Committed to conducting samples as part of lab network, provided initial guidance on sample handling, coordinated interlaboratory comparisons, and developed standard data collection formats for entire network

- **Public Utilities** – Research collaboration with Cincinnati MSD, reached out to Ohio utilities organizations (i.e., AOMWA, OWEA) early in the pandemic, participated in initial meetings with potential participating utilities in Ohio, presented on status/progress to California WEA

- **States** – Provided technical assistance to Arkansas, Maryland, New Jersey and New Mexico as they developed their wastewater surveillance efforts

- **Research Community** – Participated in the Water Research Foundation International Virtual Summit on the topic in April and subsequent interlaboratory comparison of methods organized by WRF, shared results with Global Water Research Coalition’s Workgroup on SARS-CoV2 sewage surveillance
Final Summary

• **Analytical Method Development**
  – No standard method, but many options available (useful to address supply chain)
  – Quality Control for assessing method performance (recovery efficiency, inhibition control)

• **Dilution/Degradation in Sewer System**
  – Ongoing comparison of different approaches to normalize for dilution
  – Use existing temperature dependent rates, targeted studies on industrial wastes

• **Relation of Sewer Signal to Infection rates**
  – Accounting for recovery efficiency, dilution, degradation
  – Need better data on shedding rates
Final Summary

• Developing a network
  – Linking wastewater utilities, environmental analytical labs, public health agencies
  – Network of labs to increase capacity if needed; build in QA/QC

• Translating data to public health decisions
  – Focus on trends or significant changes in the concentration to reinforce public messaging
    • As models to predict infection are refined
  – Early warning?
    • Relative turnaround time of individual and wastewater data key
    • Sentinel sites might be very useful, but attributes of these sites may vary across pandemic cycle
  – Targeted sampling to direct individual testing/actions
    • e.g., university dormitory monitoring
Jay L. Garland, PhD
Research Scientist
Center for Environmental Solutions and Emergency Response
US EPA Office of Research & Development
569-7334 | Cell (513) 680-9264
garland.jay@epa.gov

The views expressed in this presentation are those of the author(s) and do not necessarily represent the views or policies of the US EPA. Any mention of trade names or commercial products does not constitute EPA endorsement or recommendation for use.
Research Team and Partners

EPA ORD
Laura Boczek
Jacob Botkins
Ana Braam
Nichole Brinkman
Dave Feldhake
Alison Franklin
Chloe Hart
Michael Jahne
Leah Julifs
Scott Keely
Brian Morris
Maitreyi Nagarkar
Sarah Okrum
Randy Revetta
Eunice Varughese
Emily Wheaton
Barry Wiechman

Hamilton County Public Health Department

Chris Griffith

Ohio Water Resources Center

Zuzana Bohrerova

Ohio Department of Health

Rebecca Fugitt

Ohio EPA

Brian Hall

Tiffani Kavalec

University Labs

Ohio State University

University of Toledo

Kent State University

University of Akron

Utilities

Metropolitan Sewer District of Greater Cincinnati

Bruce Smith

City of Dayton

Chris Clark, Walter Schroder

City of Marion

Steve Morris

City of Portsmouth

Tommy Stewart

Montgomery County

Jim Davis

City of Hamilton

Mark Smith

City of Springfield

Jeff Yinger