Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN-MADISON

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

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**Wisconsin State Laboratory of Hygiene**

*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 ☺

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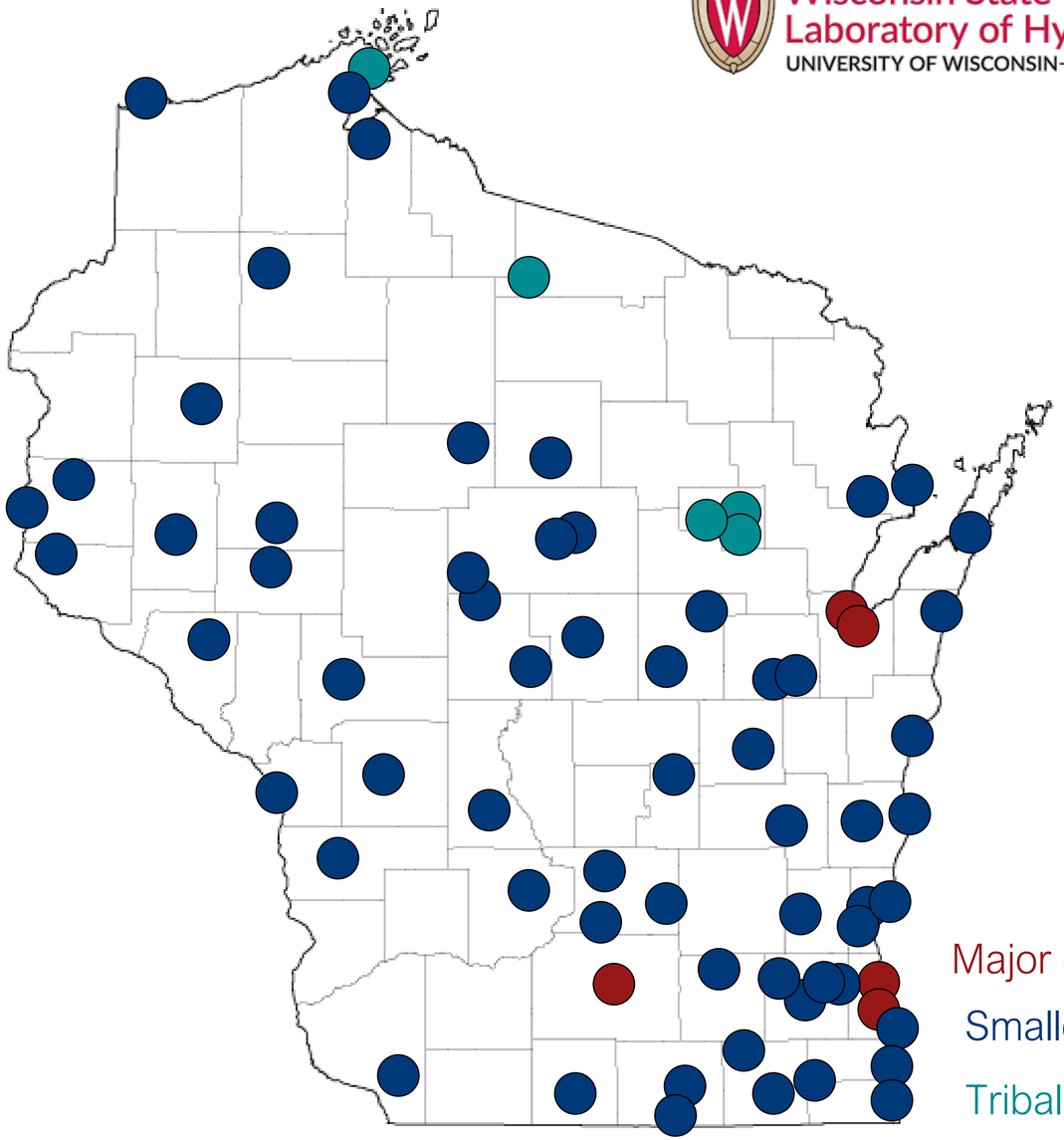
# Overview of Wisconsin Wastewater Surveillance

# Wisconsin Wastewater Surveillance

Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

WISCONSIN DEPARTMENT of HEALTH SERVICES

UNIVERSITY of WISCONSIN
UWMILWAUKEE



Major cities

Smaller cities

Tribal nations

Mostly 2x per week

## Fall 2020

**72 WWTFs**

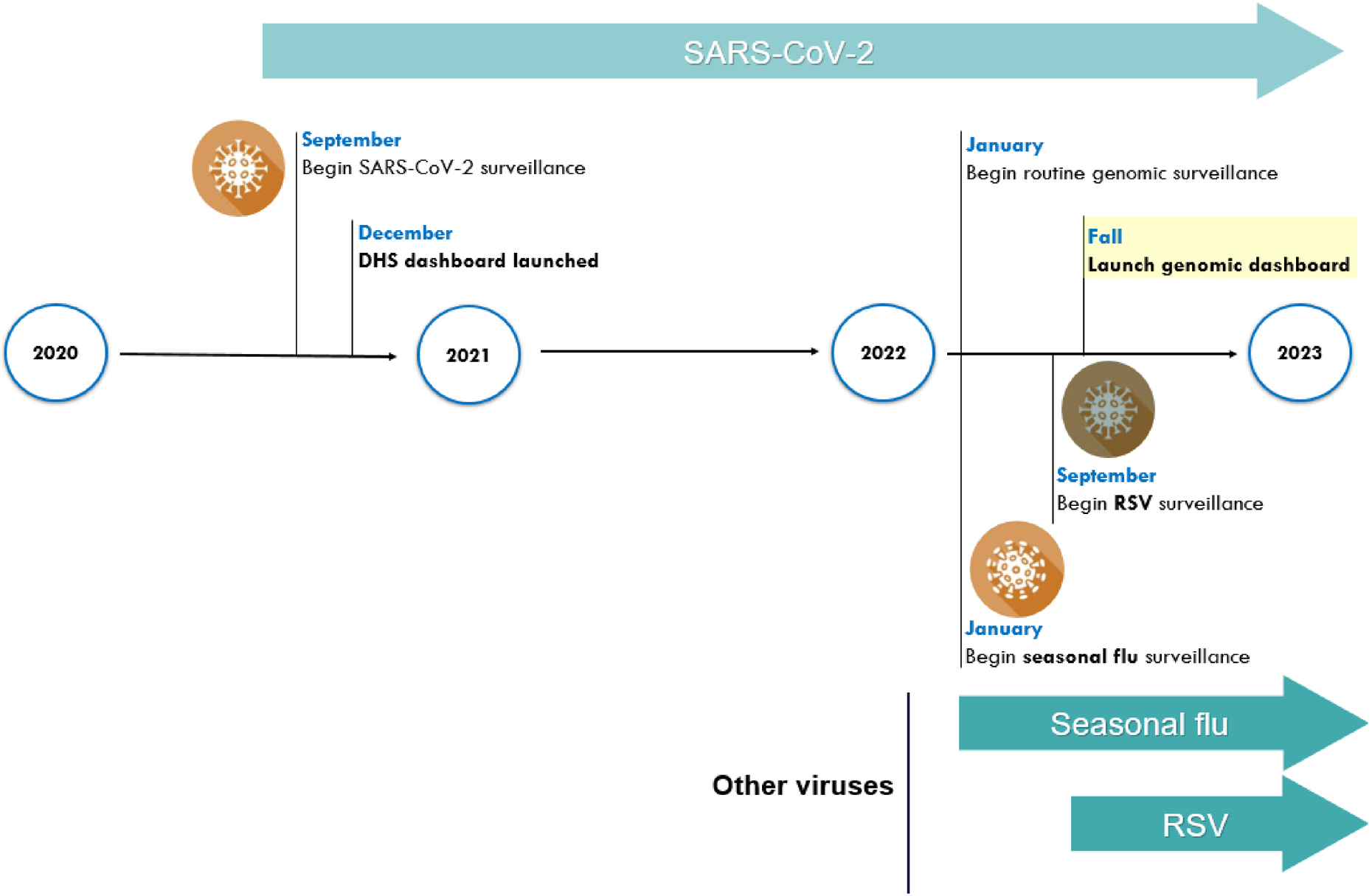
49 out of 72 counties

60% population

**UW-dorms**

Funded by CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

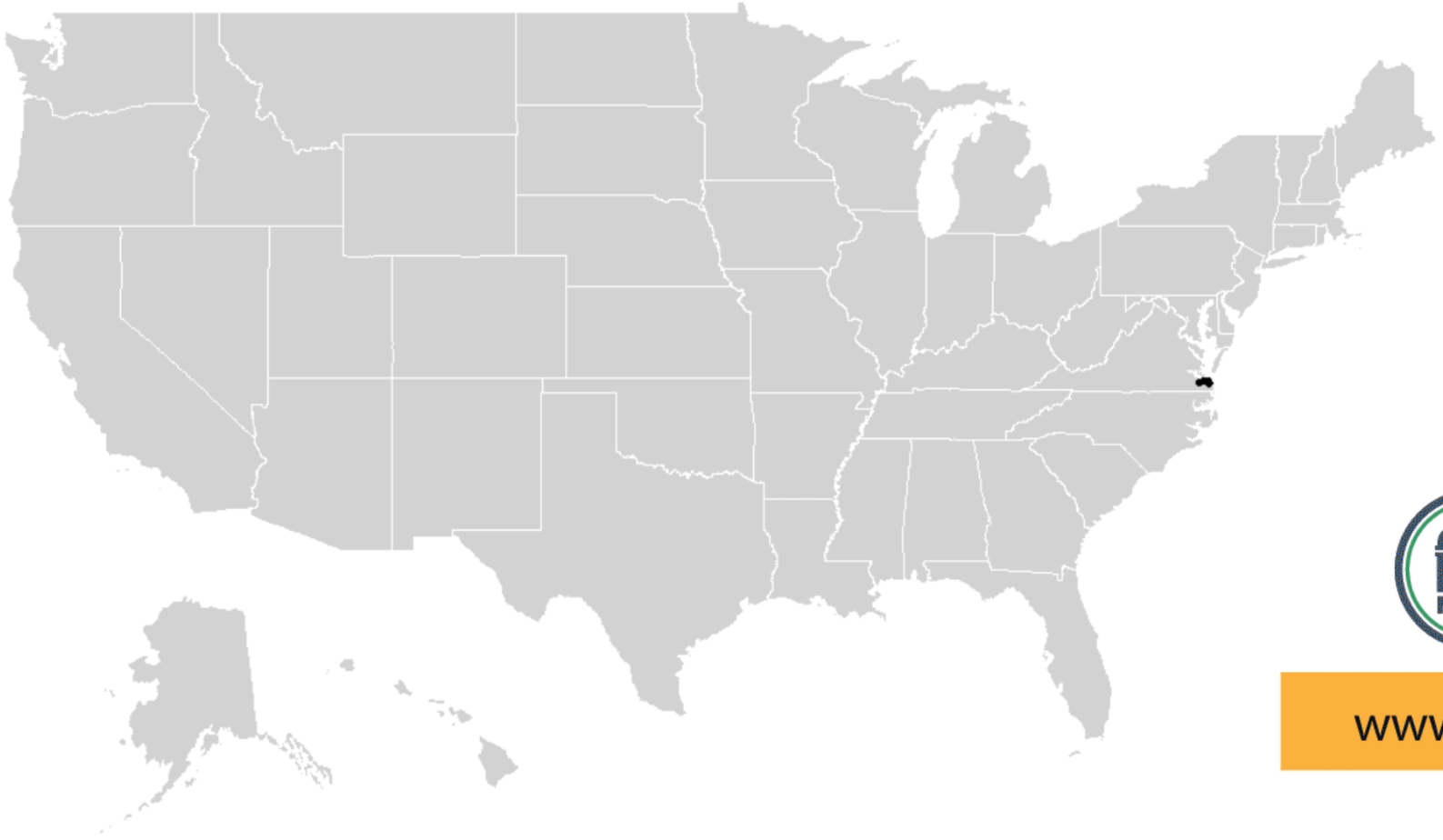
# Wastewater Surveillance Timeline



# BUILDING NATIONAL WASTEWATER SURVEILLANCE | COVID-19 |

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



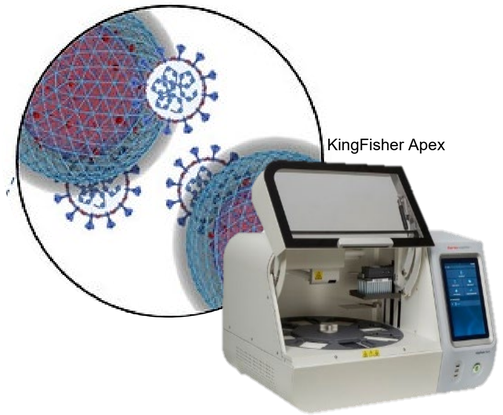
NATIONAL ™ WASTEWATER SURVEILLANCE SYSTEM

www.cdc.gov/nwss


# Sample Processing Workflow

WWTFs → WSLH/UWM → DHS

Collection 24h Composite Samples → Concentration → RNA Extraction → Quantification → Data Analysis & Quality Control → WI Dashboard → National Dashboard

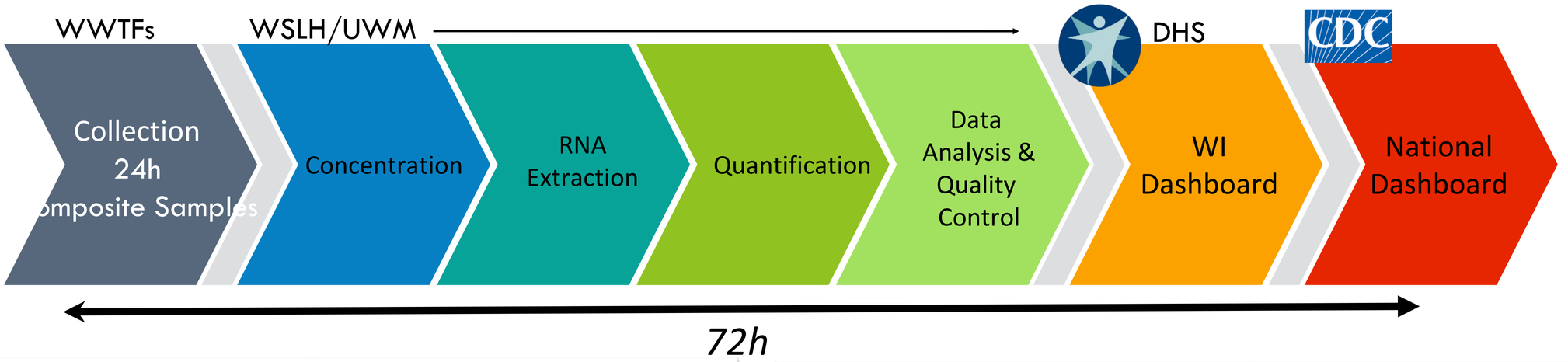


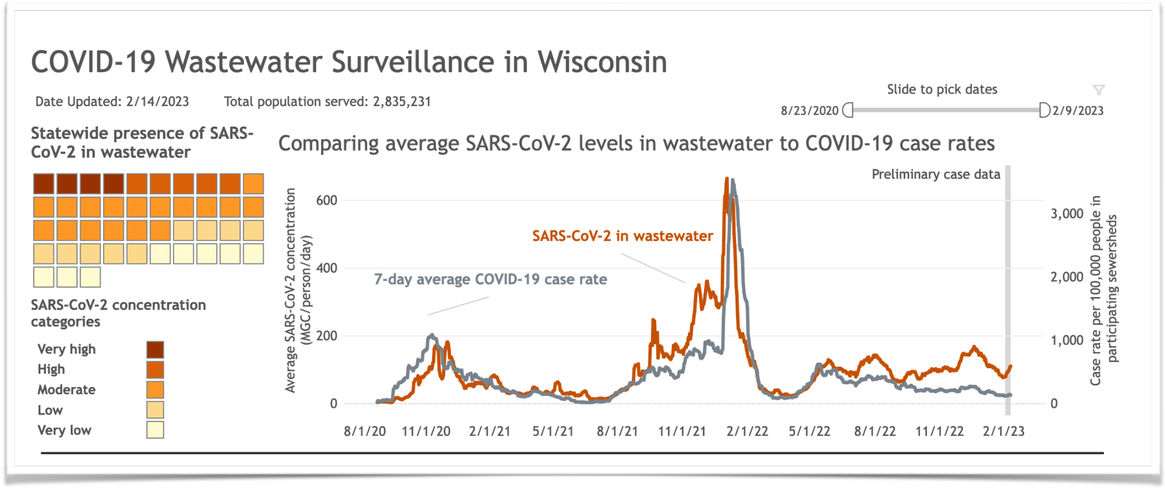

**Automated concentration**
Nanotrap ER2 (Ceres)
= nanoparticle capture


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

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

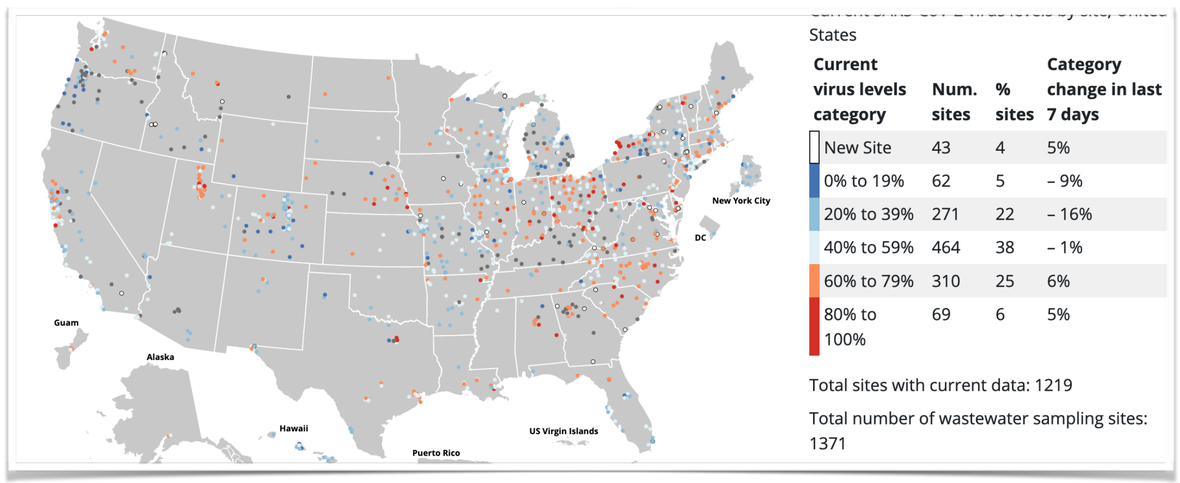
# Sample Processing Workflow




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

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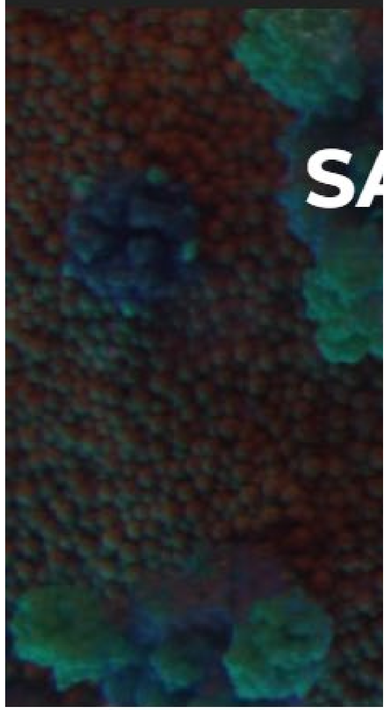
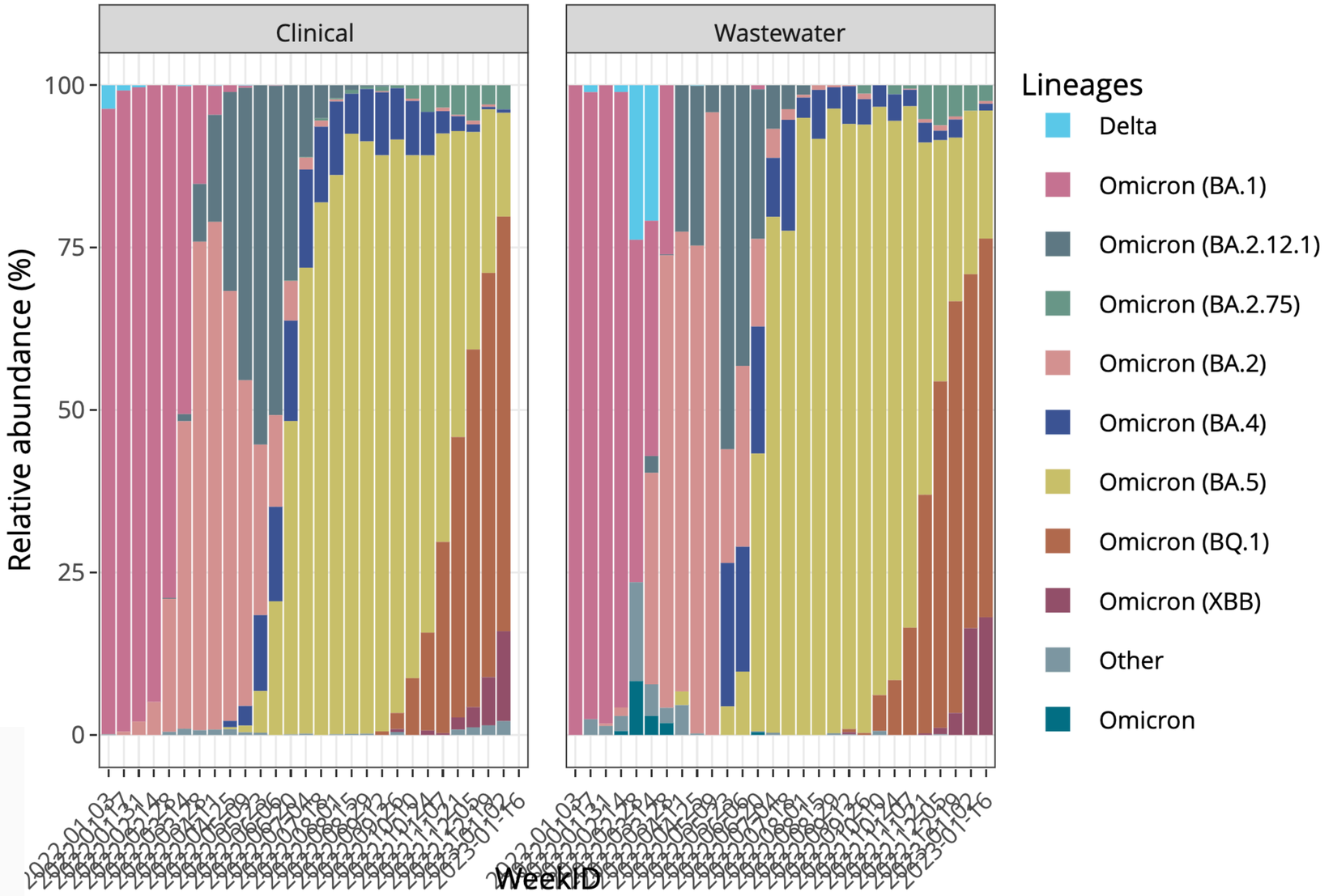


# Proportion of variants trends over times




Last update: 2023

Similar clinical vs wastewater variant profiles

sequencing, we can even identify which COVID-19 variants are circulating in a community. This is important to understand because

https://dataportal.slh.wisc.edu/sc2-ww-dashboard

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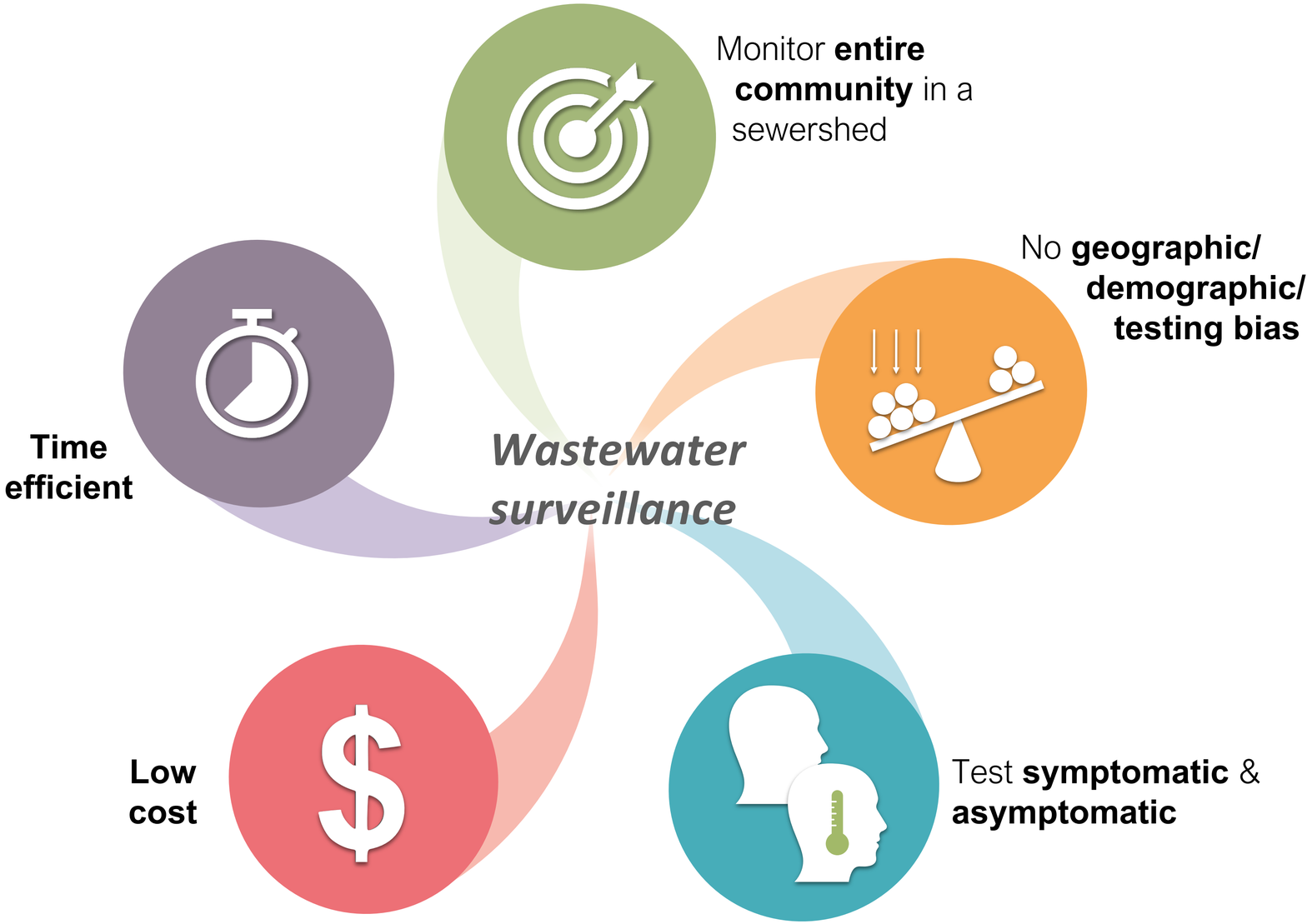
# Advantages of Wastewater-Based Epidemiology (WBE)

# WBE vs. Traditional Epidemiology



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# WBE
# Key Challenges

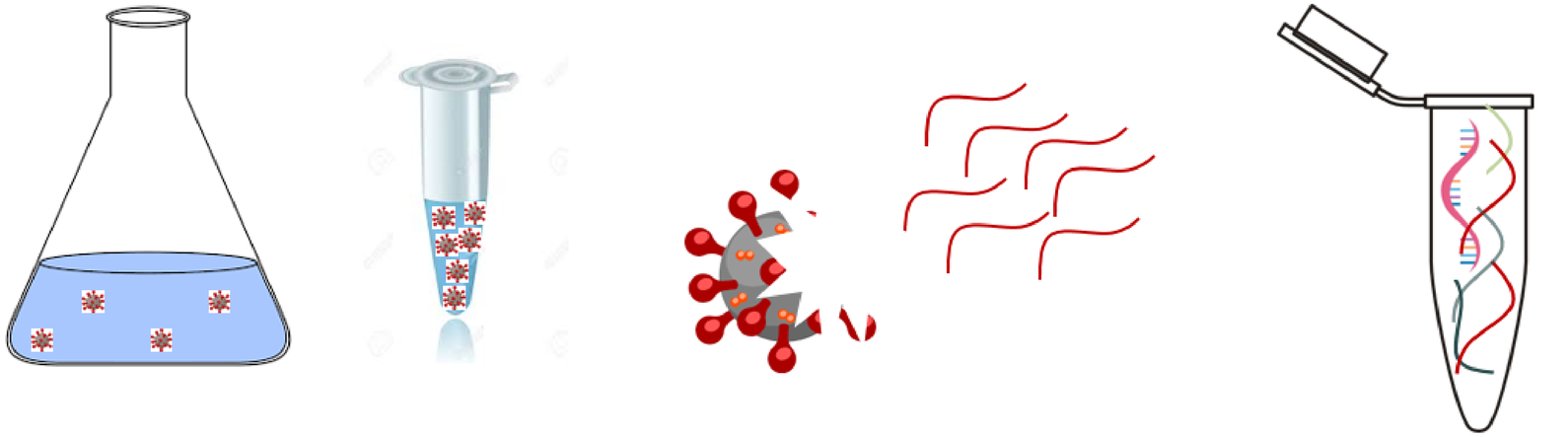
# WBE as Epidemiological Tool

- 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

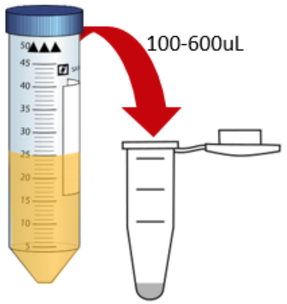
Extract nucleic acids

Quantify RNA
(Gene copies)

*Consider & evaluate each step for optimal sensitivity and precision for every pathogen*

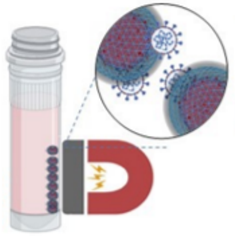
# Sensitivity / Signal Recovery

***Most Common Viral Concentration Methods***




Direct Extraction

HA Filtration



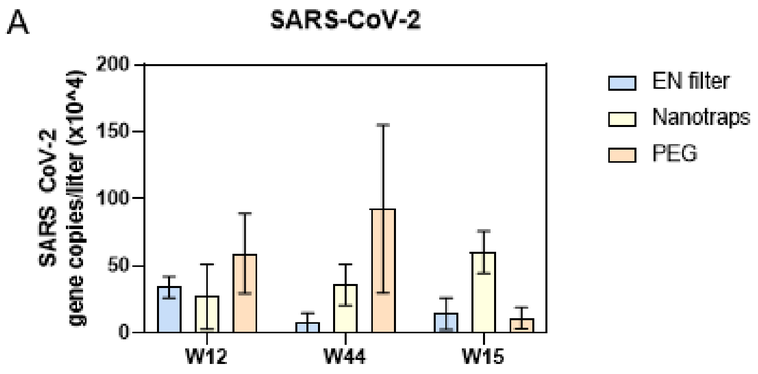
Magnetic Particles
(Nanotraps)

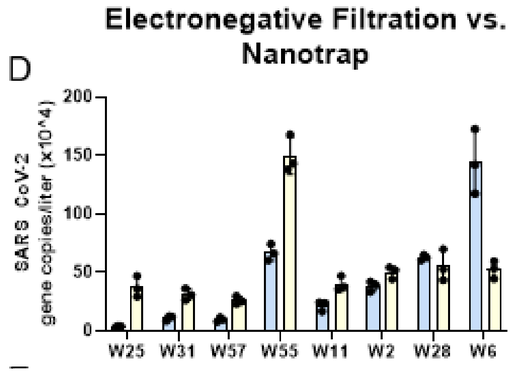
Direct Capture

Centricon size selection

PEG Precipitation

# Sensitivity / Signal Recovery

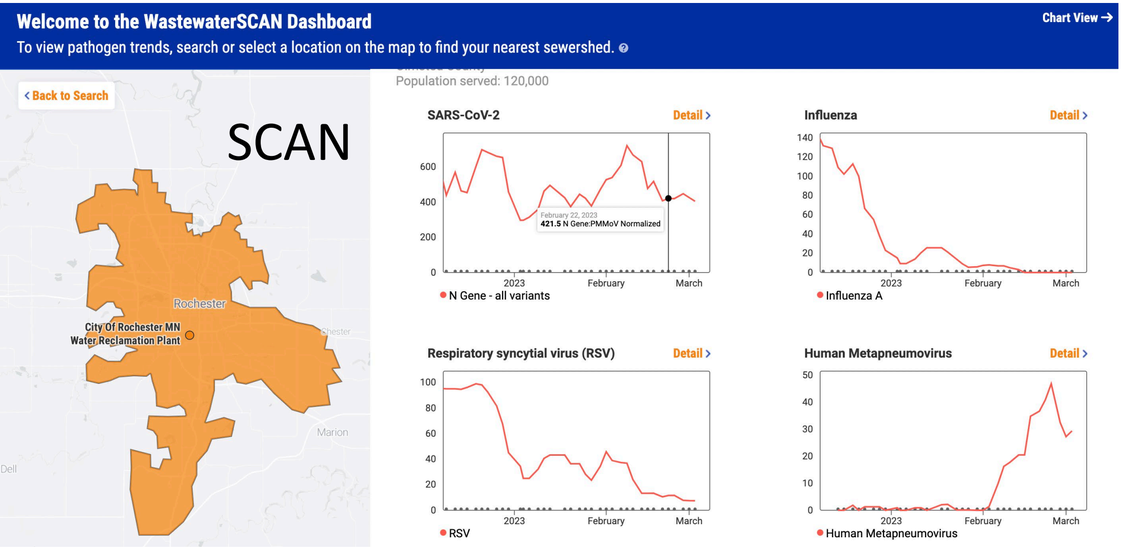


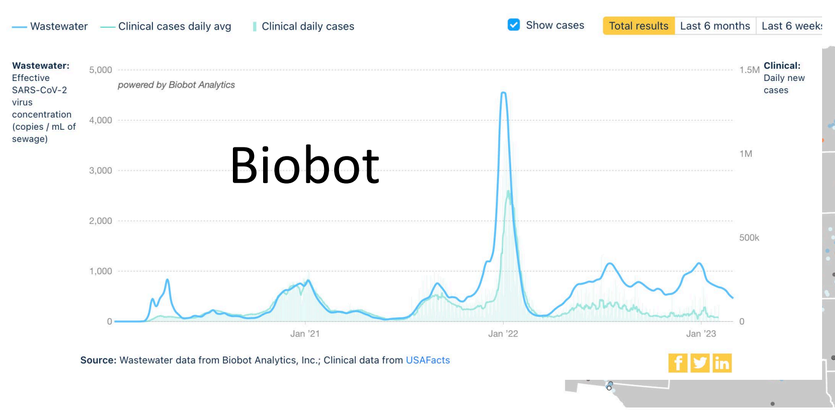



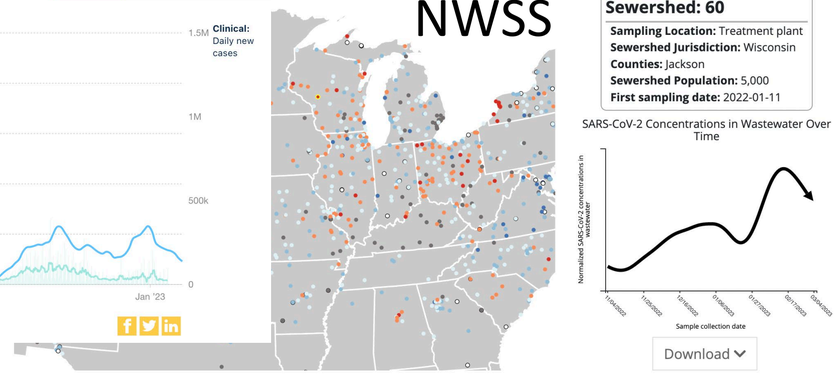

- Recovery is method AND sample matrix dependent
- Highest recovery can also be more variable (LOD vs. precision)
- Viral recovery control (BCoV) and fecal markers (PMMoV) need to be also evaluated
- BCoV and PMMoV are not great surrogates SARS-CoV-2

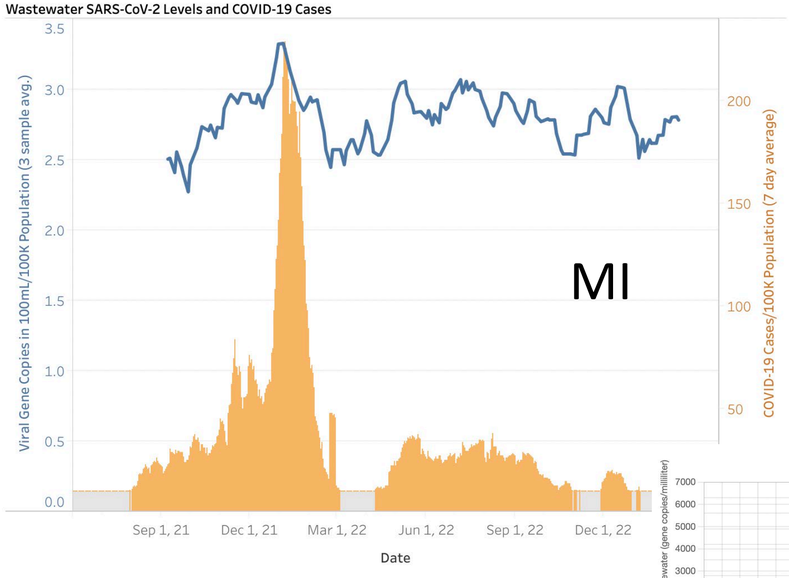
*To normalize or not to normalize?*

# Signal Normalization to Improve Trends



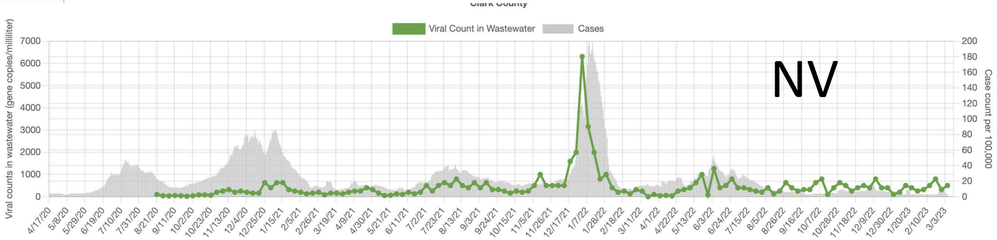



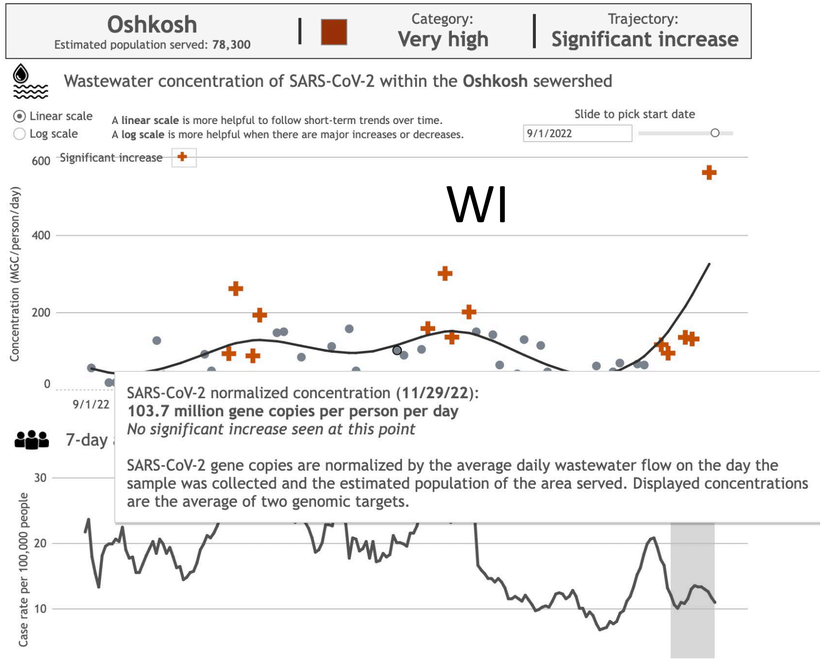



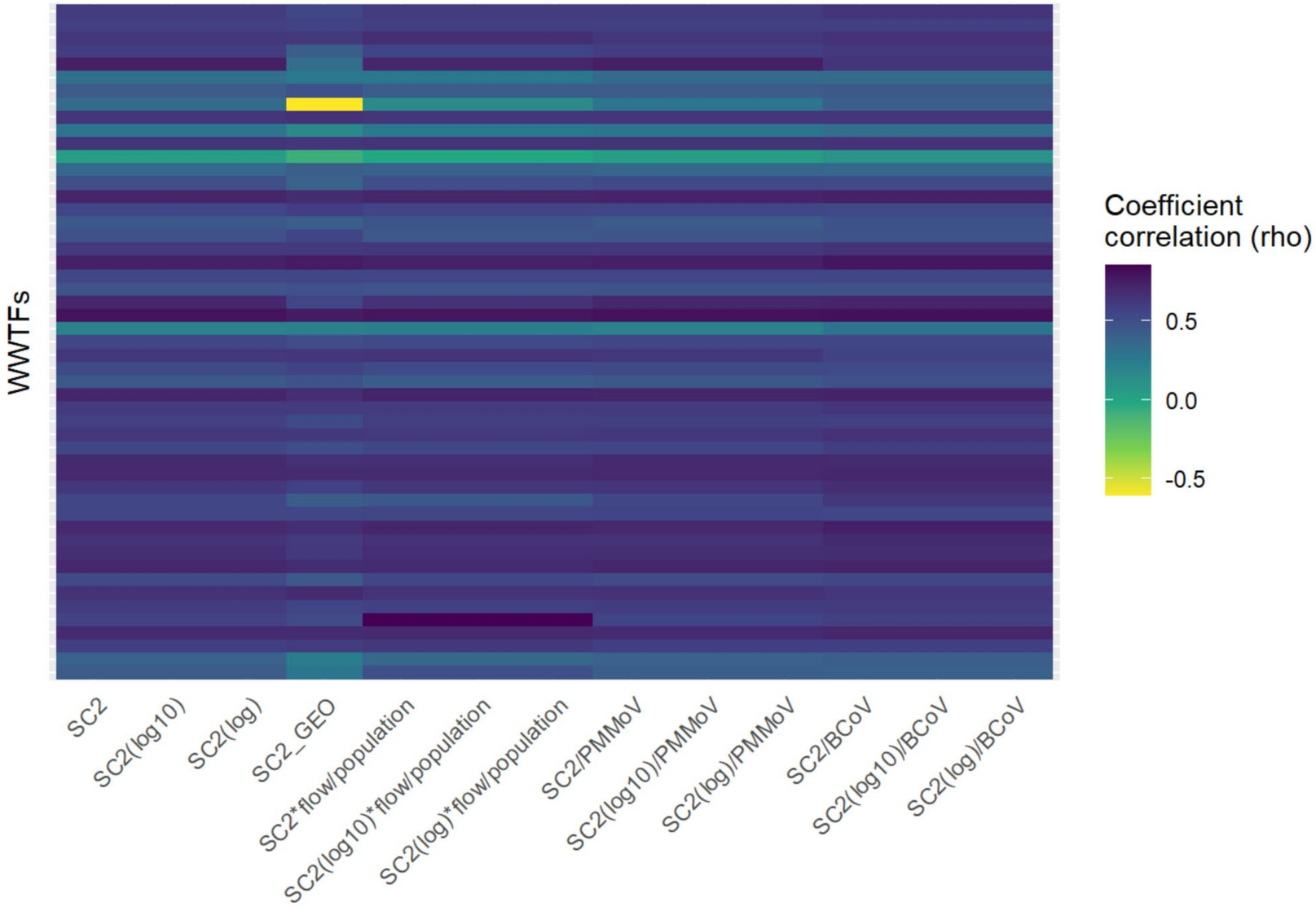

The blue line on the graph shows the levels of SARS-CoV-2, the virus that causes COVID-19, in the waste collected from Kalamazoo WWTP. Each data point is calculated by averaging the number of viral gene c 100mL of wastewater in the 3 most recent samples. The orange bars on the graph show the COVID-19 c MDHHS from the zip codes that the wastewater treatment plant serves (7-day average). Both the virus cases are calculated per 100,000 people. Case data will not be shown on the graph when the average nu than 10 per 100,000 people to protect the confidentiality of individuals with infections. This will be represented by an orange dashed line with gray shading below.

- Flow & population
- Fecal strength marker
- Viral Recovery Control
- Dry mass & fecal marker
- Population only



# Signal Normalization?




# Sampling / Matrix Variability

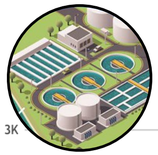
- **Variability in wastewater data** (larger for small WWTF)
- **Wastewater** SARS-CoV-2 levels **reflect COVID-19 clinical trends**



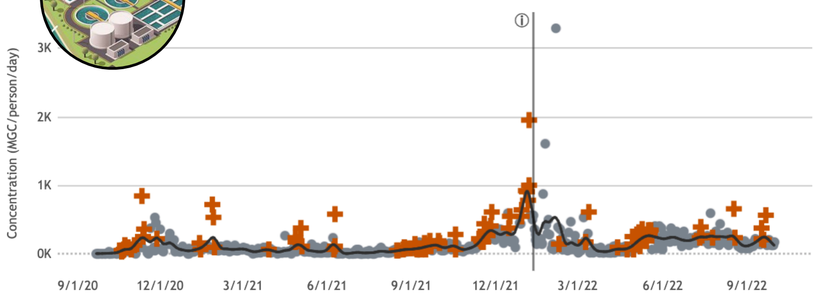
**City size**

**2 samples/week wastewater trend**

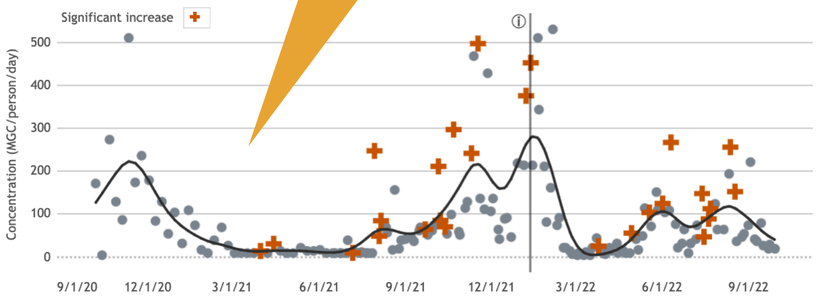
**Madison**
Estimated population served: **345,000**
Category: **Low**
Trajectory: **No alert**



**Large cities**: signal less sensitive to small variations



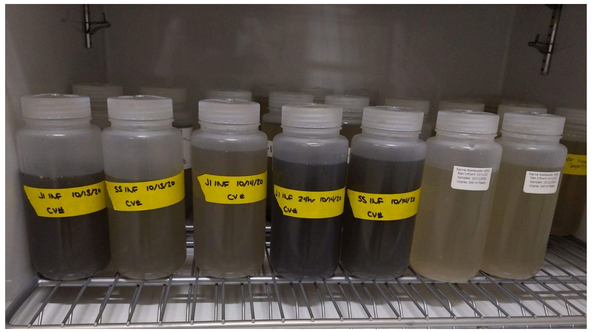

**Black River Falls**
Estimated population served: **5,000**
Category: **Low**
Trajectory: **No alert**



# Overall Signal Variability

**Improving data accuracy & decreasing variability – better match with clinical trends**

## i. Reduce variability within samples



**Wastewater matrices are complex**

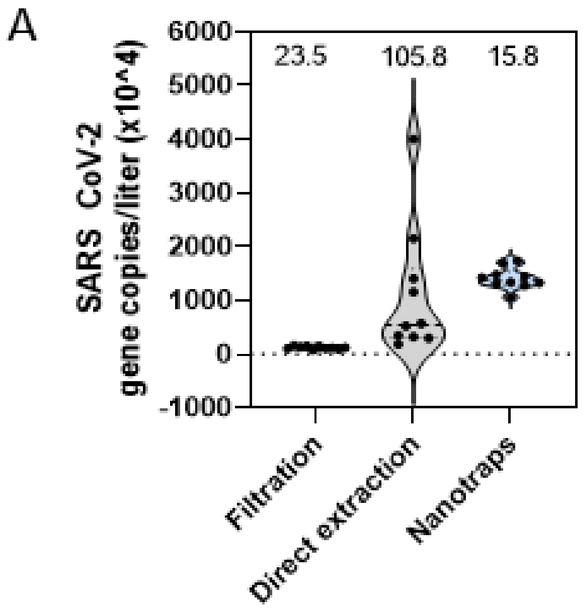
© Jeff Miller

- 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**



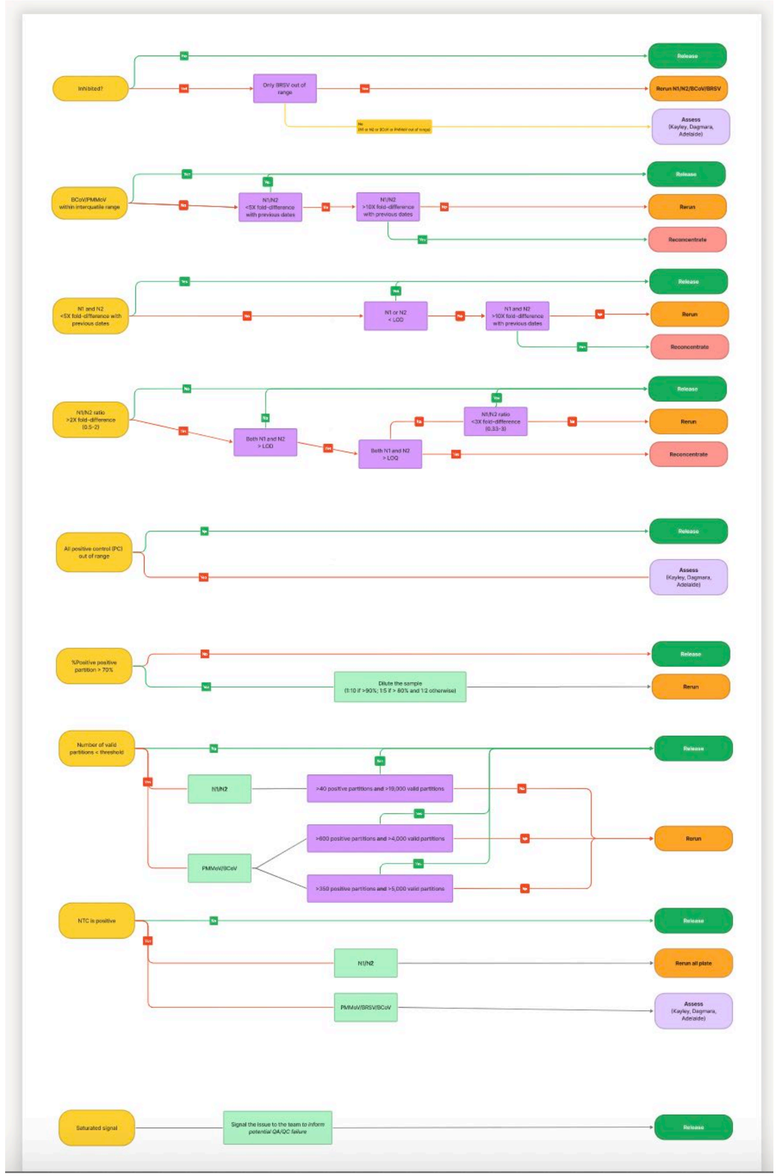

**Can be lessened by:**

- Step-wise method evaluation (isolate steps with high variability)
- Inhibition assessment
- Stringent QAQC – rerun & reconcentration criteria

i. qPCR depends on good standard curves

ii. Jumps in signal > 3-fold

iii. Viral recovery or fecal marker loss, etc.

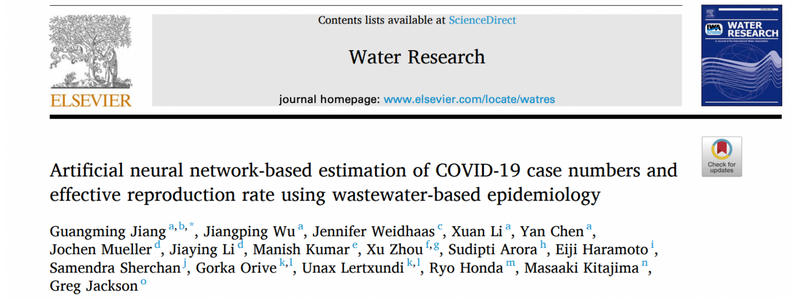


# Overall Signal Variability

i. Reduce variability within samples

ii. Reduce variability of laboratory analysis

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



Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Artificial neural network-based estimation of COVID-19 case numbers and effective reproduction rate using wastewater-based epidemiology

Guangming Jiang [a,b,*], Jiangping Wu [a], Jennifer Weidhaas [c], Xuan Li [a], Yan Chen [a], Jochen Mueller [d], Jiaying Li [d], Manish Kumar [e], Xu Zhou [f,g], Sudipti Arora [h], Eiji Haramoto [i], Samendra Sherchan [j], Gorka Orive [k,l], Unax Lertxundi [k,l], Ryo Honda [m], Masaaki Kitajima [n], Greg Jackson [o]

**Better models**

PLOS ONE

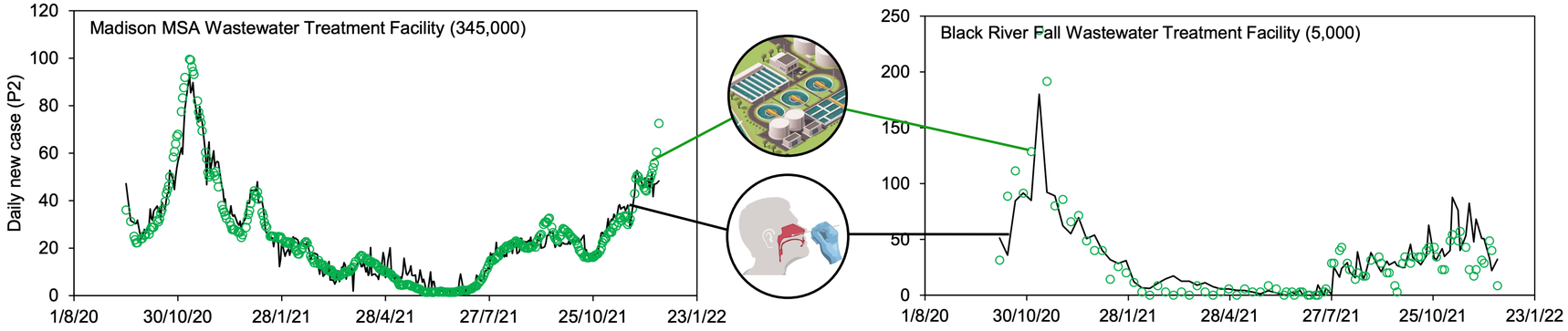
RESEARCH ARTICLE

Application of machine learning for multi-community COVID-19 outbreak predictions with wastewater surveillance

Yuehan Ai[1], Fan He[1], Emma Lancaster[2,3], Jiyoung Lee[1,2,4]*

1 Department of Food Science and Technology, The Ohio State University, Columbus, OH, United States of America, 2 Division of Environmental Health Sciences, College of Public Health, The Ohio State University, Columbus, OH, United States of America, 3 Environmental Science Graduate Program, The Ohio State

Machine learning (wastewater levels + vaccination rate + temperature)



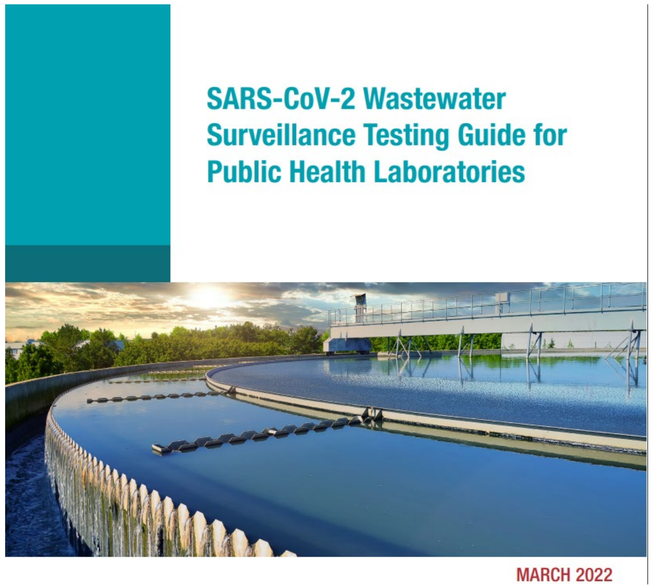
# 4

# Conclusions
# Current
# Recommendations

# Best Practices


## Sample

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



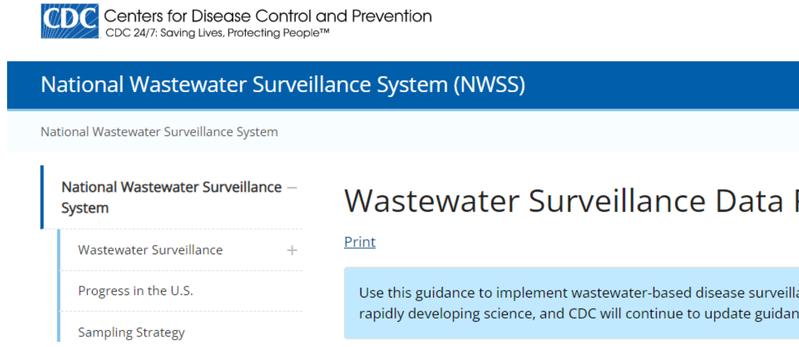

## Laboratory

- 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

*https://www.aphl.org/aboutAPHL/publications/Documents/EH-2022-SARSCoV2-Wastewater-Surveillance-Testing-Guide.pdf*

## 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




*https://www.cdc.gov/nwss/reporting/index.html*

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# 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

Wisconsin State Laboratory of Hygiene
UNIVERSITY OF WISCONSIN–MADISON

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CDC
CENTERS FOR DISEASE CONTROL AND PREVENTION

# Questions?

# Flu/RSV surveillance in Wisconsin

## 2022-2023 season

Statewide level

RESPIRATORY VIRUS SURVEILLANCE REPORT