IOWA

State Hygienic Laboratory

COVID-19 Wastewater Surveillance: Sampling Frequency and Hospitalization Trends

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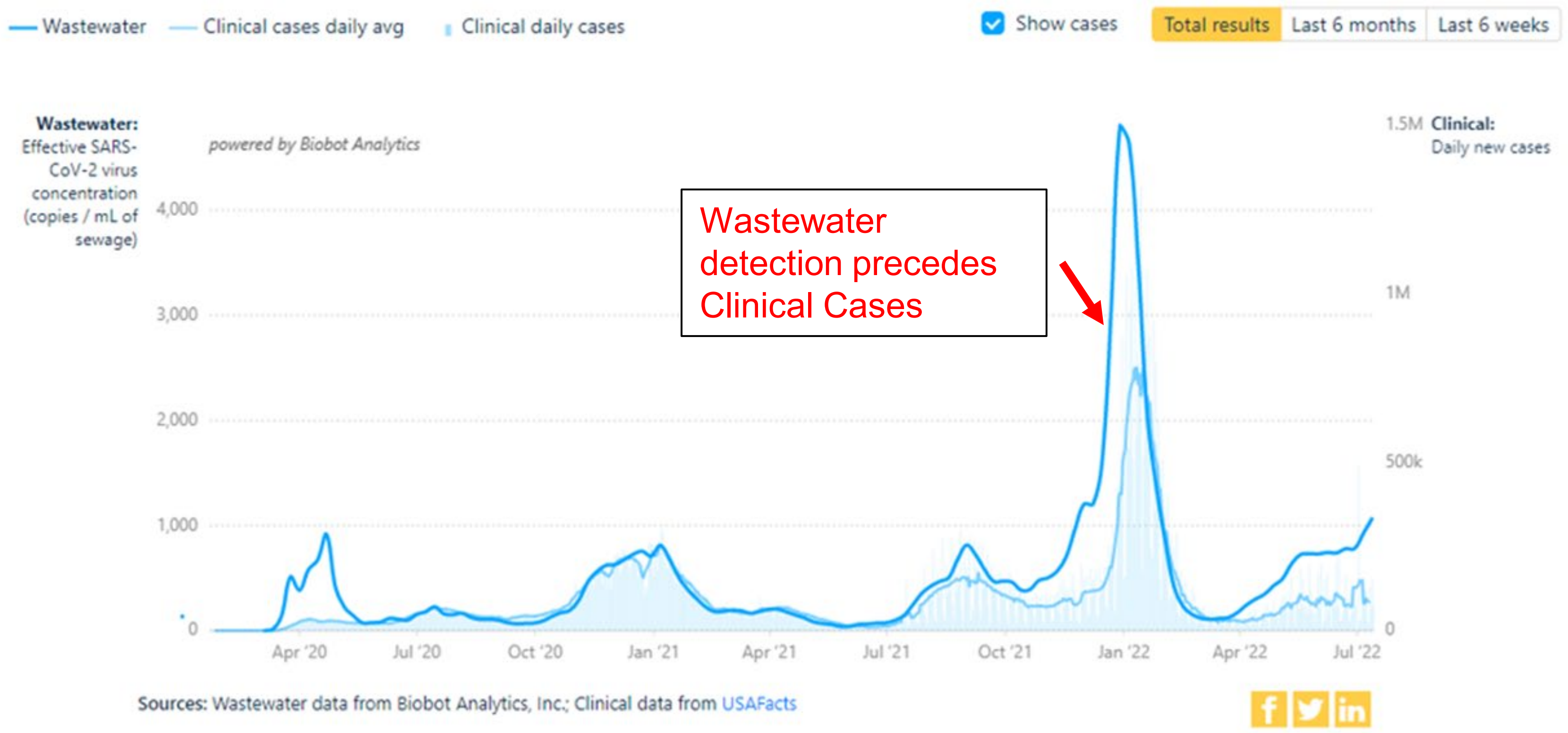
March 26, 2025

Introduction

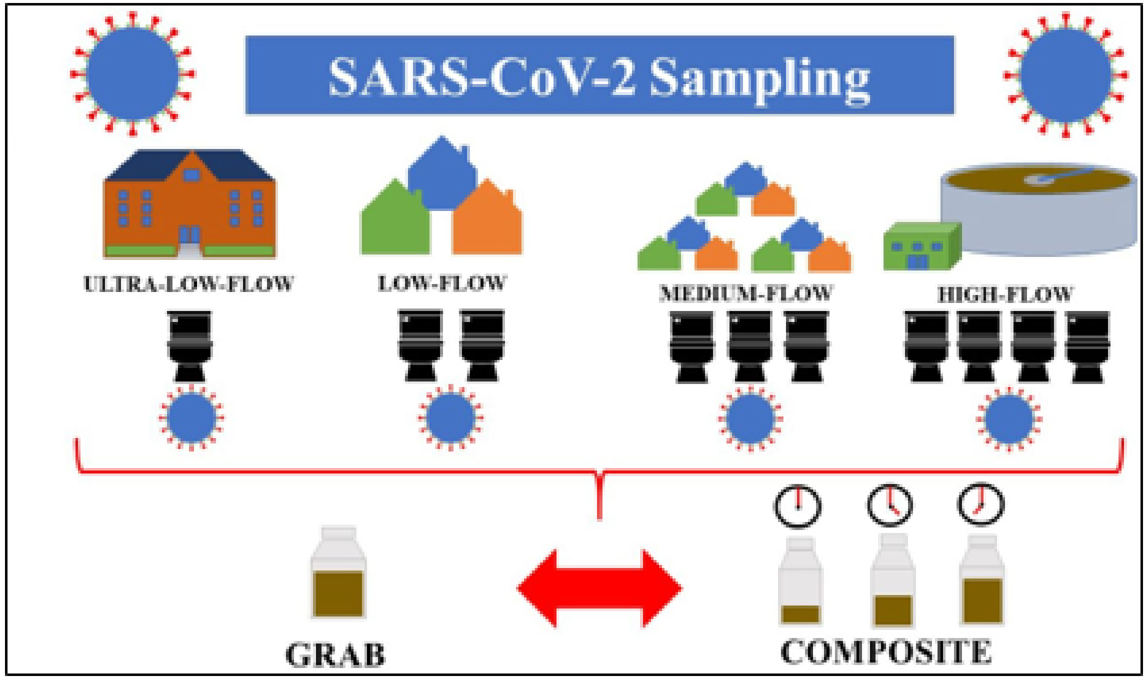
Wastewater Detection Precedes Clinical Cases

- Representative National Wastewater Surveillance System (NWSS) Biobot data



Sampling Strategy

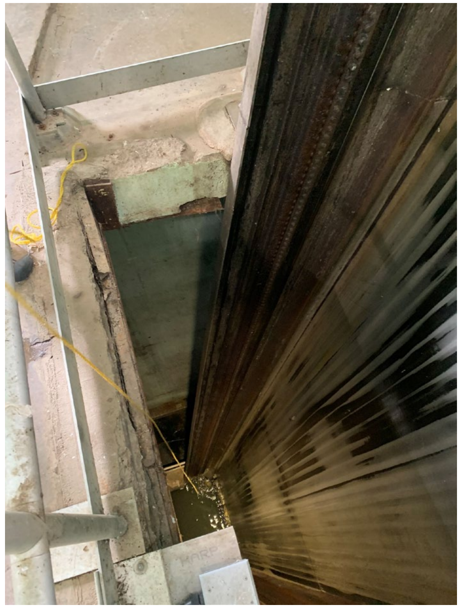
- CDC recommends a “minimum of three samples to detect wastewater trends over time” (Centers for Disease Control and Prevention, 2023).
- High-flow = WWTP
- Medium-flow = neighborhood
- Low-flow = city block



Manual composite sample

Bucket on a rope

Toss into pit



Retrieve wastewater, pour into collection container.

Combine composites, mix, and aliquot.

Time weighted composite sample

ISCO Autosampler

Autosampler hose enters manhole

Wastewater collection

Collection carboys

Grab sample

Manhole

Bart Everson - https://www.flickr.com/photos/editor/48728796337/

Dry manhole

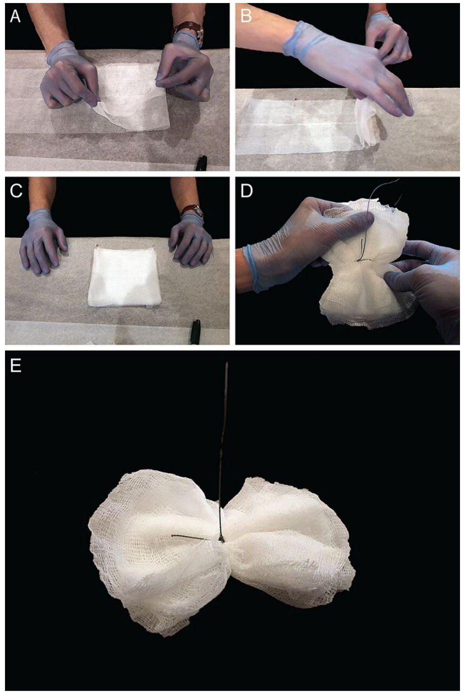
Todd Hubbard, SHL

Detergents and detritus can affect downstream detection

Todd Hubbard, SHL

Moore swab sample

Constructing a Moore Swab



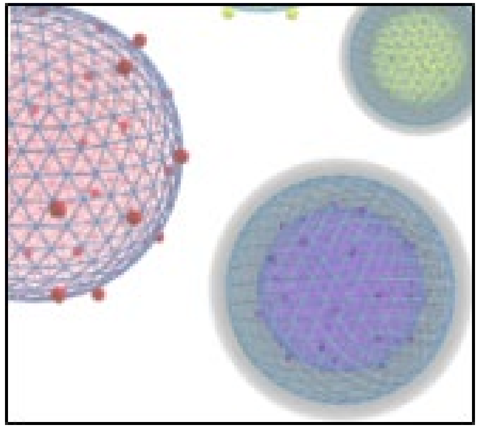
- "A classic environmental surveillance tool involving filtration of flowing surface water and sewage water to recover typhoidal Salmonella."

- Scientific Figure on ResearchGate. Available from: https://www.researchgate.net/figure/Constructing-a-Moore-swab-A-and-B-A-length-of-gauze-6-inches-by-48-inches-is-folded_fig2_340900006 [accessed 24 Mar 2025]
- Sikorski, Michael & Levine, Myron. (2020). Reviving the "Moore swab": a classic environmental surveillance tool involving filtration of flowing surface water and sewage water to recover typhoidal Salmonella. Applied and Environmental Microbiology. 86. 10.1128/AEM.00060-20.

Current wastewater test methods

Processing

Concentration



Ceres Nanotrap Particles

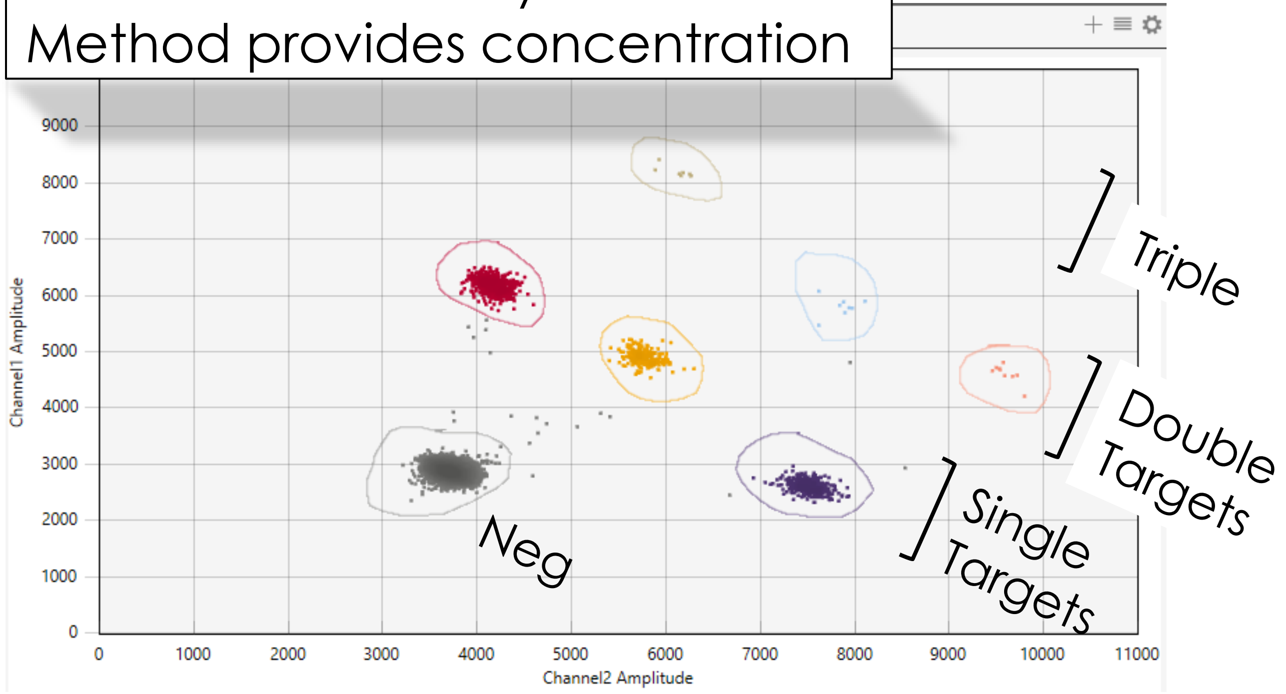
Extraction

Amplification

Droplet digital PCR and digital PCR
- Improved sensitivity
- Quantitative result for targets

Droplet Digital PCR Analysis

Bio-Rad PREvalence Assay
3 targets = 2 dyes
Full Validation Study
Method provides concentration



<u>Two COVID-19 gene targets:</u>

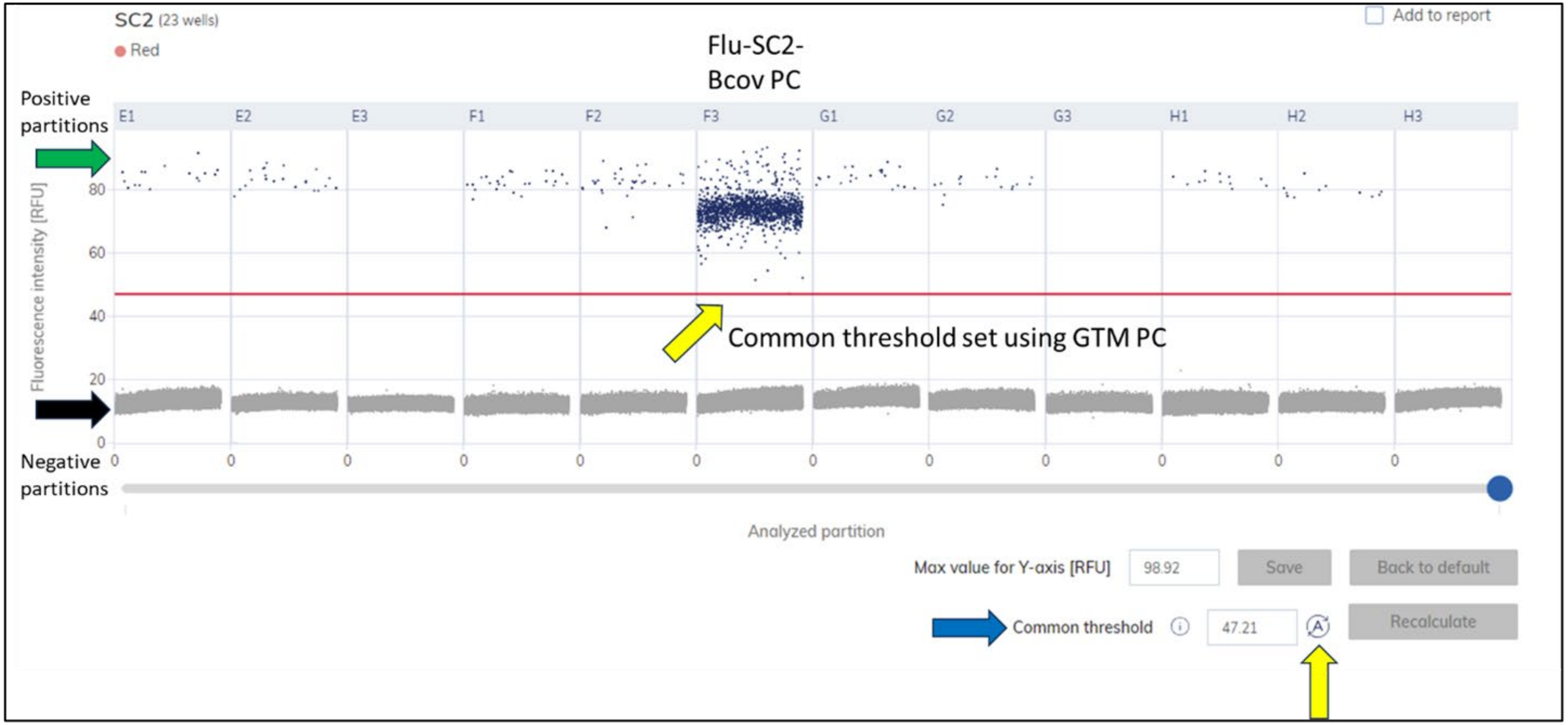
- N2 gene
- E gene

<u>Spike amplification target:</u>

- MHV (murine hepatitis virus)

Results reported through SHL LIMS system to publicly accessible dashboard

Digital PCR Analysis



Wastewater Reports

- Wastewater Sequencing
- Wastewater PCR
  - Qualitative Result
    - Pathogen target
  - Quantitative Result
    - Pathogen target
    - Fecal indicator
  - Normalized Result

Sample info

Results

Notes

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ANALYTICAL REPORT

1-800-421-IOWA (4692)

Collection Location	Collector and Phone	Client Reference	Accession #
IA	Collected: 2024-10-30 13:00	Received: 2024-10-31 10:00	2517426
Report To			Project: covid ww wwtp
			Sample Description: wastewater
			Sample Type: Non-Drinking Water
			Sample Source:
			Sample Note(s): 1

ADDITIONAL SAMPLE INFORMATION

Flow Rate (mgd): 0.661
Zip Code:
NWSS Sample Location: wwtp
NWSS Sample Location Specify: Not Applicable
Institution Type: not institution specific
WWTP Name:
NWSS Sample Type: 24-hr time-weighted composite
NWSS Sample Matrix: raw wastewater

RESULTS OF ANALYSIS - FINAL REPORT

TEST	RESULT	ANALYSIS NOTE(S)
Wastewater SARS-CoV-2, Next Generation Sequencing		
Most Abundant Lineage	KP.3.1.1	
Lineage %	41.0	
Coverage Breadth %	90.4	
QC pass	Yes	
SARS Flu RSV in Wastewater, Digital PCR		2
Influenza A	Not Detected	
Influenza A (M1 gene) copies/L	0	
Influenza A Normalized to Fecal Material	0	
Influenza B	Not Detected	
Influenza B (NS1 gene) copies/L	0	
Influenza B Normalized to Fecal Material	0	
SARS-CoV-2	Detected	
SARS-CoV-2 (N gene) copies/L	59000	
SARS-CoV-2 Normalized to Fecal Material	0.00019	
RSV	Not Detected	
RSV (L gene) copies/L	0	
RSV Normalized to Fecal Material	0	
Fecal Material (PMMoV) copies/L	310000000	

SAMPLE AND ANALYSIS NOTES

1. Unless otherwise noted, the sample met container and preservation requirements for the analysis requested. Please review carefully your sample results for additional analyte comments or method exceptions.
2. SHL Wastewater Surveillance link: https://covidtesting.shl.uiowa.edu/wastewater-surveillance-covid-19

   Normally, a quantitative result (copies/L) for each analyte would not be reported if the value is below the Limit of Quantitation (LoQ) because, at very low levels of analyte, precision is reduced. However, to analyze trends, values are reported if they are above the Limit of Blank (LoB). LoB is the highest apparent analyte concentration expected to be found when samples containing no analyte were tested.

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Interpreting wastewater PCR results

Test	Result	Units	Definition
SARS-CoV-2	Detected	Not applicable	SARS-CoV-2 (N gene) value >= Limit of Detection (LoD)
SARS-CoV-2 (N gene)	120000	copies/liter	PCR Gene Target
SARS-CoV-2 Normalized to Fecal Material	0.0014	Unitless	The mean of SARS-CoV-2, or Influenza A/B, or RSV in copies/L wastewater, divided by the amount of the fecal matter indicator virus (pepper mild mottle virus) in copies/L wastewater.
Fecal Material (PMMoV)	310000000	copies/liter	Amount of pepper mild mottle virus in wastewater sample in copies/L wastewater

Objective

Hypothesis

Null hypothesis: $\beta = 0$

Increasing wastewater sample collections from one sample to two samples per week will have no effect in predicting hospitalizations. (Slope = 0)

Alternative hypothesis: $\beta \neq 0$

Increasing sample collections from one sample to two samples per week will predict hospitalizations. (Slope ≠ 0)

Sampling Frequency and Hospitalization Trends

Simple Linear Regression Model

$$y = \alpha + \beta x + \epsilon$$

Fitted Simple Linear Regression Line

$$Y = a + bx$$

Hospitalization = α + log10 normalized SARS-CoV-2 virus x β

Methods

Test Methods

- January 2023 – November 2023
  - Concentration: Ceres Nanotrap Particles
  - Instrument: Bio-Rad QXOne
  - Assay: Bio-Rad PREvalence ddPCR SARS-COV-2 Wastewater Quantification Kit
  - Limit-of-Detection = 11,000 copies/L
- December 2023 – April 2024
  - Concentration: Ceres Nanotrap Particles
  - Instrument: QIAcuity 4
  - Assay: GT Molecular GT-Digital Influenza and SARS-Cov-2 Wastewater Surveillance Multiplexed Assay Kit
  - Limit-of-Detection = 10,000 copies/L

Wastewater Samples

- 24-hour composite wastewater samples
- Davenport Wastewater Treatment Plant
- Collection dates
  - One wastewater sample per week
    - January 9, 2023 – September 18, 2023 (n = 39)
  - One wastewater sample
    - September 25, 2023 – April 8, 2024 (n = 51)

Hospitalization Data

- Inpatients at Genesis Healthcare in Davenport, Iowa with a positive COVID-19 PCR test from January 1, 2023 – April 22, 2024 (n = 2440)
  - No patient information collected
    - Date of PCR positive
    - Number of inpatients

Results

Hospitalizations Statistics

7-day rolling average of daily PCR positive COVID-19 inpatients

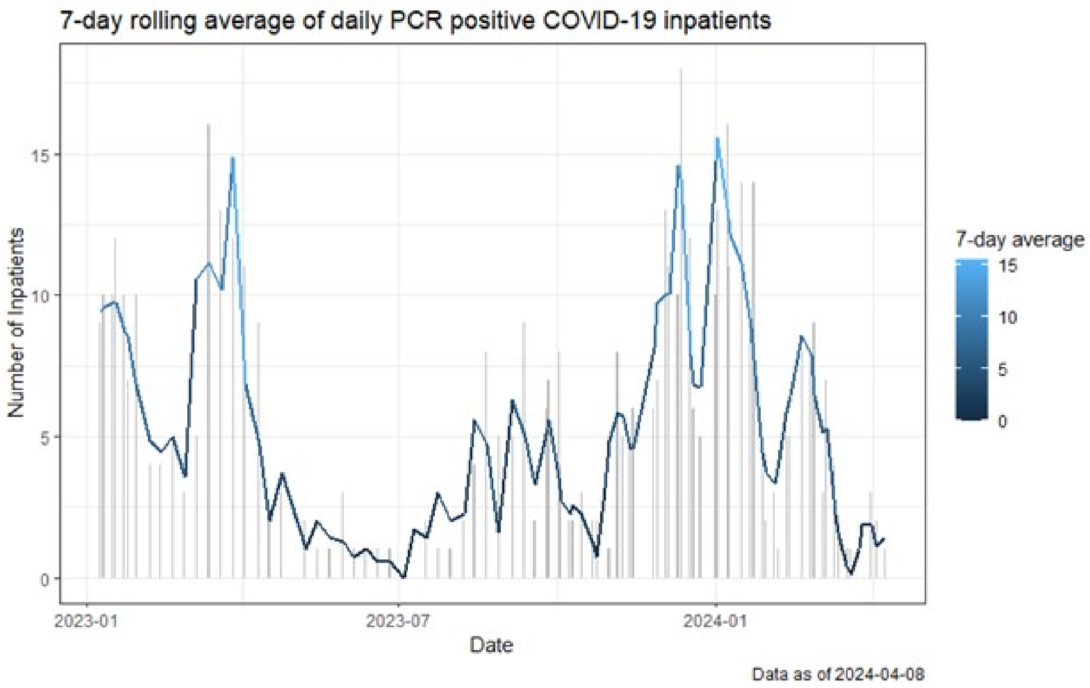


Figure 1 Graph of daily hospitalized patients with the 7-day rolling average overlay.

14-day rolling average of daily PCR positive COVID-19 inpatients

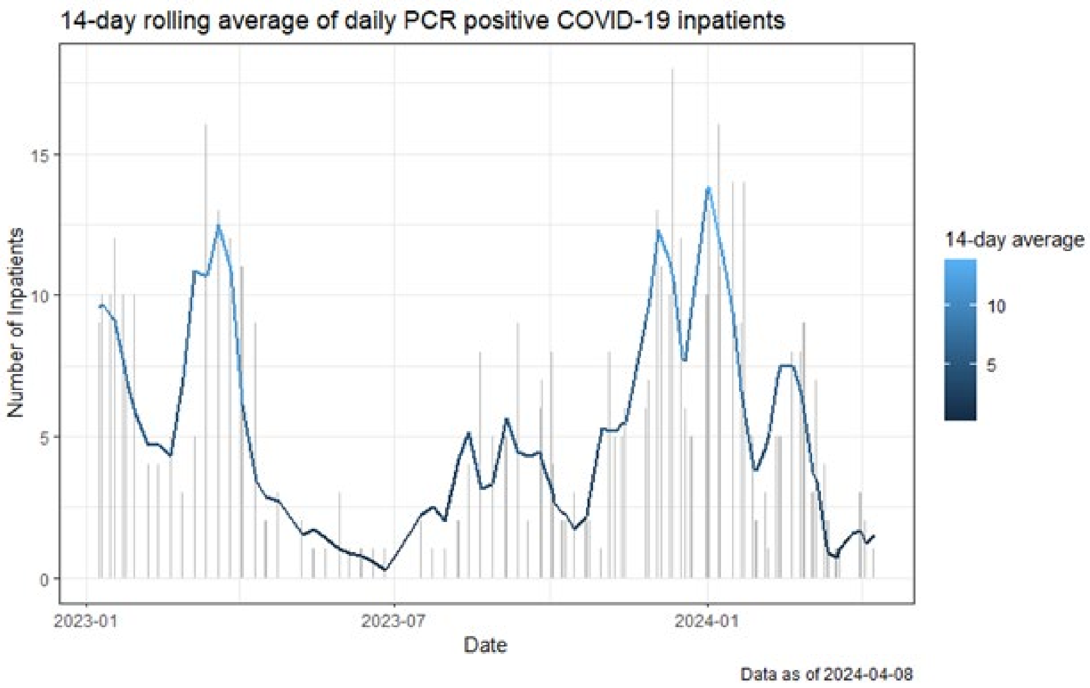


Figure 2 Graph of daily hospitalization patients with the 14-day rolling average overlay.

Hospitalizations by sample(s) per week

One sample/week N=39

7-day Hospitalizations	
Mean (SD)	4.9 (3.7)
Median (IQR)	4.4 (1.6, 7.7)
Range	0.0, 14.9

14-day Hospitalizations	
Mean (SD)	4.8 (3.7)
Median (IQR)	4.3 (1.9, 7.0)
Range	0.3, 12.5

Two samples/week N=51

7-day Hospitalizations	
Mean (SD)	5.8 (4.1)
Median (IQR)	5.3 (2.4, 8.0)
Range	0.1, 15.6

14-day Hospitalizations	
Mean (SD)	5.7 (3.7)
Median (IQR)	5.2 (2.5, 7.7)
Range	0.7, 13.9

Normalized wastewater by sample(s) per week

One sample/week N=39

Normalized SARS-CoV-2	
Mean	0.00050
Median	0.00058
Range	0.00000, 0.00110

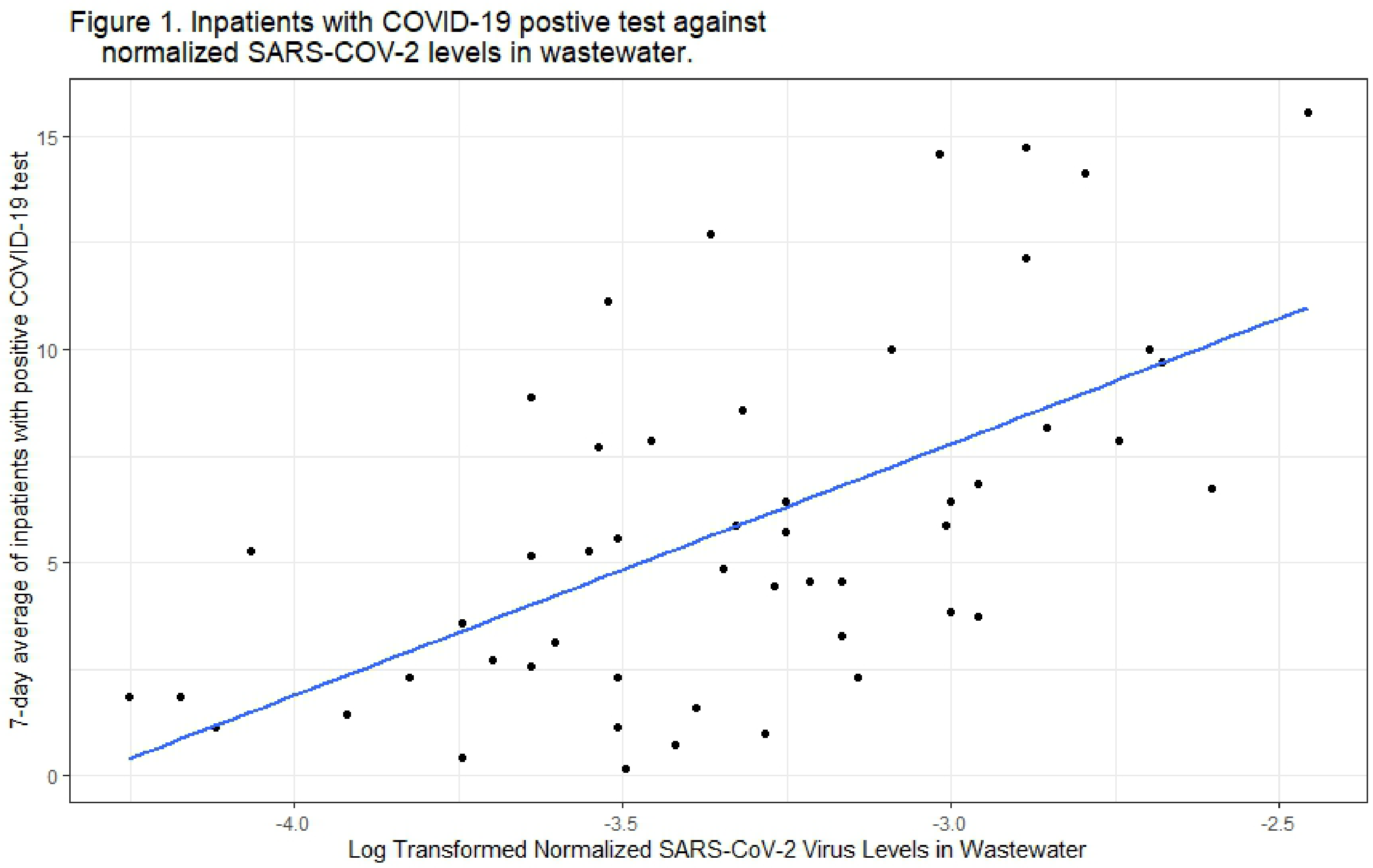
Normalized SARS-CoV-2 x10,000	
Mean (SD)	5.0 (3.7)
Median (IQR)	5.8 (1.3, 8.6)
Range	0.0, 11.0

Two samples/week N=51

Normalized SARS-CoV-2	
Mean	0.00072
Median	0.00047
Range	0.00006, 0.00350

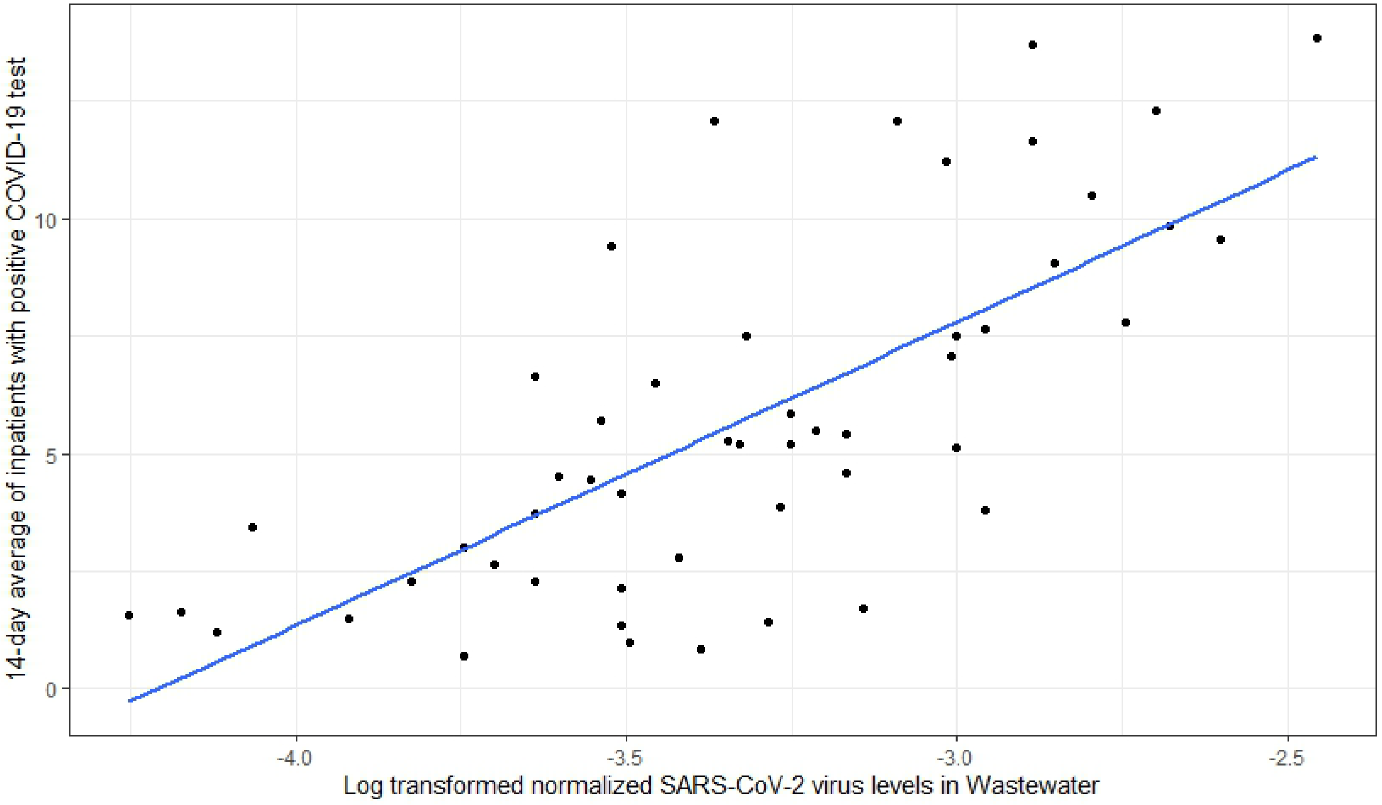
Normalized SARS-CoV-2 x10,000	
Mean (SD)	7.2 (7.0)
Median (IQR)	4.7 (2.5, 10.0)
Range	0.6, 35.0

7-day, two-sample, linear regression model



14-day, two-sample, linear regression model

Figure 2. Inpatients with COVID-19 postive test against normalized SARS-COV-2 levels in wastewater.



7-day, regression coefficients & test statistics

Model	F	Df	P-value	R^2 adjusted
7-day inpatients, log10 normalized wastewater levels	27.63	1, 49	< 0.001	0.35

Coefficients	Estimate	Standard Error	P-value
7-day inpatients	25.50	3.76	< 0.001
Log10 Wastewater	5.90	1.12	<0.001

14-day, regression coefficients & test statistics

Model	F	Df	P-value	R^2 adjusted
14-day inpatients, log10 normalized wastewater levels	52.61	1, 49	< 0.001	0.51

Coefficients	Estimate	Standard Error	P-value
14-day inpatients	27.20	2.99	< 0.001
Log10 Wastewater	6.46	0.89	<0.001

Discussion

Summary

- Based on the analysis of hospitalizations for the largest healthcare system in Scott County, Iowa there is a significant relationship between SARS-CoV-2 wastewater levels predicting hospitalization rates at 7-days and 14-days when sample collection is increased to two samples per week.
- Hospitalization numbers only include inpatients with a positive COVID-19 PCR test and do not include total inpatients per day.
- Screening of all inpatients for COVID-19 ceased on January 1st, 2023, so testing for COVID-19 was ordered because symptoms were present that indicated COVID-19 infection.
- Multiplying normalized SARS-CoV-2 wastewater levels, log transforming, improved normality and could be included in future studies with the goal of improving model fit.
  - Shapiro-Wilk test was performed using model residuals
  - Normality was rejected for every one-sample and two-sample regression models except for the two sample/week, 14-day hospitalizations regression model.
- Thank you to Dr. Cynthia Cass and Dr. Michael Pentella.
- Thank you to the Association of Public Health Laboratories.

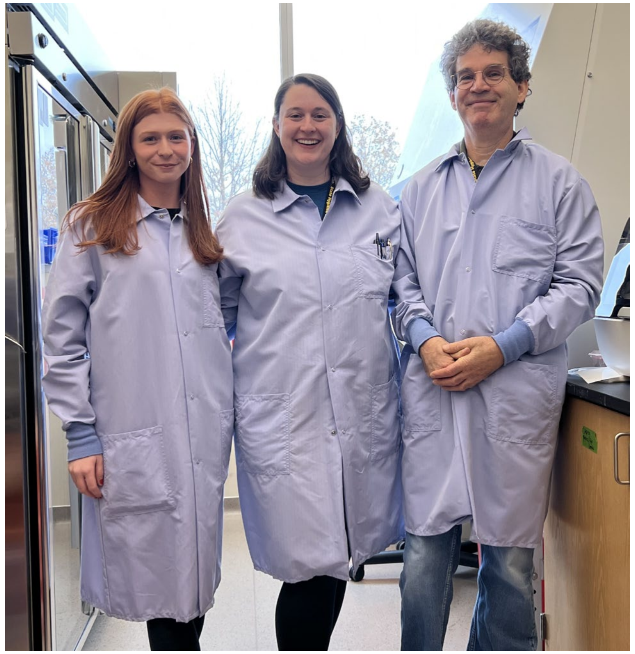
Iowa Wastewater Team

Limnology

Wastewater Collection

Environmental Microbiology

Wastewater dPCR/ddPCR



Molecular Biology

Sequencing/Bioinformatics

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Thank you

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