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Executive Summary

As the SARS-CoV-2 pandemic quickly spread from country to country and continent to continent in 2020, governments and scientists needed a way to track COVID-19 through populations in order to position public health interventions in the most impactful locations.

Having a decision-based risk framework may help to guide policy creation that could minimize or prevent possible outbreaks and surges of infection within communities. The University of Louisville in partnership with Louisville's Department of Public Health and Wellness tested this strategy in 2021 and 2022. This Wastewater-Informed Public Health Intervention Playbook describes the decisions and actions of that academic and public service partnership to develop an epidemiological-based, public wastewater surveillance system to monitor community infection and spread. This playbook details the cooperative processes between academic, public, and governmental organizations in the creation of a decision-risk framework, informed by wastewater-based community surveillance, that guided decision-making about public health interventions.

This Playbook will provide an action-oriented framework for policy intervention in three areas:

01. How to use wastewater and other data to identify “hotspots” and develop a health risk scoring system and decision-making framework applicable to neighborhood-scale geographies.

02. How to develop and implement targeted communication interventions to increase participation in available public health services such as vaccination and testing.

03. Leveraging the Rockefeller Foundation cities network forum, Kentucky State Department of Public Health, Louisville Metro, and related avenues to ensure other cities may adopt relevant practices.

This Playbook is organized in three parts:

Part 1: History of wastewater-based epidemiology and the partnerships and inputs needed for the creation of the risk framework.

Part 2: Details the iterative development process as the risk framework was refined through weekly touchpoints with the University of Louisville and Louisville’s Department of Public Health and Wellness. This section also describes the outcomes of implemented public interventions.

Part 3: Shares lessons learned throughout the development process, ending with construction of this playbook itself.

Part 1 / Context: Wastewater in the time of COVID-19 & Louisville’s Pandemic Response

>> Introduction

The COVID-19 pandemic brought many uncertainties and challenges to the world. Given its magnitude, novelty, and rapid spread many nations struggled with proper outbreak preparedness and timely responses.

When infection rates and hospitalizations increased during the initial wave in February and March 2020, there were no established protocols or models to estimate or predict how many people had been or would be infected until and unless symptom presentation occurred. Later, COVID-19 testing was limited and inaccessible to many people and symptoms were not specific and sometimes not present. This highlighted the need for largescale surveillance systems that could gather timely, cost-effective data on city-wide populations to estimate true prevalence and track the spread of illness. Wastewater-based surveillance systems were presented as a potential solution. However, while wastewater-based epidemiology had been used to track other pathogens, a problem of this magnitude had not yet tested its bounds.

The University of Louisville took on the task of testing this potential solution in Louisville. This playbook describes the process to develop decision-based risk framework using wastewater epidemiology for Louisville, Kentucky in 2021 and 2022. It outlines the partnerships, strengths, and limitations of this collaborative project that aimed to layer academic research, public health monitoring, and geographically targeted response activities. This playbook is informed by the real and varied experiences from multiple departments within the University of Louisville and Louisville Metro Government as well as community partners, commercial laboratories, and municipal wastewater utility providers.

NOTE: In this playbook wastewater-based epidemiology, sewer monitoring, and sewer surveillance are used interchangeably. Sewershed and catchment area are used interchangeably as well.

Figure 1: Geographic Target for the Wastewater-Informed Public Health Intervention Project
**Wastewater Based Epidemiology in the time of COVID-19: A light in the underground tunnel**

**What is WBE?**

Wastewater-based epidemiology (WBE) is a field that identifies biomarkers of human disease in community sanitary sewer systems. Analyzing which microbes and chemicals are present, and their amounts, within wastewater is a non-invasive way to learn about the health of a community. WBE is an efficient public health surveillance tool because it uses pooled samples instead of individual samples. A pooled sample represents many individuals and is less costly and time consuming than collecting and analyzing individual samples. Figure 1 shows how pooled samples reduce cost and effort sampling a central location which represents many unique entities.

**History of WBE**

John Snow (1813-1858) is considered the Father of Epidemiology through his work in solving a mysterious cholera outbreak in 1854 in England by tracing sickness to specific public water sources. This was the first documented instance of tracing pathogens through a water supply although the term “wastewater-based epidemiology” would not be coined until 2001. Since then, wastewater monitoring has played a role in the detection of illicit drugs, prescriptions, polio and Hepatitis A outbreaks, food and chemical consumption, and antibacterial resistance. Figure 2 shows a historical timeline of WBE and its major uses.

**WBE as a Public Health Surveillance Tool During the COVID-19 Pandemic**

Most recently, wastewater data has been a critical tool in identifying COVID-19 infection rates, spread, and variant emergence within specific communities. In March 2020, SARS-CoV-2 was first detected in public sewage in the Netherlands. Throughout 2020 and 2021 levels of SARS-CoV-2 in wastewater informed public decision making and the arrival of variants of interest were identified in communities through wastewater before clinical tests. By 2022 the Center for Disease Control (CDC) had launched a National Wastewater Surveillance System. This national system was created to support more local level pathogen tracking through wastewater. Figure 3 shows the process for how pooled samples relay important information to researchers and decision makers about the upstream impacts of COVID-19 infection.

Wastewater testing has many advantages that complement clinical testing methods that were important during the COVID-19 pandemic. It is not limited to the availability of clinical tests, avoiding both supply chain and staffing shortages, and can capture evidence of asymptomatic populations which may be missed by clinical testing. Additionally, clinical testing normally occurs after symptom onset. Therefore, sewage monitoring can provide valuable early warnings for new infection waves or new variant emergence that could overload a hospital system. Finally, COVID-19 positive individuals can shed SARS-CoV-2, the virus that causes COVID-19, particles through feces days to weeks prior to symptom development.

**Figure 1: Pooled Sample Example**

**Figure 2: Timeline of Wastewater-Based Epidemiology**

**Figure 3: WBE Process for the Detection of COVID-19**

Christina Lee Brown Envirome Institute at the University of Louisville
Louisville's Community Testing Response to the COVID-19 Pandemic

Department of Public Health & Wellness, Louisville Metro-Jefferson County Government

“3-Legged Stool” Testing Plan

Early in pandemic Louisville Metro’s Department of Public Health and Wellness (LMPHW) designed a “3-legged stool” testing program to gain much needed knowledge of the scope of infection within Louisville Metro-Jefferson County. The three legs of the testing stool were testing for vulnerable and symptomatic populations, free public testing for potentially asymptomatic individuals, and randomized community testing through the Envirome Institute’s Co-Immunity Project.

Adequate testing for vulnerable and symptomatic individuals was a difficult task to complete early in the pandemic due to limited testing capabilities and supply chain interruptions. However, later in the pandemic such constraints eased.

Testing for potentially asymptomatic individuals allowed for estimations of infection that were not included in the clinical case load. This testing was focused on minority communities and those who were disproportionately affected by the outbreak including individuals in high-risk jobs (e.g., as healthcare professionals and those working at meatpacking warehouses or within other essential functions).

Working with the Envirome Institute’s Co-Immunity Project, LMPHW gained “community testing insight” through randomized community testing, the only way to capture true incidence and prevalence rates. Randomized testing allowed for better understanding of how widespread COVID-19 was within Louisville. LMPHW’s COVID-19 Consultant commented that the Co-Immunity Project enabled LMPHW to make generalizations about the extent of virus spread considering both active positive cases and resolved cases.

By partnering in the Co-Immunity Project, LMPHW gained access to scientific data and supplemental metrics to make more evidence-informed policy judgments. Additionally, the University of Louisville met with LMPHW leadership weekly to provide qualitative and quantitative data and research translation services that helped to guide the city’s decision-making processes. This activity later became the Wastewater-Informed Public Health Intervention Project.

COLLABORATOR SPOTLIGHT:
LOUISVILLE METRO DEPARTMENT OF PUBLIC HEALTH & WELLNESS

MISSION
To achieve health equity and improve the health and well-being of all Louisville residents and visitors.

ROLE OF LMPHW IN THE WASTEWATER-INFORMED PUBLIC HEALTH INTERVENTION PROJECT
LMPHW WAS THE PRIMARY CUSTOMER FOR ANALYZED WASTEWATER DATA. Representatives participated in weekly meetings to review wastewater results, recommended improvements to the risk framework, and to use wastewater data in decision making processes.

LMPHW Organization Chart

Christina Lee Brown Envirome Institute at the University of Louisville

In May 2020 the Co-Immunity Project, a large scientific research study led by the Christina Lee Brown Envirome Institute (EI) and the Center for Predictive Medicine at the University of Louisville with support from several hospital systems through the Hospital CEO Council, launched. The Co-Immunity study tracked COVID-19 infection among healthcare workers, the general public, and water. See Figure 4, the 4 phases of the Co-Immunity Project. This layered approach combining randomized community testing and wastewater results over time provided the theoretical basis for estimating clinical infection risk based on viral concentrations in wastewater.

HEALTHCARE WORKER STUDY
Phase 1 focused on Louisville's healthcare community and began COVID-19 infection and antibody testing for healthcare workers within the Louisville health systems. This phase ended in late 2020.

Surface Water Study:
This phase monitored Beargrass Creek at sites known for combined sewer overflows near areas of recreation. However, after detecting very low levels of SARS-CoV-2, this study ended in December 2020.

Community Study:
Phase 2 involved a series of eight randomized community testing to estimate the true prevalence of SARS-CoV-2 in Jefferson County. By testing a representative sample of individuals from different areas in Louisville a reliable estimate of the breadth and spread of COVID-19 infection across the city is possible. Random sampling within different neighborhoods allowed for the identification of high-risk areas with sustained high prevalence of infection.

Wastewater Study:
This phase collected and analyzed wastewater from 17 sewersheds twice weekly. The Envirome assessed the amount of SARS-CoV-2 RNA in sewer samples and compared it to community and health care worker testing results to understand infection hotspots. Genomic analysis monitored for the emergence and virulence of variants of interest. Testing wastewater allowed the Co-Immunity Project to increase the resolution of its clinical findings, begin to assess potential geographic and demographic factors that contribute to risk of infection, and refine predictive models through consideration of local, seasonal, and sporadic variables that affect infection rates.

COLLABORATOR SPOTLIGHT:
UNIVERSITY OF LOUISVILLE’S CHRISTINA LEE BROWN ENVIROME INSTITUTE
The Envirome Institute is a part of the University of Louisville’s School of Medicine and the primary research institute within the Division of Environmental Medicine.

MISSION
Envirome Institute is dedicated to creating a new understanding of how individual health is maintained and affected by the environment. The Envirome’s research unites a multidisciplinary group to turn scientific discovery into actionable knowledge to build healthier cities.

ROLE IN THE WASTEWATER AND COVID-19 COMMUNICATION PROJECT
The University of Louisville Envirome Institute’s role was to coordinate field and laboratory processes, translate the results of sample analysis, convene the primary collaborators weekly to review results and discuss appropriate public health responses, and to develop a risk framework to assist city leaders in public health decision making.
After the Co-Immunity Project’s individual testing program ended and with reduced clinical testing occurring across the city, wastewater monitoring (WBE) was positioned as the best way to share timely data with LMPHW and Louisville Metro Government for ongoing risk management and effective pandemic response. However, there was no national guidance for using wastewater surveillance for risk assessment or decision making at the city scale. The Envirome recognized the opportunity to collaborate with LMPHW to develop and implement a wastewater-based framework to guide public health decisions and interventions. A collaboration between the Envirome Institute and the Bioinformatics Laboratory at the University of Louisville, Louisville’s Metropolitan Sewer Department (MSD), and Louisville Metro Department of Public Health and Wellness launched in October 2021.

### Project Overview

**Project Goal**

To co-create a risk framework with primary public health stakeholders for decision making that incorporates sewer monitoring data with traditional clinical data (clinical cases, new hospitalizations, and ICU bed occupancy) to create replicable processes for the identification of disease hotspots and intervention deployment at the neighborhood scale.

**Core Objectives**

To learn best practices for communicating WBE results and data, to create a risk framework to guide decision making at neighborhood level, and to foster collaboration between Louisville Metro’s Department of Public Health and Wellness and the University of Louisville’s Envirome Institute.

**Project Schedules**

**Overall Project Schedule:**

The Wastewater and COVID Communications project started in September 2021. Initial activities included developing a detailed project and communication plan and creating avenues to access public health data. From November 2021 to March 2022, project activities were centered around aligning wastewater data and clinical data, adjusting the risk framework according to LMPHW’s feedback, and coordinating public health interventions for areas identified as high risk by the framework. In June 2022, the project was closed with the completion of the final deliverable.
Weekly Project Schedule

Over the course of 18 months, MSD, UofL’s Envirome Institute, UofL Bioinformatics, and LMHPHW settled into a weekly routine to collect and analyze over 1,000 wastewater samples.

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<th>TUESDAY</th>
<th>WEDNESDAY</th>
<th>THURSDAY</th>
<th>FRIDAY</th>
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<td>• DATA ANALYSIS IS CONDUCTED</td>
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<tr>
<td>• LAB ANALYSIS CONTINUES</td>
<td>• LAB ANALYSIS CONTINUES</td>
<td>• RESEARCH TRANSLATION IS CONDUCTED</td>
<td>• INTERNAL REVIEW OF TRANSLATED RESULTS</td>
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<tr>
<td>• WASTEWATER RESULTS ARE SHARED</td>
<td></td>
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</tbody>
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Key Inputs

Partnership with Louisville's Metropolitan Sewer District (MSD)

The Envirome first partnered with Louisville’s Metropolitan Sewer District (MSD) in 2017 for environmental analysis in a separate study.

In 2020 the team pivoted and MSD provided much-needed equipment and human resources to collect wastewater samples from across the city. MSD field technicians were trained by UofL to collect 24-hour composite samples and deliver them to UofL for laboratory analysis.

MSD’s single point of contact, the Operations Director, coordinated communication between the project leaders and the field manager who coordinated the field sampling crews. MSD’s partnership was essential and valuable to the operations of this city-wide surveillance project.
Prior to this project, the Envirome Institute developed a place-based sampling process which tracked SARS-CoV-2 viral material in 17 sewersheds of various sizes. These 17 sewersheds include 2 major and 3 minor Water Quality Treatment Centers (WQTC); Morris Foreman and Derek G. Guthrie; and Cedar Creek, Floyds Fork, and Hite Creek, and 12 smaller catchment areas. Refer to Figure 5 to see overall coverage of Jefferson County and how these sewersheds overlay each other.

Existing sewer data, location and attributes of sewer lines and access points were collected, in geographic information systems (GIS) format from MSD through the Louisville-Jefferson County Information Consortium. Countywide socioeconomic data was collected from the U.S. Census Bureau, including information on the distribution of income, race, ethnicity, and population characteristics. This information was used to ensure representative sampling of minority and low socioeconomic status communities. Demographic estimates were based on the 2018 American Community Survey block group data and aggregated to the wastewater catchment areas with overlapping block group centroids.

Ideal sample sites were identified using a stepwise approach, see Figure 6. Predominately residential areas were prioritized over large commercial areas due to predictable outflows from residential locations and the ability to quantify area population and demographics with census and property data. Because commercial areas may demonstrate greater day-to-day variability and workers commuting from many places, it is significantly more difficult to trace COVID-19 in these sectors. Sampling of lines with effluent from hospitals or other facilities with high chemical effluent was also minimized. When dividing sewer lines to exclude non-residential areas or capture more resolute spatial coverage areas, sample sites were strategically placed upstream to points before two larger sewer lines joined. Finally, sample sites were added at each treatment facility in the county, representing nearly complete coverage of residences within the county.

MSD reviewed the sites selected by UofL and assessed field sampling logistics based on their internal occupational safety standards for field activities. Practical obstacles were identified including inaccessible manholes (requiring 4-wheel drive vehicles to access or on steep banks) and manholes that were located along high traffic roadways. If an ideal site was not logistically feasible, the sampling point would be moved to the next feasible location upstream from the ideal location in order to capture most of the catchment area. The total number of sampling sites was limited by MSD staff availability. Two field teams were deployed to collect 12-16 samples over two full days. Ultimately, 17 sample sites were selected, 12 reflecting local neighborhood catchment areas collecting from manholes or pump stations and five WQTCs. Refer to figure 6 to see the final catchment areas.
Field Coordination & Sample Collection

UofL trained MSD’s field technicians to prepare sampling equipment, follow research protocols for collecting samples, track chain of custody information, and deliver the samples.

Sampling personnel from MSD wore standard personal protective equipment for wastewater sampling, including Tyvek coveralls, boots, hard hats, face shields, and gloves.

Samples consisted of 24-hour portable composite wastewater samples using a 4-L GLS Sampler (60-2954-001 Model). The sampler collected 30 ml of wastewater into a larger container every 15 minutes. Wastewater samples were collected one to four times per week and usually included a weekend sample to increase the likelihood of residential attribution. Samplers were placed based on site-specific considerations, below a manhole and suspended with rope or on the ground adjacent to a sampling site.

After being drawn from the composite sampler, samples were placed on ice and transported to the University of Louisville. Samples were typically collected within a 5-hour time frame to minimize temperature variations and virus particle decay. After sampling, the portable pump and tubing were rinsed with bleach water and double rinsed with deionized water before reuse.

MSD’s field technicians experienced occasional delays in gathering wastewater samples due to inclement weather and flooding. Setting up sites and sampling procedures took time to coordinate, therefore when adjustments were requested by the project leaders, one to two weeks was needed to fully implement changes.

Lab Coordination & Wastewater Data

The University of Louisville analyzed overall infection levels for communities by assessing the amount of SARS-CoV-2 RNA through N1 values and genomic variation in wastewater samples. Upon delivery to UofL, viral particles where concentrated using PEG precipitation methods, RNA was isolated and inspected, and N1 Ct values were generated. Next, cDNA was generated. Samples were indexed through PCR with the SNAP Unique Dual Indexing Primers and the library concentration was measured using the Qubit dsDNA HS Assay Kit. The libraries’ size distribution was checked on the Agilent Bioanalyzer using the DNA High Sensitivity Kit. Sequencing reads were analyzed using a custom bioinformatics pipeline.

Wastewater is a valuable resource to investigate the genetic diversity of SARS-CoV-2 in communities. UofL used next-generation sequencing to determine the sequence diversity of the circulating SARS-CoV-2 populations. Through this analysis, a loose coupling emerged between UofL’s wastewater findings and KY State’s clinical genomic surveillance which provided an excellent opportunity for shared learning. Because community wastewater sampling is a form of pooled testing, it effectively samples 100% of the population connected to the wastewater system. Thus, it is far more likely to detect an emerging variant than would be found through sequencing of clinical nasal swabs.
For instance, when wastewater genomic surveillance identified the Gamma (P1) SARS-CoV-2 variant, UofL communicated that to the state for geographically and demographically targeted public health response. The variant was confirmed one week later with a clinical case of the same variant within the same catchment area. Since then, there have been numerous comparisons between the catchment areas and clinical trends.

**SCREENING OF GEOGRAPHICALLY RESOLVED WASTEWATER SAMPLES WITH GENOMIC SURVEILLANCE**

Wastewater sample reveals a variant and triggers an increase in the number of clinical tests for sequencing in geographic area.

**DETECTION**

Individual clinical sample reveals a variant and triggers a deep review of wastewater catchment area genomic surveillance screening data.

**CONFIRMATION OF VARIANT PRESENCE IN INDIVIDUAL CLINICAL SAMPLE WITH GENOMIC SURVEILLANCE**

Hold clinical PCR swab tests which are positive.

**HOLD CLINICAL PCR SWAB TESTS WHICH ARE POSITIVE**

Community level wastewater sample genomic sequencing.

**COMMUNITY LEVEL WASTEWATER SAMPLING**

Figure 14: Community and Individual Genomic Sequencing Process (Smith, 2021)

**Measurement Normalization**

General guidelines in sewer surveillance of SARS-CoV-2 in wastewater calls for minimum meta-information in the form of fecal indicators.

Fecal indicators are used to estimate the occurrence of fecal-borne pathogens in wastewater, such as the COVID-19 virus. Standardized reporting of fecal indicator concentrations was decided upon by the UofL team. A positive control measure used to calibrate results came from the detection of the pepper mild mottle virus (PMMoV) in fecal samples. PMMoV is a plant virus most associated with peppers found in the human diet. This virus persists in feces through the wastewater system and allows for the quantification of human signals that can be used as a control for testing against other pathogens.

### Two calculations were used during in the formation of the risk framework:

**01.** The first version was more of an adjustment than a normalization. It made a unitless ratio comparing current PMMoV result to the median of all PMMoV results at the site, then used that ratio to scale the SARS-CoV-2 N1 results. So, the result was still in units of ‘N1 copies/mL’:

\[
N_1 \times \left( \frac{\text{current PMMoV}}{\text{median of all PMMoV at the site, ever}} \right)
\]

**02.** The second version is a normalization that results in ‘N1 copies/mL per copy of PMMoV’.

\[
\frac{(N_1^2)}{\text{PMMoV}}
\]

Figure 15: Pepper Mild Mottle Virus Presented on Bell Pepper.
CLINICAL CASES: LMPHW provided COVID-19 clinical testing results, test date, and geographic location for both positive and negative test outcomes to the Envirome Institute. The Envirome geocoded these clinical cases to sewersheds to assess the distribution of infection.

VACCINE RATES: LMPHW provided COVID-19 vaccination rates, date of vaccination, and geographic location for all COVID-19 vaccinations given to residents of Louisville. The Envirome geocoded vaccination rates to sewersheds to assess the distribution of vaccination.

KEY DEMOGRAPHICS: The Envirome used American Community Survey (ACS 2019) and US Census (2020) data to outline key demographics and vulnerability of sewersheds. Refer to Figure 8 to see how these different types of data were combined in the final risk framework format.

SOUTH CENTRAL LOUISVILLE

Research Translation

Research data is not always easily understood to people outside of the academic environment. Moving reporting from a formal technical document to a presentation format allowed for the rapid understanding of wastewater results even among non-genomics and non-bioinformatics professionals. The process of translating collected data into actionable information took many months to develop. The presentation started as two distinct parts, a color-coded map that showed virus levels overall in each catchment area by order of virus magnitude and individual per-catchment slides that layered key metrics and demographics with vaccine rates, case rates, and PMMoV adjusted N1 count from that sewer catchment (see Figure 9). Color-coding allowed for quick identification of hotspot areas that could need more specific attention from LMPHW.
The risk framework combines multiple data types and trends to assess risk at the neighborhood scale. The initial risk framework incorporated wastewater levels of the SARS-CoV-2 virus (copies of N1), clinical data (case rate), vaccine rate, and key demographics (median household income, population, and racial makeup) to develop a weekly risk score for each sewershed. Fifteen sites were ranked weekly based on their risk score. Catchment areas were labeled by the utility-based sewershed nomenclature used by MSD. Morris Foreman and Derek R. Guthrie WQTCs were not included in risk analysis because of their large size and because the multiple smaller catchment areas nested within these two WQTCs provided a more granular assessment. Refer to figure 5 to see the relationships between catchment areas.

**Initial Risk Framework**

**Figure 18: Individual sewershed risk framework slide**

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**Roundtable Stakeholder Meetings**

Every Friday afternoon UofL guided the interpretation of the weekly risk framework to public health stakeholders and collaborators.

The purpose of these meetings was to share information and create awareness of how wastewater can inform public health decision-making. These meetings provided proof of concept around the University’s ability to assess presence of COVID-19 in wastewater in Louisville, connect wastewater levels to infection, and demonstrate that levels of SARS-CoV-2 are variable between sewersheds and over time.

Each week the University and LMPHW leadership would review and discuss the latest wastewater data, identify action targets, assess community spread and risk, and evaluate the value of the previous week’s iterations. The areas of highest risk were highlighted and potential intervention responses were considered. Further adjustments and refinements to the risk framework itself were recommended to fully implement changes.

**DISCUSSIONS LED TO DEVELOPING VALUE-ADD FOR LMPHW:**

01. Clinical cases
02. Under testing
03. Age / Demographics
04. Hospitalization metrics
05. Panvirome Analysis

**APPLYING MONITORING FOR ACTION:**

01. Clinical testing sites
02. Call to action ad campaigns
A risk management score tracked points and trends among infection variables, wastewater PMMoV corrected N1 levels, case rates, and vaccination. Each sewershed was assigned values for each variable and they were combined to arrive at the risk score. LMPHW requested doubling the weighting the case rate as it reflected their primary metric of interest. Later, the rubric was normalized to give equal weight to all variables except for case rate which remained more highly weighted.
Once risk scores had been calculated, the sewersheds were placed in rank order on a table for easy review by LMPHW (Table 3). Areas of high, sustained risk were identified by its inclusion in one of the top three positions on the ranked list for more than three consecutive weeks (see Table 3). When this occurred, the Envirome cultivated partnerships with local nonprofit and faith leaders to collaboratively develop appropriate messages about the research findings and a call to action for vaccination and testing. Neighborhood interventions included geotargeted digital advertisements, in-person learning events, and the deployment of testing resources. The risk score framework and review of multiple local catchment areas contained information that was too granular to be useful later in the pandemic and were discontinued early in 2022.

During the initial Omicron surge, LMPHW asked if the wastewater data could be used to identify areas of the community where there was insufficient testing being conducted.

The University plotted reported cases against virus concentration in the wastewater to determine if increasing levels of wastewater virus would track with positive test result and case counts (Figure 12). Areas of under-testing were identified by the appearance of outliers that showed high levels of viral concentrations yet lower-than-expected reported positive cases. This allowed LMPHW to strategically target scarce resources and increase COVID-19 testing sites where they would be most impactful.

Figure 12: Under-testing prediction model with each dot equaling a site.

Testing Analysis
Demographic Data Expansion

Demographic information was expanded for each geographic location (Figure 13). The School of Public Health and Information Sciences at UofL requested age ranges be added for identification of sensitive populations. The addition of population age ranges gave insight into what populations were becoming infected and would likely spread the virus among their peers. This request was driven by increasing breakthrough cases within family members who had school-aged children. Viewing the locations through this slide sequence showed that the places with high positivity rates also had the highest numbers of individuals who were in middle to high school during a time when that population was considered super spreaders in the community. This was an interesting anecdotal data point that suggested consistent information gleaned on what was driving breakthrough cases.

Familiar Geographic Names

Organizing the catchment areas by MSD’s nomenclature was not facilitating easy comprehension by LMPHW.

In an effort to address this the predominant zip codes within each sewershed were included to help anchor catchment areas for LMPHW. Soon, neighborhood names and landmarks were included (refer to Figure 14) to provide additional clarity as zip codes alone did not offer as much value as initially believed. The addition of neighborhood, township, and landmarks broadly known to Louisvillians helped to connect catchment areas to the populations LMPHW serves.

Vaccination Rates

Vaccination administration rates reached a plateau after many months of vaccination encouragement programs in 2022.

Everyone who was willing and able to become vaccinated had completed the process with small influxes occasionally. Vaccination administration trends were no longer helpful in risk identification.
Variant Tracking

Variant genomic data was defined by the CDC in March of 2022, where “detection” was defined as able to detect more or equal to 50% of mutations associated with a variant. Variant tracing allowed for LMHPW to see surges of infection attributed to particularly transmissible versions of SARS-CoV-2 virus and was included in the weekly presentations.

VARIANT SEQUENCING

Reflecting an anticipated reduction in resources and with the understanding that fewer locations could still offer acceptable resolution of SARS-CoV-2 information, sampling sites were reduced from 17 to 8.

The remaining sites reflect 5 water quality treatment centers and 3 local sewersheds (see Figure 14). All sites were analyzed and three were chosen for their history of either offering early warnings of future surges or emergence of variants. The Ashby Lane site was the first to register the November 2020 and December 2021 waves and the omicron variant. The Preston and South Park site was first to register the delta variant. Shawnee was chosen due to its strategic location.

Figure 25: Reduction of local sites.
Final Risk Framework

The final version of the risk framework highlights several metrics:

- PMMoV-normalized N1 averages across water quality treatment plants
- Comparison of wastewater levels and clinical cases
- Comparison of Hospitalizations and ICU bed occupancy
- Variant proportion tracking by catchment area
- Comparison of Louisville’s wastewater results to CDC NWSS, Biobot, and Verily data

City-Wide N1 Averages

The citywide trend slide sequence needed subtle changes to provide usable data to LMPHW. Rather than using the index based on orders of magnitude, the average virus concentration among all five treatment plants was plotted. Though the non-treatment plant sample sites were included in this slide sequence (Figure 15) they did not contribute to the cityside average.

PMMoV-normalized N1 (average of Treatment Plants)

Comparing Hospital Metrics to Wastewater

To more closely connect wastewater results to clinical data, wastewater trends were compared to hospital indicators, new admissions and ICU bed occupancy (Figure 16).

The CDC issued recommended thresholds that signal levels of concern. This visualization was useful for helping LMPHW to considering how trends in wastewater might predict upcoming threshold category changes in hospital indicators such as new admissions and bed occupancy.

Comparison of Wastewater to Hospitalizations

Discussion Points:
- Based on FAQs
- Shifting to CDC framework

Tracking Variant Proportions

As variant testing continued, new data visualization charts (Figure 17) were included to help show the distribution of dominant variants that were sampled from the appropriate treatment plant.

Variants at WQTPs

Figure 27: Hospital indicators layered over CDC color-coded thresholds.
**Inclusion of KY in the CDC’s NWSS Dashboard**

CDC dashboard framework (Figure 18) was screenshot into the presentation to show the 15-day trend among national sampling sites that report wastewater data. This was re-created into the presentation on the Louisville level.

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<th>Change in % Sites</th>
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<tr>
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</table>

**Neighborhood scale Interventions**

LMPHW developed successful interventions in the early stages of the pandemic and has drawn from that experience to create new interventions using wastewater data. Like many cities, Louisville had early success in vaccine uptake when vaccine supply was limited and demand was high. The primary mechanism for communication was city-wide media coverage and institutional outreach (school systems, first responders, etc.). This communication tool was highly effective with a vaccine delivery approach focused on mass vaccination sites. When vaccine supply became plentiful and demand waned, the LMPHW pivoted to a “community partner” model to overcome logistical barriers to vaccines and to proactively address equity concerns which informed the development of neighborhood scale interventions.
UofL undertook immediate action based on the wastewater risk framework and assessment and launched a geographically targeted awareness and action ad campaign.

When at-risk areas were identified by the risk framework, showing where action was best targeted, UofL collaborated with local ad agencies to create hyper-local marketing campaigns to drive awareness of wastewater monitoring and action towards getting vaccinated or tested for COVID-19. Special attention was given to the advice of community partners. In the initial rounds of advertising, UofL highlighted these community champions by including their logo on the ads and creating a section on the call-to-action webpage dedicated to these community partners. In addition, UofL featured images of landmarks concordant with target communities, highlighted the scientific nature of the project, and finally shared good news during a low period of infection. Through this work, the Envirome demonstrated that rapid-turnaround, hyper-targeted ads based on specific geographics could increase awareness in areas that were identified by the risk framework.

**Local Leader Ads**

**Scientific Discovery Ads**

**Local Landmark Ads**

**Good News Ads**

Figure 30: Selection of Advertisements
Marketing Campaign Outcomes

Over 3.3 million ads ran in four catchment areas between November 2021 and April 3022. 5,284 people interacted with the ads, clicking on the call to action and visiting a webpage where they were presented with information about testing and vaccination options. The national average for banner ad click-through-rates (CTR) across all industries is .06%. This campaign showed a .16% CTR, almost three times the national average.

<table>
<thead>
<tr>
<th>TARGET GEOGRAPHY</th>
<th>CAMPAIGN DATES</th>
<th>CAMPAIGN LENGTH</th>
<th>&quot;AD IMPRESSIONS&quot;</th>
<th>CLICKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Portland</td>
<td>Nov. 2021 - Feb. 2022</td>
<td>9 Weeks</td>
<td>1,077,268</td>
<td>2,050</td>
</tr>
<tr>
<td>Cedar Creek</td>
<td>Feb. - Apr. 2022</td>
<td>8 Weeks</td>
<td>1,038,585</td>
<td>1,509</td>
</tr>
<tr>
<td>Preston &amp; South Park</td>
<td>Mar. 2022</td>
<td>4 Weeks</td>
<td>544,389</td>
<td>720</td>
</tr>
<tr>
<td>Highlands</td>
<td>Mar. - Apr. 2022</td>
<td>4 Weeks</td>
<td>692,975</td>
<td>1,005</td>
</tr>
<tr>
<td><strong>TOTALS</strong></td>
<td></td>
<td><strong>27 Weeks</strong></td>
<td><strong>3,353,217</strong></td>
<td><strong>5,284</strong></td>
</tr>
</tbody>
</table>

The ad campaign seemed to have an impact in the Portland area, where ads ran for the longest period of time. In the month following the advertisements testing in that area decreased by 64%, the lowest of all sewersheds (range 64%-78%). In the second month and following, testing decreases were larger and the expected pattern resumed. There were no discernable testing patterns following the ad campaigns in Cedar Creek or Preston and South Park.

Following the ads, the Envirome received two requests to host in-person learning events. The first was hosted in partnership with a local sports and academic after-school program, the Delta Basketball Foundation. This event was attended by middle school students between tutoring and basketball practice. The second event was hosted at Cole’s Place and was attended by adults and livestreamed on Facebook. Dr. Mark Burns, an infectious disease specialist, was featured at both events. Dr. Burns grew up in the neighborhood where the events were hosted and has a strong family history with basketball and the neighborhood’s trust in his advice was apparent. A total of 37 individuals were reached through these two in-person events.

![Figure 31: Testing activity vs ad runs - relative activity.](image1)

![Figure 32: In Person Event Flyers.](image2)
Part 3: Lessons Learned

Monitoring wastewater for SARS-CoV-2 is a useful complement to clinical surveillance for COVID-19 and should be used as an important tool in the toolkit to inform public health action.

Wastewater surveillance data can specifically help public health departments to alert communities to increased COVID-19 prevalence and track spread, guide individual behavioral choices, target public health messaging, allocate testing resources, inform infection control policies (e.g., limiting size of gatherings, building openings, and school modalities), and evaluate the success of such interventions.

Hence, collaborating with local public health department – Louisville Metro Public Health and Wellness (LMPHW) was an important step for wastewater surveillance to have its greatest public health impact. The goal of layering wastewater surveillance data with clinical, demographic, socioeconomic and geographic data to inform public health action presented new challenges.

In this section, the University of Louisville and LMPHW outline their initial collaborative efforts and lessons learned, as well reflect on their experiences in this project.

Balancing Public Health and Academic Interests

There is a responsibility that is placed on academia to tell our results to the public. Responsibility about telling the world what we do and observe.

One of the major observations found was that wastewater surveillance is a national issue, or perhaps even a world issue, and not fully a local issue. It was found that wastewater monitoring was not as helpful to local governments as it was hoped to be, given that it did not fully address the local needs. It is important to remember that sewer water surveillance is but one tool in our toolkit. “A hammer, for example… a very important tool with a specific use. But only if you have a nail”. As viral concentrations of SARS-CoV-2 decreased at large wastewater testing sites, WBE became less important to general leadership decision making.

Is it worth the time and money to continue and broaden the wastewater surveillance system as the COVID-19 pandemic winds down?

While WBE was not as helpful for local communities, it provided a great way to track disease spread, if not severity. Though wastewater data showed positive results in tracking the spread of COVID-19 throughout the city, it became clear that perhaps it was more helpful to look at the WBE outcomes of other cities and regions. In the same way a meteorologist uses weather forecasting systems, wastewater monitoring showed how the virus moved and mutated around the world. “You don’t look at the weather right outside to predict the weather for later that day or later that week. You look thousands of miles away to see what’s coming” (Ted Smith). Our data could benefit another community to give them time to prepare, as somewhere else could help us to prepare. Taking a step back to watch the global trends in sewer monitoring results can give a better look at what might be coming towards us and what interventions would need to be placed to overcome an incoming viral storm. WBE could be one of the most important worldwide altruistic public health tools in which the data collected may not be as valuable at the local level but that is recorded to help prepare other communities, which is the goal of public health: to prevent, promote, and protect.

Hence, collaborating with local public health department – Louisville Metro Public Health and Wellness (LMPHW) was an important step for wastewater surveillance to have its greatest public health impact. The goal of layering wastewater surveillance data with clinical, demographic, socioeconomic and geographic data to inform public health action presented new challenges.

In this section, the University of Louisville and LMPHW outline their initial collaborative efforts and lessons learned, as well reflect on their experiences in this project.
Conventional Data used by LMPHW

The city of Louisville was focused on clinical data, such as hospital bed occupancy and clinical case load for SARS-CoV-2 positive patients.

The Envirome Institute and the University of Louisville brought data to the table in the form of geographic reports that were not tied to these clinical metrics. In order to bridge this gap, several solutions were tried:

- Benchmarking with LMPHW and the City of Houston in December 2021
- Creating a web-based dashboard to share data through LMPHW’s COVID Data Tracker
- Comparing city-wide trends in wastewater and clinical cases graph
- Showing early warning system, about 3 weeks between rise in wastewater and hospital admissions. Use this to train Public Health Departments in how to use wastewater.

Practice v. Research

Research involves systematic processes such as collecting, organizing, and analyzing the data to gain insight and knowledge about a topic or subject.

Research has strict validity processes including using various instruments, experiments, and procedures to test the information discovered. Research involves the collection of data through surveys, observations, questionnaires, interviews, etc.

Practice, more specifically evidence-based practice (EBP), is entirely based on the scientific evidence produced by research. Practice relies on existing data and the things that are known at that time. Practice can change as more and newer research is compiled. Outdated research can create many issues within practice.

Wastewater monitoring during the pandemic did not allow for clear cut lines between practice and research. Often the research was relied upon to implement public health practice as soon as data began to flow in efforts to initiate community interventions for quick response times. This was the fastest wastewater surveillance system creation of our time. Record timing made the surveillance system feel like it was built out of plywood, thrown together haphazardly. Some directions were dead ends. Time seemed to be wasted on trying to figure out which avenues were still open, and which had closed. For example, the “neighborhoods as dormitories” was a false narrative that was investigated and was found to be unhelpful because neighborhoods do not act as dormitories. Facility level wastewater monitoring worked well for prevalence testing for COVID-19, however, this level of testing posed ethical and privacy concerns for the public. The pandemic did not allow for in-depth research that usually requires many years to even gain a base understanding of the topic. The fast-paced learning environment created many challenges when trying to implement best practices.

The LMPHW team were interviewed by an external collaborator and some of the most commonly cited challenges by roles are reviewed below.

Key Health Practitioners and Public Health Leadership

The science of wastewater data has been rapidly evolving throughout the COVID-19 pandemic.

Initially, there were no established or standardized guidance on thresholds for detecting COVID-19 in wastewater. The research scientists and statisticians had to constantly refine the processes and analyses many times to try to develop data-driven action strategies. Some of the main challenges included detecting levels of COVID-19 in wastewater, interpreting whether those levels reflected the amount of COVID-19 in the community, correlation with clinical cases and communicating the results to important stakeholders, collaborators and community members to make informed public health decisions.

When COVID-19 incidence is high in a sampling area to produce consistently positive results, looking at the trends of COVID-19 in the wastewater and can help understand how the virus is spatially and temporally distributed in the community. However, later in the pandemic with multiple variants in circulation, it may be difficult to differentiate and interpret the data.
When COVID-19 incidence is low in a sampling it may not be captured by wastewater surveillance. The lower limits of detection (i.e., the smallest number of people shedding the virus in stool that can still be detected by current testing methods) for wastewater surveillance are not yet known. More data on fecal shedding by infected individuals over the course of disease are needed to better understand the limits of detection, as well as how it impacts the interpretation of the data.

Important factors such as new variants, variability in fecal shedding and population density may affect the validity and reliability of the wastewater data as it correlates to human infection and severity of infection. These and other correction factors such as flowrates should be applied.

Tourists or commuters especially during holidays and special occasions may make it difficult to distinguish whether the levels of virus in the wastewater is directly related to levels within the community itself or from a visitor(s) passing though.

In a perfect world, wastewater data would be used to identify trends in communities before COVID-19 clinical testing. Hence, the logistical timing of collection of wastewater data, as an important tool or predictor of public health trends in the disease course to support public health decision making is a key component.

Wastewater data can be used as one tool in your toolkit in the public health decision making process. It is most useful when used in combination other data such as overall levels of the virus in wastewater, historical wastewater data by location or geographical context and clinical cases such as vaccination data, incidence and hospitalizations. Trend data on wastewater testing over time can complement other surveillance data to develop data-driven action strategies and inform public health decision making. However, it is not possible yet to accurately predict the number of infected individuals in a community based on wastewater data.

**Feedback from Project Partners**

**Public Health Communications**

Communicating the value of wastewater monitoring to relevant stakeholders and the community can help inform the local government, public health authorities and the public to respond appropriately. However, miscommunication or using the wrong interpretation of wastewater data given its novelty can create barriers to funding, project development, and increased skepticism.

**Laboratory Technicians**

Collection system logistics: Heavy rainfall and other factors that may be more logistically challenging can affect the samples and should be taken into consideration when interpreting the results.

Developing laboratory methods: Many challenges were presented setting up the system within a university setting including developing appropriate techniques for concentration, extraction, and data analysis, which involved different laboratories.

Variant sequencing: Kentucky started variant sequencing in August 2021 and detecting the variants in real time when circumstances were changing very quickly was inherently challenging. Interpreting the significance of variant data with the wastewater results also proved to be difficult.

Academic core facility vs non-academic core facility: Undertaking this project within an academic facility presented some minor challenges that may not arise in other setting such as slow turnovers due to holidays breaks and the administrative process involved with doing research in a university setting.

**Data Scientists**

**CDC’s NWSS framework guidelines:**

Given that that CDC guidelines were evolving with the pandemic, it was very difficult to integrate the constant changing data in real time in order to develop a data-driven decision-making framework including case data and hospitalization data for the target population.

Communication with the state public health department and national agencies: When working with multiple organizations such as the local and state health department as well as national agencies to retrieve data or share data, there needs to be communication plan in place. For example, if a definition or variables had been changed or did remove, this should be fully disclosure to external parties that are receiving the data for analysis.

**Public Health Liaisons**

**Communication Plan:**

When collaborating with external organizations, clear roles and concise communication tools are needed to effectively explain wastewater data and how it correlates with community exposure and clinical infections, for it to then be shared with others in an immediate application in a public setting.

Developing an ongoing feedback loop in real-time to ensure open and effective communication: Trusted public health experts can create tailored responses to wastewater signals,
Wastewater-based epidemiology is a useful tool that has shown great promise in increasing the overall sample of the population to gain valuable data that previous recording (such as hospital data) and traditional surveillance techniques (such as public testing sites) were unable to completely capture. As Figure 19 shows, WBE can gather more information about populations that are either pre- or post-symptomatic and asymptomatic in nature than individual testing and reporting capabilities are able to perform at current date. The more information local and national governments have on incoming pathogens and other human health indicators, the faster and more effective their intervention responses will be such as in mitigating and preventing community spread of infection and preparing hospitals and other healthcare services for influxes of symptomatic cases. Sewer system surveillance technique improvements are vital for increasing the knowledgebase of public leaders about the community they serve. Public health and epidemiology-based decisions are central to leadership from initial disaster response through community recovery.

Major Strengths

LMPHW offered several insights into the key elements that contributed to progress in wastewater surveillance and the common themes are summarized below.

Collaboration: This was a large multidisciplinary collaboration including experts from environmental sciences, epidemiology, biostatisticians, public health department (Louisville Metro Public Health and Wellness), laboratory-based analyst and genomics. The concerted cooperation among public health organizations, university faculty, staff, and administration, and the wastewater treatment facilities under the leadership of the Envirome Institute was a major strength of the project. There has been other collaborations and research opportunities that came out of this initial project which has helped to create successful workflows.

Weekly meetings with detailed illustrative slides: Meeting weekly provided the right number of updates that was effective and not burdensome. There was always a quick follow up within a day or two, hence data lag was quite minimal.

Highly motivated staff: The possibility of being able to see and understand the trends within the community, as well as be prepared for an increase was very beneficial. It can provide a short, advanced warning of an increase, enough to mobilize testing and efficiently allocate additional resources.

Impact & Conclusion

Wastewater-based epidemiology is a useful tool that has shown great promise in increasing the overall sample of the population to gain valuable data that previous recording (such as hospital data) and traditional surveillance techniques (such as public testing sites) were unable to completely capture. As Figure 19 shows, WBE can gather more information about populations that are either pre- or post-symptomatic and asymptomatic in nature than individual testing and reporting capabilities are able to perform at current date. The more information local and national governments have on incoming pathogens and other human health indicators, the faster and more effective their intervention responses will be such as in mitigating and preventing community spread of infection and preparing hospitals and other healthcare services for influxes of symptomatic cases. Sewer system surveillance technique improvements are vital for increasing the knowledgebase of public leaders about the community they serve. Public health and epidemiology-based decisions are central to leadership from initial disaster response through community recovery.

This was the fastest wastewater surveillance system creation of our time due to the necessity the pandemic required. Record timing made the surveillance system feel like it was built out of plywood, thrown together haphazardly. There was no time for the creation of solid system foundation processes and analysis. This would come with more familiarity of WBE surveillance systems. Some directions led to dead ends. Time seemed to be wasted on trying to figure out which avenues were still open or had closed after following a direction that led to one of those dead ends. For example, the “neighborhoods as dormitories” model was a false narrative that was investigated and was found to be unhelpful because neighborhoods do not, in fact, act as dormitories. Facility level wastewater monitoring worked well for prevalence testing for COVID-19, however, this level of testing posed ethical and privacy concerns for the public.

Though this project was faced with many challenges, positive information was gleaned. In a resource scarce environment, as we found ourselves to be with shipping interruptions during the pandemic, wastewater monitoring was much better than individual testing due to using a lot fewer testing supplies and materials for a much larger population sample. This allowed engage in community meetings to explain what wastewater sampling is, how it is done, and how it can serve as an early detection system help educate community members and generate interest in receiving regular wastewater updates. Developing a review and approval process for local leaders and other stakeholders involved in the implementation of the wastewater monitoring program is equally beneficial.

It is important to provide transparency when publicly sharing wastewater results to build trust and encourage healthy behaviors. However, there is some concern that these results could also incite unnecessary concern and create stigma against communities from a particular demographic or interest group who resided in areas with high levels of COVID-19 in the wastewater.

Targeted marketing interventions of areas that exhibited increased viral concentrations in wastewater did not deliver the value that was estimated.

Changing dialog as pandemic evolves.
The recommendation of changing the focus of WBE from COVID-19 to include full panels of pathogens would be very helpful to governments in monitoring the health of the public. The impact of increasing city, state, and national WBE systems would have multifaceted effects into multidisciplinary interests not just for infectious disease monitoring. Decision making leaders may be interested to know that monitoring of metabolites from antiviral drugs can shed light into whether the levels noted are consistent with antiviral drugs prescribed as well as providing information on environmental issues of antiviral release from public sewer systems altering ecosystems with the development of antiviral resistance which can later negatively affect human populations.

Overall, the effort put into the Louisville Metro and Jefferson County sewer monitoring program was a great first phase. Further WBE application is encouraged for increasing the purity of the information gained from sewer surveillance and to smooth out the decision-making processes needed for correct interpretation of the data collected. Wastewater systems offer leaders a reservoir for a wealth of knowledge about human populations which can be applied to many faces of public health interventions to create healthier communities.

Resources


Additional support for the Wastewater Research Program came from:

**Acknowledgments**

**CHRISTINA LEE BROWN**
**ENVIROME INSTITUTE:**
Aruni Bhatnagar, Ph.D. FAHA
Ted Smith, Ph.D.
Ray Yeager, Ph.D.
Shesh Rai, Ph.D.
Joe Moore, Ph.D.
Lauren Anderson, M.P.A.
Heather Ness
David Hoetker
Rick Strehl
Jacob Ziegler
Mamata Chaudhari
Dan Riggs

**CENTER FOR PREDICTIVE MEDICINE:**
Joshua Fuqua, Ph.D.
Kenneth Palmer, Ph.D.
Saurabh Kumar, Ph.D.

**GENOMICS CORE:**
Wolfgang Zacharias, Ph.D.
Mei Zhang, Ph.D.
Sabine Waigel

**BIOINFORMATICS CORE:**
Eric Rouchka, D.Sc.

**MICROBIOLOGY & IMMUNOLOGY:**
Kevin Sokoloski, Ph.D.

**INTERDISCIPLINARY STUDIES:**
Tamara Sluss, Ph.D.
Cullen Hunter, Ph.D.

**ARIZONA STATE UNIVERSITY:**
Rolf Halden, Ph.D.
Arvind Varsani, Ph.D.
Erin Driver, Ph.D.

**THE OHIO STATE UNIVERSITY:**
Mark Weir, Ph.D.
Grzegorz Rempala, Ph.D.

**LOUISVILLE METROPOLITAN SEWER DISTRICT:**
Daymond Talley

**LOUISVILLE METRO PUBLIC HEALTH & WELLNESS:**
Sarah Moyer, M.D.
William Atman, J.D.

**KENTUCKY STATE LABORATORY:**
Vaneet Arora, Ph.D.

**FUNDING PROVIDED BY:**
Louisville Metro Government LMPHW
Commonwealth of Kentucky DPH/DOC
James Graham Brown Foundation
Owsley Brown II Family Foundation
Foundation for a Healthy Kentucky
Newman’s Own Foundation
Center for Disease Control