Technical Challenges to Enhanced Integration of Wastewater-Based Pathogen Data into Epidemiological Practice

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3/14/2023
Outline

1. Overview of the Wisconsin wastewater surveillance program
2. Advantages of Wastewater-Based Epidemiology (WBE)
3. Challenges of WBE
   a. Technical challenges
   b. Examples of key types & how to assess them
4. Current recommendations
5. Existing gaps = future directions 😊
Overview of Wisconsin Wastewater Surveillance
Wisconsin Wastewater Surveillance

Fall 2020

72 WWTFs
49 out of 72 counties
60% population

UW-dorms

Mostly 2x per week

Major cities
Smaller cities
Tribal nations

Funded by

Centers for Disease Control and Prevention

Wisconsin State Laboratory of Hygiene
Wisconsin Department of Health Services
University of Wisconsin-Madison
University of Wisconsin-Milwaukee
Wastewater Surveillance Timeline

- **2020**
  - September: Begin SARS-CoV-2 surveillance
- **2021**
  - December: DHS dashboard launched
- **2022**
  - January: Begin routine genomic surveillance
  - Fall: Launch genomic dashboard
  - September: Begin RSV surveillance
  - January: Begin seasonal flu surveillance
- **2023**
  - Autumn: Other viruses
  - Seasonal flu
  - RSV
Since 2020, hundreds of communities have started reporting wastewater data to CDC’s National Wastewater Surveillance System to track COVID-19.

March 11, 2020

– larger points represent larger communities

www.cdc.gov/nwss
Sample Processing Workflow

**WWTFs**
- **Collection**: 24h Composite Samples
- **Concentration**

**WSLH/UWM**
- **RNA Extraction**
- **Quantification**
- **Data Analysis & Quality Control**

**DHS**
- **WI Dashboard**
- **National Dashboard**

**Automated concentration**
Nanotrap ER2 (Ceres) = nanoparticle capture

**Automated extraction**
Maxwell® HT Viral TNA kit (Promega)

**Digital PCR**
QIAcuity (Qiagen) N1/N2 CDC assays
Sample Processing Workflow

- **WWTFs**
  - Collection
  - 24h Composite Samples

- **WSLH/UWM**
  - Concentration

- **Data Analysis & Quality Control**

- **DHS**
  - WI Dashboard

- **National Dashboard**

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**COVID-19 Wastewater Surveillance in Wisconsin**

Comparing average SARS-CoV-2 levels in wastewater to COVID-19 case rates

- **Statewide presence of SARS-CoV-2 in wastewater**
- **SARS-CoV-2 concentration categories**
  - Very high
  - High
  - Moderate
  - Low
  - Very low

- **7-day average COVID-19 case rate**

**WI Department of Health Services (DHS) dashboard**
https://dhs.wisconsin.gov/covid-19/wastewater.htm

**Centers for Disease Control and Prevention (CDC) dashboard**
https://covid.cdc.gov/covid-data-tracker/#wastewater-surveillance
Similar clinical vs wastewater variant profiles

Proportion of variants trends over times

- Clinical vs Wastewater
- Lineages:
  - Delta
  - Omicron (BA.1)
  - Omicron (BA.2,12.1)
  - Omicron (BA.2,75)
  - Omicron (BA.2)
  - Omicron (BA.4)
  - Omicron (BA.5)
  - Omicron (BQ.1)
  - Omicron (XBB)
  - Other
  - Other
- WeekID

Last update: 2023

sequencing, we can even identify which COVID-19 variants are circulating in a community. This is important to understand because
Advantages of Wastewater-Based Epidemiology (WBE)
WBE vs. Traditional Epidemiology

- **Low cost**
- **Time efficient**
- **Monitor entire community in a sewershed**
- **No geographic/demographic/testing bias**
- **Test symptomatic & asymptomatic**
WBE

Key Challenges
WBE as Epidemiological Tool

- CDC National & State / Local dashboards
- Success stories of WBE → wider acceptance of data
- APHL and CDC → guidelines for WBE
- Correlation with clinical trends, early warning, etc.

- No standard methodology
- Sensitivity – what is sufficient recovery?
- Understanding sources of variability
- Minimization of signal variability – sample and analytical
- Data analysis – normalization?
- Turnaround time – what is sufficient?
Sensitivity / Signal Recovery

Basic Steps of Pathogen Quantification in Wastewater

- Concentrate Viral Particles
- Extract nucleic acids
- Quantify RNA (Gene copies)

Consider & evaluate each step for optimal sensitivity and precision for every pathogen
Sensitivity / Signal Recovery

Most Common Viral Concentration Methods

Direct Extraction

Magnetic Particles (Nanotrap)

Direct Capture

HA Filtration

PEG Precipitation

Centricon size selection
Sensitivity / Signal Recovery

- Recovery is method AND sample matrix dependent
- Highest recovery can also be more variable (LOD vs. precision)
- Viral recovery control (BCoV) and fecal markers (PMMoV) need to be also evaluated
- BCoV and PMMoV are not great surrogates SARS-CoV-2

To normalize or not to normalize?
Signal Normalization to Improve Trends

- Flow & population
- Fecal strength marker
- Viral Recovery Control
- Dry mass & fecal marker
- Population only
Signal Normalization?
Sampling / Matrix Variability

- Variability in wastewater data (larger for small WWTF)
- Wastewater SARS-CoV-2 levels reflect COVID-19 clinical trends

City size

2 samples/week wastewater trend
Improving data accuracy & decreasing variability – better match with clinical trends

i. Reduce variability within samples

Wastewater matrices are complex

- Flow proportional 24h composites
- Keep samples refrigerated
- Flag samples failing QC
- Sampling frequency minimum 2x a week
- Medium to large sewersheds
- Subsample for analysis well-mixed/representative portion
Laboratory Method Variability

i. Reduce variability within samples

ii. Reduce variability of laboratory analysis

Can be lessened by:

- Step-wise method evaluation (isolate steps with high variability)
- Inhibition assessment
- Stringent QAQC – rerun & re-concentration criteria
  
  i. qPCR depends on good standard curves
  ii. Jumps in signal > 3-fold
  iii. Viral recovery or fecal marker loss, etc.
Overall Signal Variability

i. Reduce variability within samples

ii. Reduce variability of laboratory analysis

iii. Reduce data variability post measurement – statistical & modeling approaches

Better models

Machine learning (wastewater levels + vaccination rate + temperature)
**Best Practices**

**Sample**
- Influent or primary sludge
- Frequency 2-3x per week
- 24h flow composite
- Best population size?
- Refrigeration (no freezing)

**Laboratory**
- Optimize for sensitivity (LOD/LOQ)
- Minimize analytical variability → include automation
- Matrix recovery & fecal controls
- PCR inhibition testing
- dPCR platforms
- Replication
- Stringent QAQC
- PT program
- Method optimization must include sequencing

**Data Reporting**
- Automated QC flagging → re-analysis
- Automation → data entry errors & turnaround time
- LIMS system: COC & data QC
- Outlier calling and statistical smoothing for best trends
- Partnerships – WWTFs, DHS, CDC

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**SARS-CoV-2 Wastewater Surveillance Testing Guide for Public Health Laboratories**

MARCH 2022


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[https://www.cdc.gov/nwss/reporting/index.html](https://www.cdc.gov/nwss/reporting/index.html)
Knowledge Gaps & Future Directions
Future Directions

- Standardization of sampling approaches depending on end-goal
- Improved standardization of laboratory analytical processes:
  * Standard Reference Materials?
  * better method performance assessment approaches and inter-laboratory evaluation programs
  * a toolbox of customizable method options that fit different objectives
  * improve accuracy, decrease variability, decrease turnaround time
  * SOP for how to deal with methodological changes

- Improved understanding of appropriate data normalization approaches
- Optimized data modeling for best trends
- Improved statistical approaches for trend calling
  * actionable thresholds that are universally accepted?
- A case-book of successful uses of WBE data
Acknowledgments

Martin Shafer
Adelaide Roguet
Jocelyn Hemming
Kayley Janssen
Angellica, Becca, Devin, Evelyn, Griffin,
Hannah, Kaitlyn, Paige, Ellie, Dash

Clinical Division:
Florek Kelsey
Jossart Christopher
Shockey Abigail

Sandra McLellan
Melissa Schussman
Angie Schmoldt
Shuchen, Melinda, Brooke

Jon Meiman
Nathan Kloczko
Matthew Schinwald
Peter DeJonge

Questions?
Flu/RSV surveillance in Wisconsin
2022-2023 season
Statewide level

Influenza A
Influenza B
RSV

Sample classification (%)

% Positive by PCR

Week ending

Statewide level