SARS CoV-2  Wastewater Monitoring: Research & Application to Meet Immediate Needs

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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 Noninvasive Tool to Heighten Public Awareness of Societal Use of Illicit-Abused Drugs and Their Adverse Health Effects
Christian G. Daughton

Estimating Community Drug Abuse I
Ettore Zuccato, Chiara Chiabrandi, Sara Castiglioni, Riccardo Grotti

Israel's Silent Polio Epidemic Breaks All the Rules
Leslie Roberts
+ See all authors and affiliations

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

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

• Adjusting the three “knobs”
  – Analytical method development
  – Understanding dilution and degradation in the sewer
  – Relating the sewer signal to community case rates

• Building a statewide network of sampling & linking to public health decisions

• Next Steps
  – Monitoring this pandemic
  – Preparing for the next potential pandemic
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

EPA Sample Processing and Analysis

24-hr composite sample, 225 ml

Centrifuge 3000 x g, 15 min

Membrane filtration, 0.45µm

Ultrafiltration, 30 kDa MWCO

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 markers
• **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%)
Recovery in Different Fractions

~ 90% measurable virus in pellet and filter fractions
Alternative RNA Extraction

Trizol-Chloroform Extraction
RNA precipitation

Phase separation
Isopropanol precipitation

Aqueous phase
Interphase
Organic phase
RNA pellet

Ana Braam
New extraction approach increased recovery efficiency 10 fold
Metropolitan Sewer District of Cincinnati

<table>
<thead>
<tr>
<th>Sewershed</th>
<th>MGD</th>
<th>% Industrial</th>
<th>% Combined</th>
<th>Dilution</th>
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<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
Sub-Sewershed Sampling – Lick Run

Combined Sewer Overflow

Dry Weather Flow Within Structure

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

Access to Sewer
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

- Mill Creek
- Taylor Creek
- Little Miami
- Muddy Creek
- Dayton
- Western Regional
- Eastern Regional
- Hamilton
- Springfield
- Portsmouth
- Marion
- Lick Run
Individual Site Example (Mill Creek)

From Dashboard
<table>
<thead>
<tr>
<th>Site_name</th>
<th>R2 Load</th>
<th>p value Load</th>
<th>Lab</th>
<th>Population Served</th>
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<tbody>
<tr>
<td>Mill Creek WWTP</td>
<td>0.689</td>
<td>5.46E-13</td>
<td>USEPA</td>
<td>488,000</td>
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<td>Eastern Regional WRF</td>
<td>0.674</td>
<td>2.85E-11</td>
<td>USEPA</td>
<td>36,150</td>
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<td>Hamilton WRF</td>
<td>0.584</td>
<td>6.02E-09</td>
<td>USEPA</td>
<td>65,000</td>
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<tr>
<td>Little Miami WWTP</td>
<td>0.530</td>
<td>3.16E-08</td>
<td>USEPA</td>
<td>143,000</td>
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<td>Muddy Creek WWTP</td>
<td>0.469</td>
<td>2.08E-07</td>
<td>USEPA</td>
<td>76,000</td>
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<tr>
<td>Springfield WWTP</td>
<td>0.495</td>
<td>5.83E-07</td>
<td>USEPA</td>
<td>60,000</td>
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<tr>
<td>Taylor Creek WWTP</td>
<td>0.442</td>
<td>1.14E-06</td>
<td>USEPA</td>
<td>34,000</td>
</tr>
<tr>
<td>City of Marion WPC</td>
<td>0.515</td>
<td>5.53E-06</td>
<td>USEPA</td>
<td>36,000</td>
</tr>
<tr>
<td>Dayton WWTP</td>
<td>0.318</td>
<td>1.97E-05</td>
<td>USEPA</td>
<td>269,850</td>
</tr>
<tr>
<td>Portsmouth Lawson Run WWTP</td>
<td>0.482</td>
<td>8.32E-05</td>
<td>USEPA</td>
<td>20,366</td>
</tr>
<tr>
<td>Western Regional WRF WWTP</td>
<td>0.222</td>
<td>1.42E-03</td>
<td>USEPA</td>
<td>79,000</td>
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</tbody>
</table>
Summary

• **Analytical Method Development**
  – Predominantly particle associated
  – RNA extraction is critical step; standard kits show relatively low recovery
  – Quality Control for assessing method performance (recovery efficiency, inhibition control)

• **Dilution/Degradation in Sewer System**
  – Ongoing comparison of different approaches to normalize for dilution, flow adjusted can be used in standard composite sampling
  – Use existing temperature dependent rates, targeted studies on industrial wastes

• **Relation of Sewer Signal to Infection rates**
  – Statistical models show good correlation
  – Mechanistic models still under development to predict specific # of infected individuals (need better data on shedding rates)
Next Steps

- Continue monitoring the current pandemic
  - Density of SARS-CoV-2
  - Distribution of different variants in wastewater
Actual Distribution of Variants within a Community

Range of Existing Variants of SARS-CoV-2
Estimating Variants in the Community by Analyzing COVID Patients

Clinical samples with enriched, clonal type of SARS-CoV-2*

* $10^4$-$10^7$ copies per ml in throat/sputum samples

Estimating Variants in the Community by Analyzing Wastewater

Single, diverse, unenriched wastewater sample

Up to $10^5$ per L N1 or N2 gene copies

$\sim 10^8$ gene copies per L of crassphage

Many other human and non-human related viruses

Extraction (if threshold levels)

Targeted PCR for mutations in variants of concern

Enrichment of SARS-CoV-2 Genome (multiplexed PCR)

Sequencing
Ohio Wastewater Monitoring Network

https://coronavirus.ohio.gov/wps/portal/gov/covid-19/dashboards/other-resources/wastewater
**Initiation of Network**

May 2020

Governor DeWine initiates wastewater SARS-CoV-2 monitoring project

**Funding of Network**

June 2020

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

**First Sites Onboarded**

July 2020

7 large cities
15 locations sampled
3 laboratories

**Additional Sites**

August-October 2020

Medium and smaller cities
4 added laboratories
21 sites and adding 25 other sites

**Technical Aspects**

November-December 2020

Continue to add sites (60)
Build spatial join with sewershed cases
Add additional graphs and file downloads

**Expand the Network**

January-March 2021

Continue to add sites (65)
Add additional lab (8)
Statistical data analytics
Build sequencing framework
Ohio launches coronavirus wastewater monitoring network in 22 cities

Updated Sep 03, 2020; Posted Sep 03, 2020

By Jeremy Pelzer, cleveland.com

Wastewater analysis helping to detect COVID early in Ohio, but more communities need to participate, officials say

Dean Narciso  The Columbus Dispatch
Published 5:41

Wastewater tests detected early signs of rising COVID-19 levels

Sewer water may yield faster results on COVID-19 trends than testing people

Lou Whitmire  Mansfield News Journal
Published 8:08 p.m. ET Oct. 1, 2020

Dayton Daily News
Ohio expands wastewater tests that show early signs of coronavirus increases

LOCAL NEWS | Updated Sept 29, 2020
By Kristen Spicker
Ohio Wastewater Monitoring Network

Network Status
Twice weekly sampling based on CDC recommendations
Currently monitoring at 65 sites that account for almost 50% of the state population
• All data collected from university labs, commercial lab, and US EPA are entered into one database for upload to state Innovate Ohio Platform (IOP)
• Viral gene copy results are presented on a dashboard for public use

Participating in CDC National Wastewater Surveillance System (NWSS)
• Data is uploaded daily
Ohio Wastewater Monitoring Network Dashboard

Public Health Applications

Screening tool to drive public health action

Focus is on **trends or significant changes** in the number of viral gene copies detected

- 3-17-day lead time with wastewater

Currently action is taken when last 2-3 samples show a sustained increase of at least 10-fold (1 log)

State actions when increases are observed:

- 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 and contact tracing teams
Role of Local Health Districts

Good afternoon,

Recent results indicate an increase in viral gene copies at the wastewater treatment plant:

<table>
<thead>
<tr>
<th>Facility_Name</th>
<th>Population_Served</th>
<th>Collection_Date</th>
<th>Flowrate_Sample</th>
<th>Flowrate_Ld</th>
<th>N2_Avg</th>
<th>Load_N2_Avg</th>
<th>MGC/day</th>
<th>MGC/person,</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWTP</td>
<td>52,000</td>
<td>03032021</td>
<td>11910000</td>
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<td>926864</td>
<td>390149624664</td>
<td>900000</td>
<td>18.0</td>
</tr>
</tbody>
</table>

If you would like to schedule a COVID-19 pop-up testing event in this area, please contact TestingRequests@ODH.ohio.gov and the state Testing Team will be in contact to discuss testing options.

As always, please let us know if you have any questions.

Suggested Social Media Posts

COVID-19 Alert: Wastewater monitoring in the XXX community has indicated that COVID-19 may be on the rise. The virus can be shed in wastewater as early as 3-7 days before we see an increase in positive tests and hospitalizations. Read more: [insert link].

Increasing COVID-19 trend detected: Early monitoring of the XXX wastewater sewershed, which services XXX, XXX and XXX, has indicated that COVID-19 may be on the rise. Wastewater detection can occur as early as 3-7 days before positive tests and hospitalizations. Read more: [insert link].

Higher levels of COVID-19 detected in XXX: Wastewater monitoring in the XXX community has indicated that COVID-19 may be on the rise. The virus can be shed in wastewater as early as 3-7 days before we see an increase in positive tests and hospitalizations. Read more: [insert link].

Role of Local Health Districts

Local Health Districts have received over 100 notifications of increases
• Utilized additional testing services offered by the state
• Used data for own analysis
• Conducted community outreach and messaging

Local Health Districts are continually interested in this data and how it can be used to drive public health actions
Using the Data

Challenging!

• Began in the thick of the pandemic
• Lack of baseline measurements
• Lack of biological data needed to inform predictive models
  • Asymptomatic infection rates
  • Fecal viral shedding rates
  • Duration of viral shedding
  • Transient populations
• Variability in wastewater
  • Fluctuations in sewage usage
Using the Data

Modelling/Statistics Workgroup
Collaboration between ODH, OSU, and US EPA that meets weekly to discuss best data analysis practices

Develop methodologies to determine trends
- Four-period moving averages
- Regression analyses
Using the Data

Modelling/Statistics Workgroup

Determine lead time wastewater data provides
  • Initial analysis suggests **3-17-day lead time** with wastewater ahead of case data

Develop methodologies/predictive models to use wastewater data to predict number of case rates/infected individuals
  • Viral Loads detected in wastewater and current case data can be used to inform the model and make predictions/forecast
  • Currently a lot of work focused on this around the nation
SARS-CoV-2 Variants

Variant of Concern

A variant for which there is evidence of an increase in transmissibility, more severe disease (increased hospitalizations or deaths), significant reduction in neutralization by antibodies generated during previous infection or vaccination, reduced effectiveness of treatments or vaccines, or diagnostic detection failures.

Possible attributes of a variant of concern:

In addition to the possible attributes of a variant of interest:

- Evidence of impact on diagnostics, treatments, and vaccines
  - Widespread interference with diagnostic test targets
  - Evidence of substantially increased resistance to one or more class of therapies
  - Evidence of significant decreased neutralization by antibodies generated during previous infection or vaccination
  - Evidence of reduced vaccine-induced protection from severe disease
- Evidence of increased transmissibility
- Evidence of increased disease severity

Variants - Sequencing the Wastewater

Whole genome sequencing is the most recent application of wastewater surveillance

- Sequencing clinical samples = costly and time consuming
- Sequencing wastewater = early indication of variant spread

Goal: Use wastewater as a leading indicator of variant spread

- Identify key locations
- Sequence wastewater weekly
- Wastewater sequencing results can drive clinical sequencing
Ohio’s first variant case was detected in a clinical sample taken on January 6
  • Preliminary sequencing at 16 key sites in the first week of January revealed no
    variants of concern

ODH is collaborating with university laboratories and US EPA to sequence wastewater
  • Three laboratories will sequence for the first few weeks
  • Key sentinel sites will be identified
  • Weekly sampling of key sentinel sites
The Future of Wastewater

• Continue to onboard wastewater treatment sites – good coverage around the state
• Build sequencing network and begin sequencing wastewater readily
• Utilize wastewater as a screening tool to drive focus for additional data
• Better understand factors affecting disease spread
• Determine impacts on disproportionately affected communities

• Utilize wastewater monitoring network for things other than SARS-CoV-2 – CDC National Wastewater Surveillance System
  • Seasonal influenza
  • Chemical markers – opioids
  • Future disease outbreaks
Next Steps

• Continue monitoring the current pandemic
  – Density of SARS-CoV-2
  – Distribution of different variants in wastewater

• Become better prepared for the next potential pandemic
  – Standardized methods
    • Extracting, purifying DNA & RNA from wastewater
    • Normalizing for population size
    • Screening methods for potential threats
  – Define sampling networks
    • Large scale networks for detecting emergence
    • City level networks for defining hotspots
  – Improving wastewater based epidemiological models
    • Fecal shedding rates
    • Decay rates in sewer
Research Team and Partners

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**Ohio Department of Health**
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- 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
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