THE Water Research FOUNDATION®

International Water Research Summit

Environmental Surveillance of COVID-19 Indicators in Sewersheds

OPENING SESSION

April 27, 2020

3:00 PM – 5:00 PM EDT USA

Welcome!

Christobel Ferguson, PhD

Chief Innovation Officer

The Water Research Foundation

Agenda

Perspective

Peter Grevatt, PhD, CEO
The Water Research Foundation

Best Practices and Considerations for Collection and Storage of Wastewater Samples

Dan Gerrity, PhD, Southern Nevada Water Authority
Chuck Gerba, PhD, University of Arizona

Use of Molecular Genetics Tools to Identify the Concentration of Indicators of COVID-19 in Wastewater Samples

Scott Meschke, PhD, JD, University of Washington
Krista Wigginton, PhD, University of Michigan

Use of Indicator Concentrations to Inform Trends and Estimates of Community Prevalence

Doug Yoder, PhD, Miami-Dade Water and Sewer
Gertjan Medema, PhD, KWR Water Research Institute in Nieuwegein, Netherlands

Communication of Environmental Surveillance Results with the Public Health Community, Elected Officials, Wastewater Workers, and the Public

Jim McQuarrie, Denver Metro Wastewater Reclamation District
Dan Deere, PhD, Water Futures Australia

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Peter Grevatt, PhD

Chief Executive Officer

The Water Research Foundation

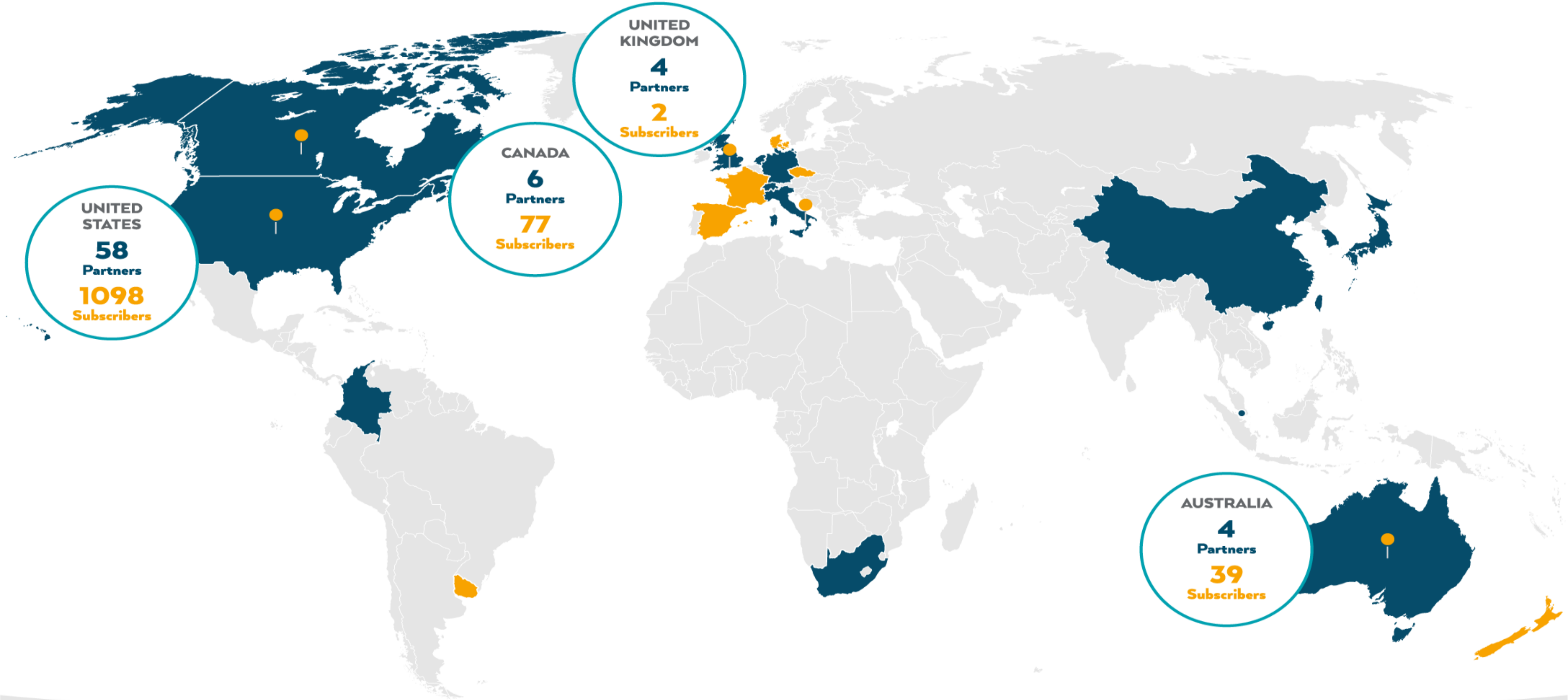
ABOUT

MISSION

Advancing the science of water to improve the quality of life.

VISION

To create the definitive research organization to advance the science of all things water to better meet the evolving needs of subscribers and the water sector.



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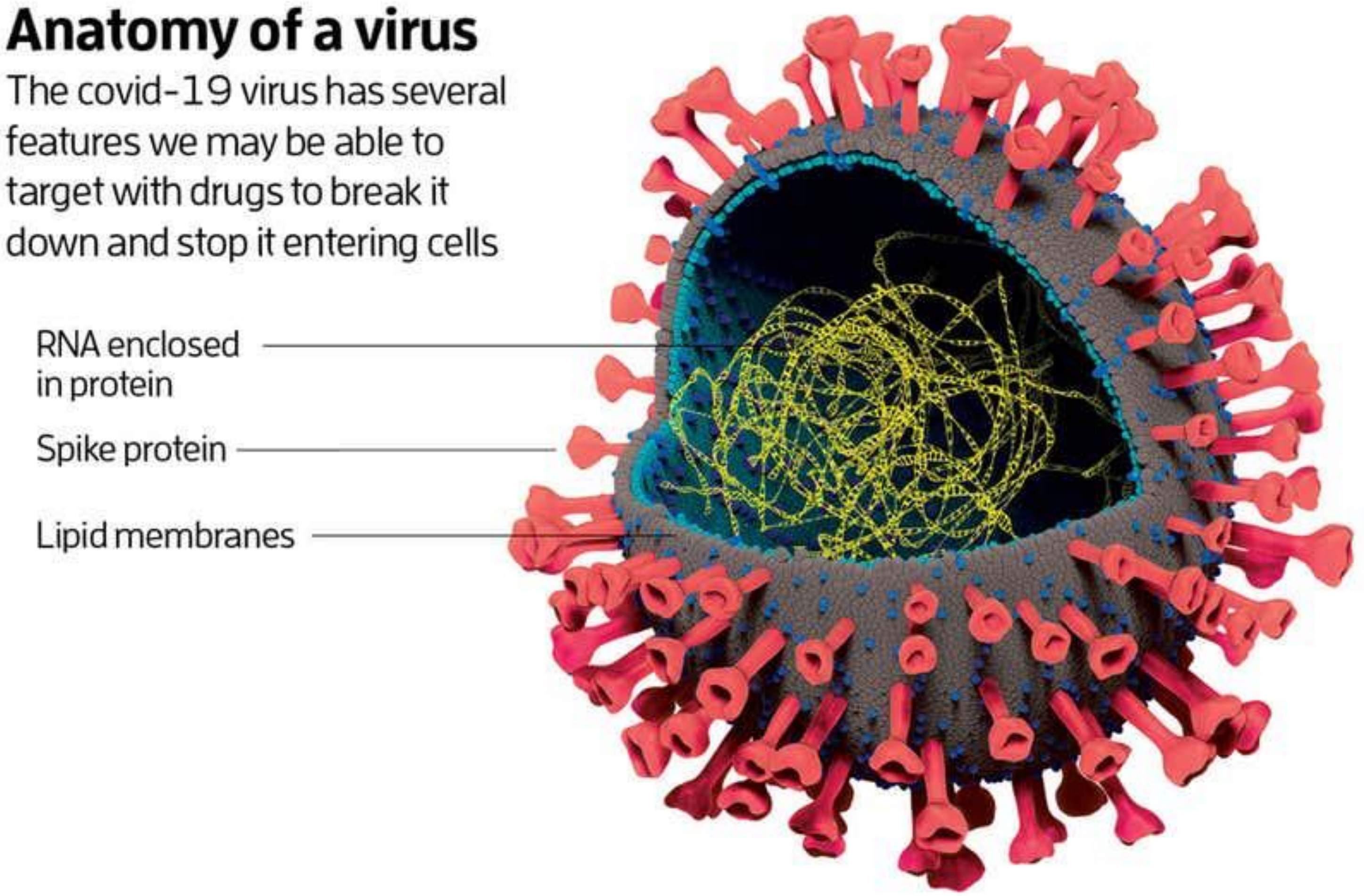
Example of Genes, RNA, and Remnants of Inactive Virus



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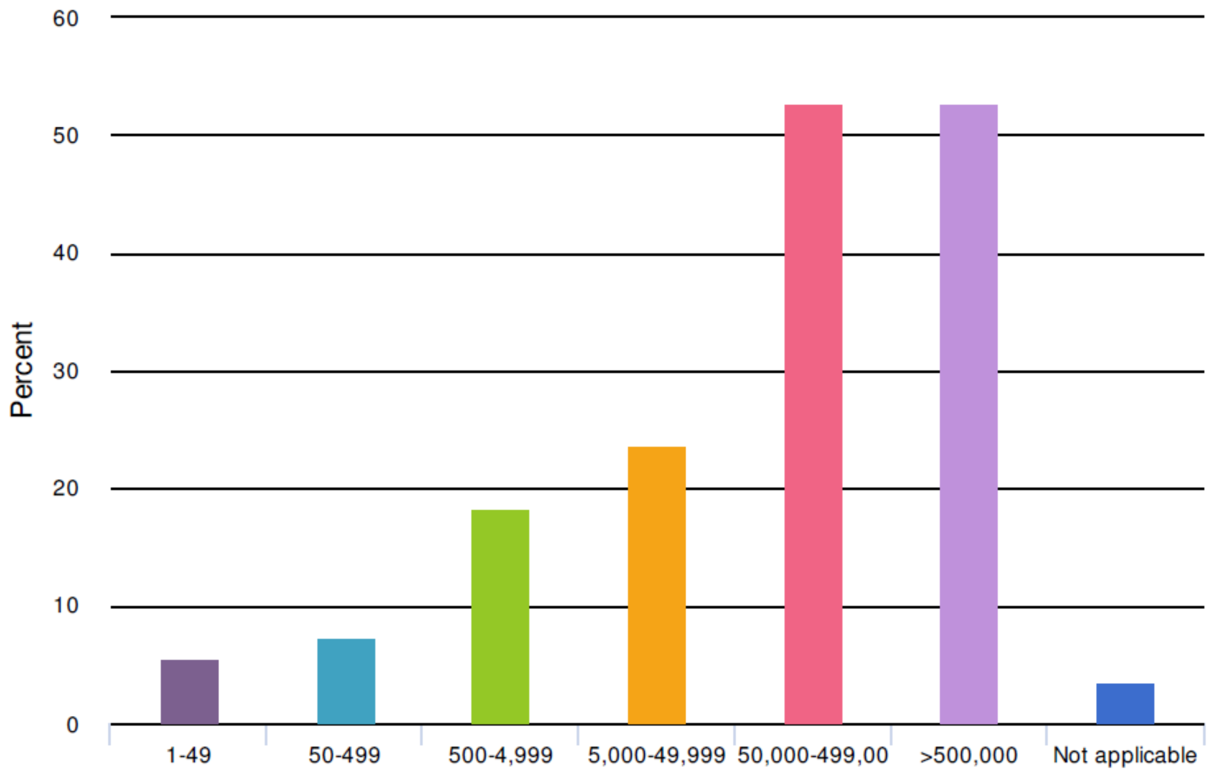
Environmental Surveillance of COVID-19 Indicators in Sewersheds

WRF Survey of Microbiological Methods for Wastewater Surveillance

Survey Results - What size service areas do they represent?

Testing at a wide variety of system sizes but mostly large urban populations of 50,000 people or more (total n=90, representing 140 systems)



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Daniel Gerrity, PhD

Principal Research Scientist

Southern Nevada Water Authority

WORKING GROUP 1

GOAL

To develop best practices and considerations for sample design, collection and storage of wastewater

Working Group 1 - Sample Collection

Co-Chairs

Chuck Gerba, University of Arizona

Jim Pletl, Hampton Roads Sanitation District

Dan Gerrity, Southern Nevada Water Authority

Participants

Mark Sobsey, University of North Carolina at Chapel Hill

Amy Pickering, Tufts University

Mark Jones, UK Water Industry Research (UKWIR)

Katrina Charles, Oxford University

Kelly Hill, Water Research Australia

Christoph Ort, Eawag – Swiss Federal Institute of Aquatic Science and Technology

Matt Burd, New York City Dept. of Environmental Protection

Kaylyn Patterson, Metropolitan Water Reclamation Dist. of Greater Chicago

Amy Kirby, Centers for Disease Control and Prevention (CDC)

Group 1 Scope

1. Study design
2. Sample collection
3. Sample storage (and preservation)
4. Research needs and knowledge gaps related to these topics

Opening Session: Current perspectives of group participants on critical aspects of study design, sample collection, and sample storage

Closing Session: Deliver recommended best practices for sample collection and sample storage in the context of study design

Study Design

- What are the goals of the study?
  - Specific 'use cases' will be presented throughout the opening session
  - Some examples addressed in this presentation
- **How do the goals impact the required methodology?**
- Who should be represented on the team?
  - Academia, industry, public health
  - How can needs/data/information be communicated in all directions?
- What is the best use of a limited number of samples/resources?

Study Design: Use Case Example
Assessment of Community Infection

Sample collection

- Average concentration over a 24-hour period (i.e., composite sample) might be more appropriate
- Metadata will be critical for understanding factors that impact SARS-CoV-2 concentrations
- Different approaches required for developing countries vs. rural areas vs. urban areas vs. specific facilities or ‘hot spots’

Study Design: Use Case Example
Risk Assessment

Sample collection:

- Maximum concentration from high frequency grab samples might be more appropriate
- Different sampling locations (raw sewage vs. finished effluent) and matrices (liquids vs. solids) are necessary to assess fate and exposure (occupational vs. general public)

Sample storage/preservation:

- Consider whether the samples might be used for future infectivity assays

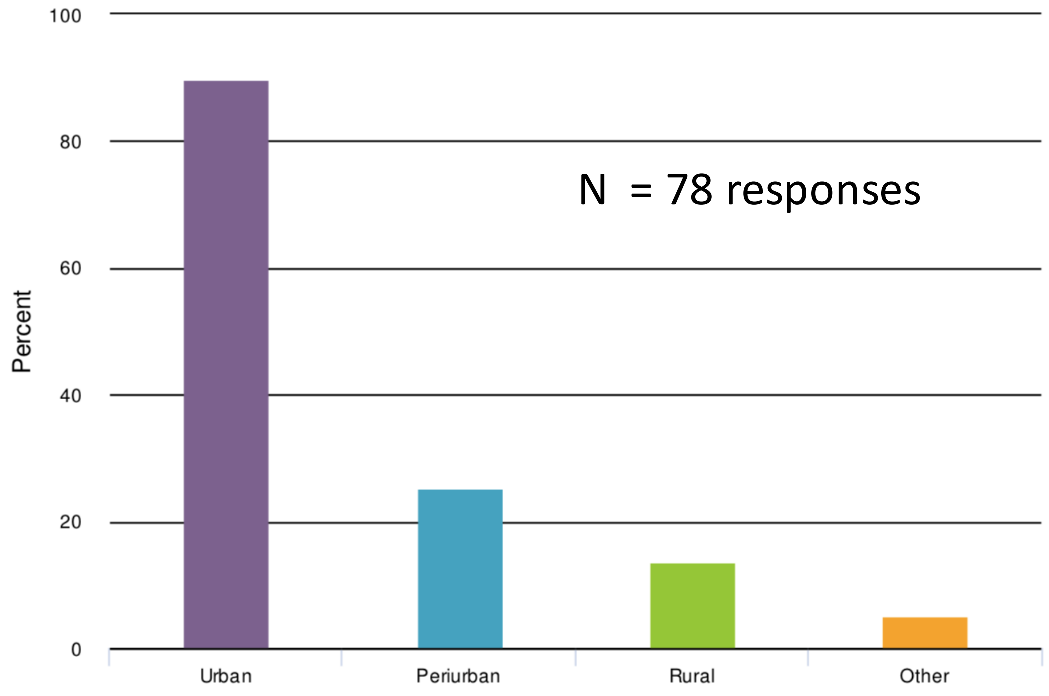
Sample Collection: Metadata

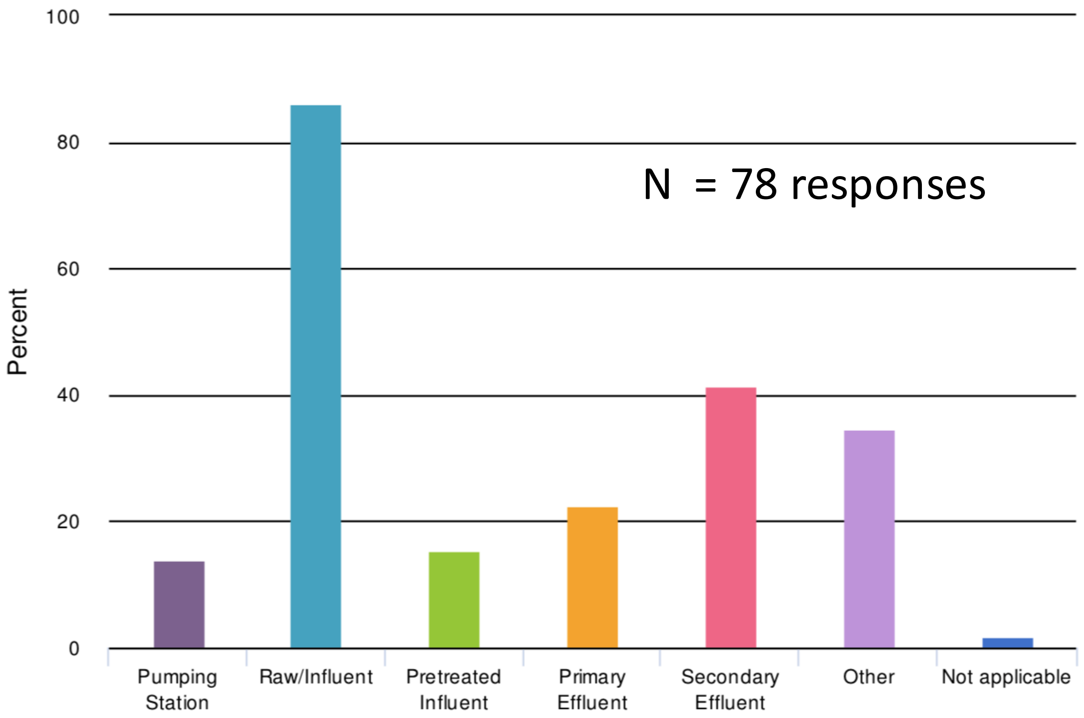
- **Basics:** Date, time, location, sampler (documentation is critical)
- **Type of sample:** Grab vs. composite (and type of composite)
- **Weather conditions:** temperature, rainfall (combined sewers)
- **Facility conditions:** flow rate, population of service area, composition of service area (residential vs. industrial), demographics (e.g., commuters), hydraulic retention times
- **Water quality:** pH, total suspended solids, biochemical oxygen demand, ammonia, chlorine residual
- **Public health:** confirmed case load, active cases

Sample Collection: Location

The size of the system and the sampling location have significant implications for indicator concentrations and data use

Example: more dilution of a 'critical' toilet flush in larger systems





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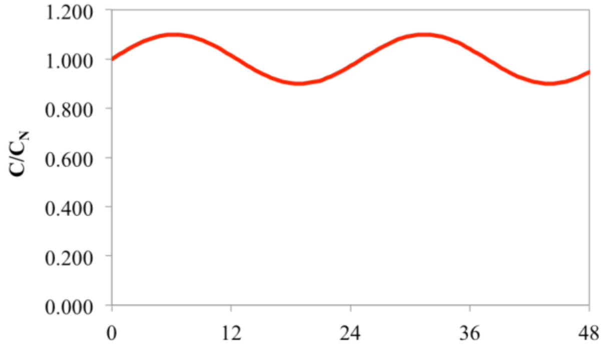
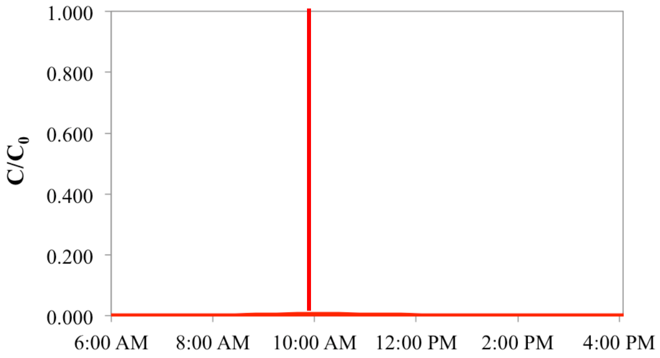
Sample Collection: Implications of Sample Type

Ubiquitous Indicator (Sucralose)



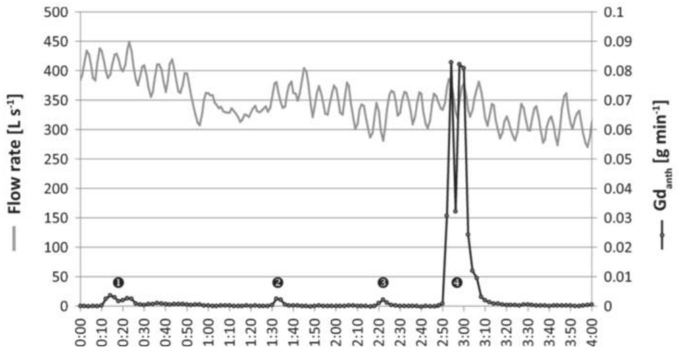
Diurnal Variation

Rare Contaminant Spike



Instantaneous Spike

Real-World Example



Grab Samples Every 2 Minutes (generally not practical)

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Sample Collection: Implications of Sample Type

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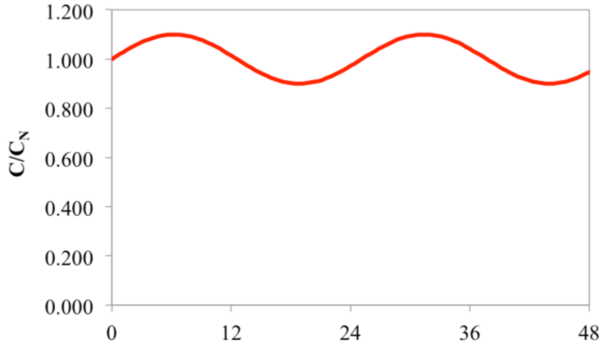
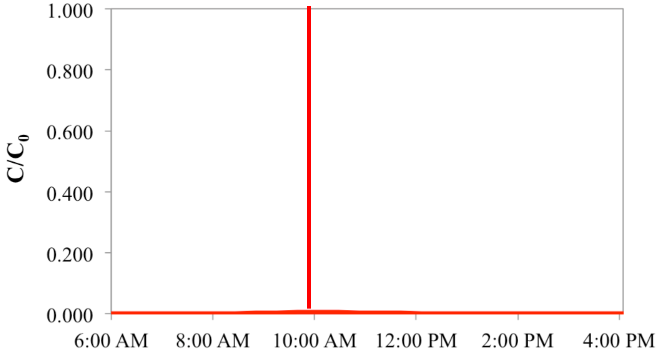


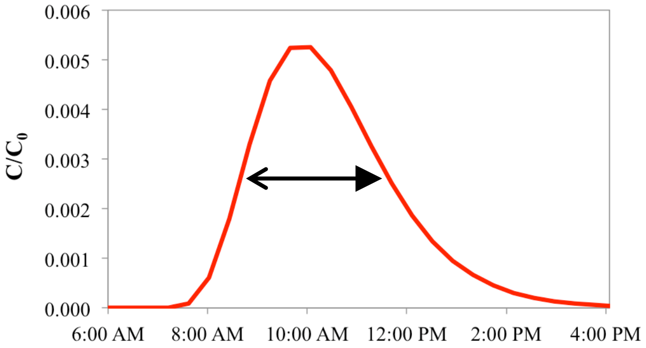
Diurnal Variation

Rare Contaminant Spike



Instantaneous Spike

Effect of Dispersion



Diluted but Distributed

How do we overcome the 'rare spike' effect?

- Continuous or high frequency composite sampling
- Sampling from locations with equalization/mixing
- Sampling downstream in the treatment facility (e.g., primary effluent)

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Sample Collection: Volume, Frequency, Duration

- Volume must balance method sensitivity requirements with practical considerations (e.g., freezer space)
  - Current consensus: **500 mL to 1 L** to account for (1) test volume requirements, (2) spiking controls, and (3) archiving for future analyses
  - Best case scenario: RT-qPCR only considers ~10% of the original sample
  - Assume limit of quantification is 100 gene copies per reaction:
    - 1 mL equivalent sample volume → required concentration > 10^5 gc/L
    - 10 mL equivalent sample volume → required concentration > 10^4 gc/L
    - 100 mL equivalent sample volume → required concentration > 10^3 gc/L
    - 1 L equivalent sample volume → required concentration > 10^2 gc/L

Sample Collection: Volume, Frequency, Duration

- Volume must balance method sensitivity requirements with practical considerations (e.g., freezer space)
  - Current consensus: **500 mL to 1 L** to account for (1) test volume requirements, (2) spiking controls, and (3) archiving for future analyses
- Frequency must balance study goals with practical considerations
  - What time resolution is needed to assess community prevalence and trends?
  - What resources are available for the study?
- Duration must balance study goals with practical considerations
  - What duration is needed to assess trends/reemergence?
  - Should the frequency of sampling change over the duration of a study?

Sample Collection: CDC Guidance

- "Standard practices associated with wastewater treatment plant operations should be sufficient to protect wastewater workers…"
- Can include personal protective equipment (PPE) among other controls, safe work practices, and precautions normally required when handling untreated wastewater
- "No additional COVID-19-specific protections are recommended for workers involved in wastewater management…"

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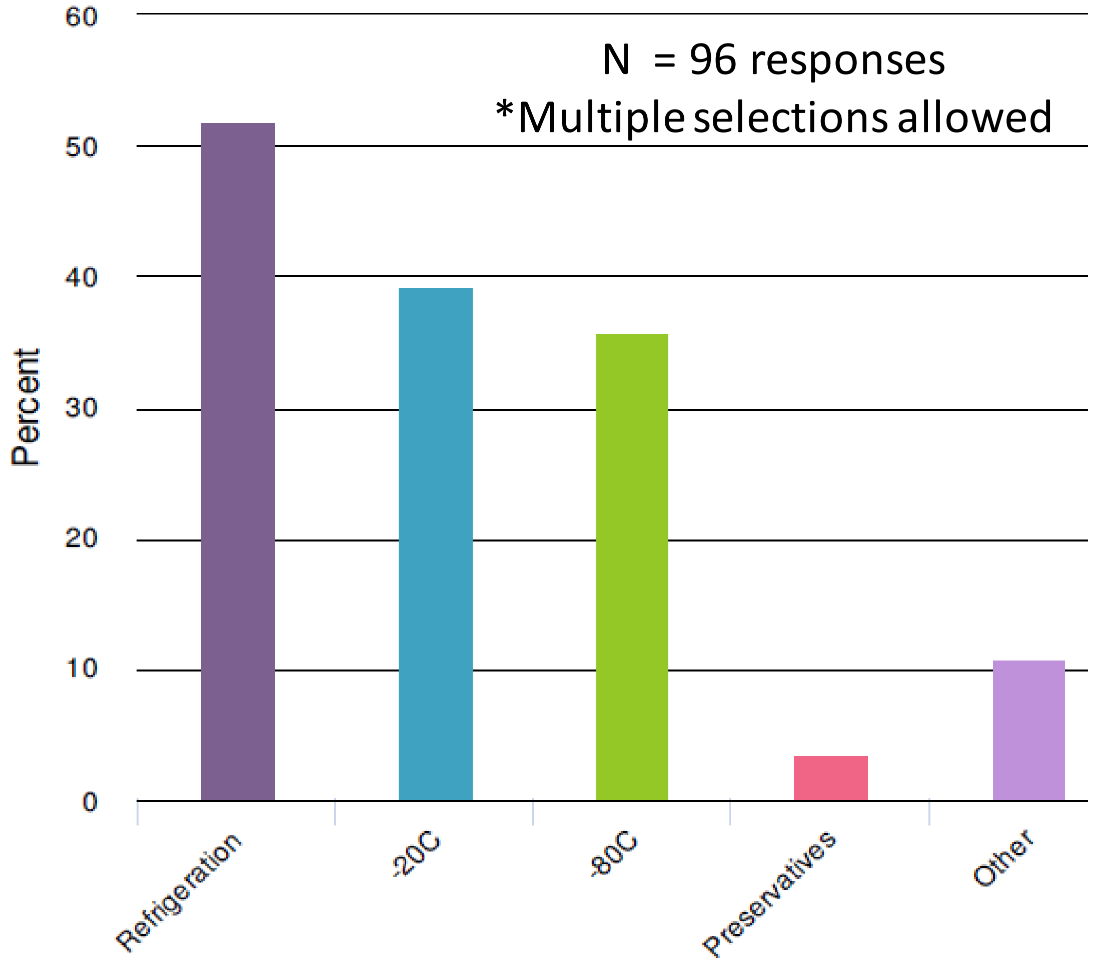
Chuck Gerba, PhD

Professor

University of Arizona

Sample Storage and Preservation

- Storage temperature
- Survey respondents asked to select all that applied
- Hold times reported were all < 48 hours
- Only preservative reported was lysis buffer



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Hold Time and Storage of Samples

SARS-CoV-1 stable in stool for 6 hrs at room temperature : 99.9% loss of infectivity in three days

Preference	Temperature	Time
Ideal	-80°C	7 years*
2nd best	-40°C (i.e., blood-bank freezers)	1 year?
3rd choice	-20°C	Several months
Minimum	4°C	4 Days

*Concentrate, not raw sewage

Note- freeze/thaw of samples may result in breakdown and release of matter that may interfere with concentration methods and detection methods

Sample Storage

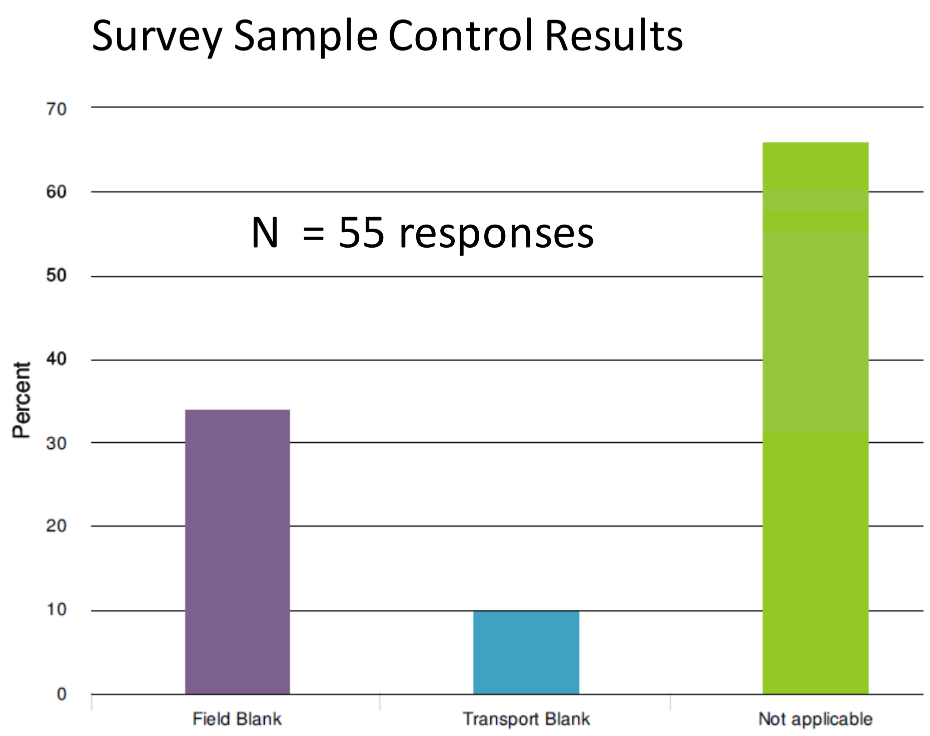
- Multiple freeze-thawing can affect detection by damaging the virus and/or release of materials that may interfere with assay or concentration methods
- Consider dividing the samples into aliquots to avoid multiple freeze thawing
- Recommended samples stored in new or autoclaved polypropylene plastic bottles leaving enough head space for expansion of liquid during freezing
  - Cleaning with bleach is a recommended alternative if above is not possible

Handling and Preservation of Samples

- Pasteurization for lab safety
  - Evidence suggests this may reduce the sensitivity of the qPCR signal
- Cryogenic preservative is usually only done after the samples have been processed or concentrated
- Additives for concentrates (such as dimethyl sulfoxide, glycerol, fetal calf sera), may enhance survival/persistence of virus for long-term storage and potential viability analyses in the future

Sample Processing Considerations

- Quality assurance/quality control (QA/QC)
  - Field blanks, transport blanks, assay blanks
  - Positive spiking controls
    - Enveloped phage (phi6)
    - Enveloped viruses (229E, OC43)
    - Non-enveloped phage
  - Internal controls
    - Non-enveloped phage (e.g., somatic, male-specific)
    - PMMoV
- Personal protective equipment (PPE)



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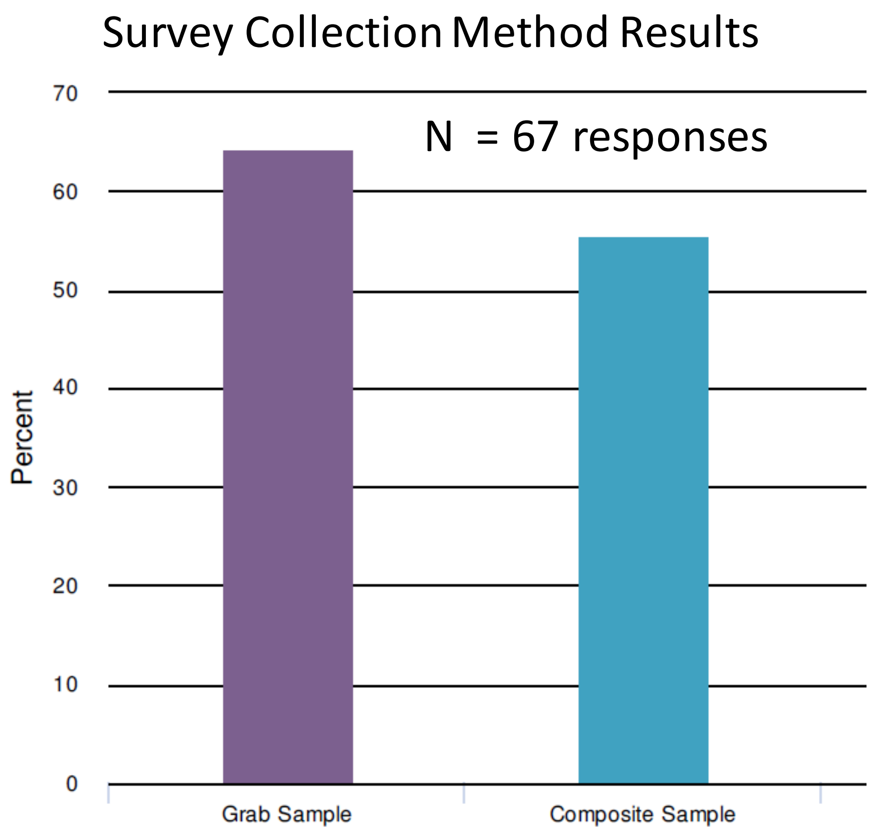
Knowledge Gaps / Discussion Items

- Metadata
  - What is needed vs. unnecessary (or "nice to have")
  - Need perspective of public health officials and other working groups
- Protocols
  - Recommendations for each use scenario
  - What study duration is needed to assess trends/reemergence?
  - Does the frequency of sampling change over the duration of a study?
  - Alternatives needed for sampling latrines, cesspits, etc.

Sample Collection Method Research Needs

Grab vs. composite

- If composite:
  - Duration of collection? 4hr vs. 8hr vs. 24hr
  - Consideration of temperature over time
  - Type of sampling device (refrigerated)
- If grab:
  - What time of day?
  - Try to capture morning flush?
  - Or would the genetic signal be more diluted in the morning?



*Respondents asked to click all that applied
Almost all composite samples were 24hr

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Research Needs

- What spike organism to use
- Shedding rates of infected people (depending on severity of illness and time since infection)
- Distribution of virus (or RNA fragments) in liquid and solid phase
  - If substantial parts are bound to particulate matter, one also has to study efficiency of routine sampling devices for their suitability to collect solids
- Transformation of virus/RNA during in-sewer transport
- Sample location: Should we target large facilities (representing the general public) or also focus on specific locations such as retirement homes or schools or other specific sub-populations?
- Persistence of the signal over time

Scott Meschke, PhD, JD

Professor

University of Washington

WORKING GROUP 2

GOAL

To develop best practices for the use of molecular genetics tools to identify the concentration of indicators of COVID-19 in wastewater samples

Working Group 2 - Analyze Results

Co-Chairs

Krista Wigginton, University of Michigan

Frederic Been, KWR

Joan Rose, Michigan State University

Participants

Raul Gonzalez, Hampton Roads Sanitation District

Kellogg Schwab, Johns Hopkins University

Scott Meschke, University of Washington

Rosina Girones, University of Barcelona

Kaye Power, Sydney Water

Sudhi Payyappat, Sydney Water

Zia Bukhari, American Water

Farida Bishay, Metro Vancouver

Tiong Gim Aw, Tulane University

Irene Xagoraraki, Michigan State University

Recommended Best Practices

- There is a need to provide credible information to decision makers
- Sense of urgency - decisions are being made and will continue to be made
- Before analyzing samples the first step is to consider what you want to use the information for:
  - Community prevalence
  - Trends in prevalence
  - Risk to operators/laboratory technicians
- Different questions require different analysis (presence/absence or quantitative)
- Availability of resources, capacity and skills will also limit which methods can be used

Current Status of Analytical Testing

- WRF conducted a survey to evaluate currently, or soon to be, employed methods to carry out environmental surveillance of wastewater for SARS CoV-2
- Survey received 169 responses primarily from academic researchers, water utilities, industry, state and federal government
- Of the responses, 54% indicated they were currently testing, and 46% were developing methods with the intention of testing wastewater
- 84% of respondents were testing for SARS CoV-2 and 39% were also testing for other organisms and chemicals
- Nearly 90% of respondents are sampling in urban areas, while only 25% were sampling in peri-urban areas and only 14% were performing sampling in rural areas
- Weekly sampling was most common scheme and raw influent was dominant matrix sampled

Sample Processing Survey Results

- **Solids Separation:**
  - Majority of survey respondents (73%) do not disassociate viruses from solids prior to concentration
  - Most used either centrifugation (41%) and/or filtration (46%) to remove solids
- **Concentration:**
  - Primary concentration methods reported were near equally split between PEG precipitation, membrane filtration, and centrifugal ultrafiltration. Some research groups reported use of Hollow fiber ultrafiltration, ultracentrifugation and skimmed milk flocculation.
  - A minority of respondents reported a secondary concentration method. The dominant methods reported were centrifugal ultrafiltration and PEG precipitation.

Preliminary Considerations for Sample Processing

- **It is difficult to recommend any particular concentration method at this point**
  - Most methods used have been developed and optimized for non-enveloped viruses
- **Sample volume is important** – analyzing too great a volume can concentrate inhibitory substances, and too small a volume reduces the sensitivity
  - WG2 recommends no more than 1 Liter of sample be analyzed
  - Concentration of inhibitor substances increases as more sample is concentrated
- **Need to understand the performance of the method used**

  -Support the use of recovery controls; just over half report use of recovery control

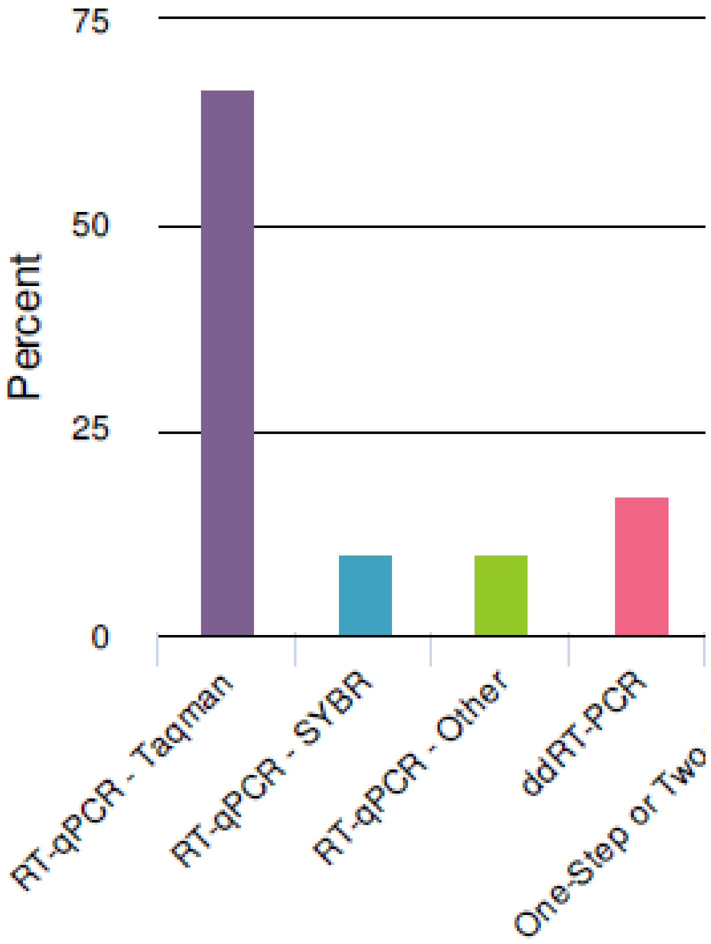
Separated Solids Analysis

- A minority (14 respondents) reported analysis of separated solids
- The dominant approach for solids analysis was direct nucleic acid extraction
  - There was no consensus regarding method applied, though use of commercial extraction kits was reported in all cases
- A few respondents reported using acid adsorption/elution or direct elution approach

Nucleic Acid Extraction Methods for Concentrates

- As for solids, there was no consensus on nucleic acid extraction method
  - Wide range of commercial kits reported, little overlap with kits reported for solids
  - Most methods appear based on guanidinium salt and/or physical disruption
  - Two thirds of respondents reported use of manual extraction methods
- Just over 60% the respondents were using an extraction control

Sample Detection Methods Survey Results



- Majority of respondents (80%) are using quantitative methods
- Molecular detection targeting viral RNA was the most frequently reported approach
- Taqman RT-qPCR was dominant method employed followed by ddRT-PCR
- 75% of respondents were using a one step PCR

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Preliminary Considerations for Molecular Methods

- The majority of analyses are using the CDC N1, N2 and N3 primers and/or the E gene primer sets described by Corman et al.
- Detection methods should be optimized for environmental samples, not clinical samples
- Some of published papers on this topic are pre-print. Pre-print are research papers shared **before peer review**. Some caution is required when citing them
- Several considerations for method development: 1) effective sample volumes; 2) variability in assays; 3) recovery efficiencies; and 4) appropriate controls

Other Potential Methods – Cell Culture Infectivity

- **WG2 does not recommend cell culture efforts at this time for routine surveillance**
- Culture of SARS-CoV-2 requires BSL3 containment
- Demand for BSL3 and PC3 facilities is high and clinical applications have priority at this time
- Further, there are very limited data to indicate the presence of viable virus in stool samples, it is therefore not a high priority to look for viable virus in wastewater at this time
- However in areas where COVID-19 infections are prevalent, a risk analysis of routine efforts to culture enteric or other viruses from wastewater should be performed to assess inadvertent risk of SARS CoV-2 culture
  - Consider whether this work should be suspended in the short term unless it can be conducted in BSL3 or PC3 facilities

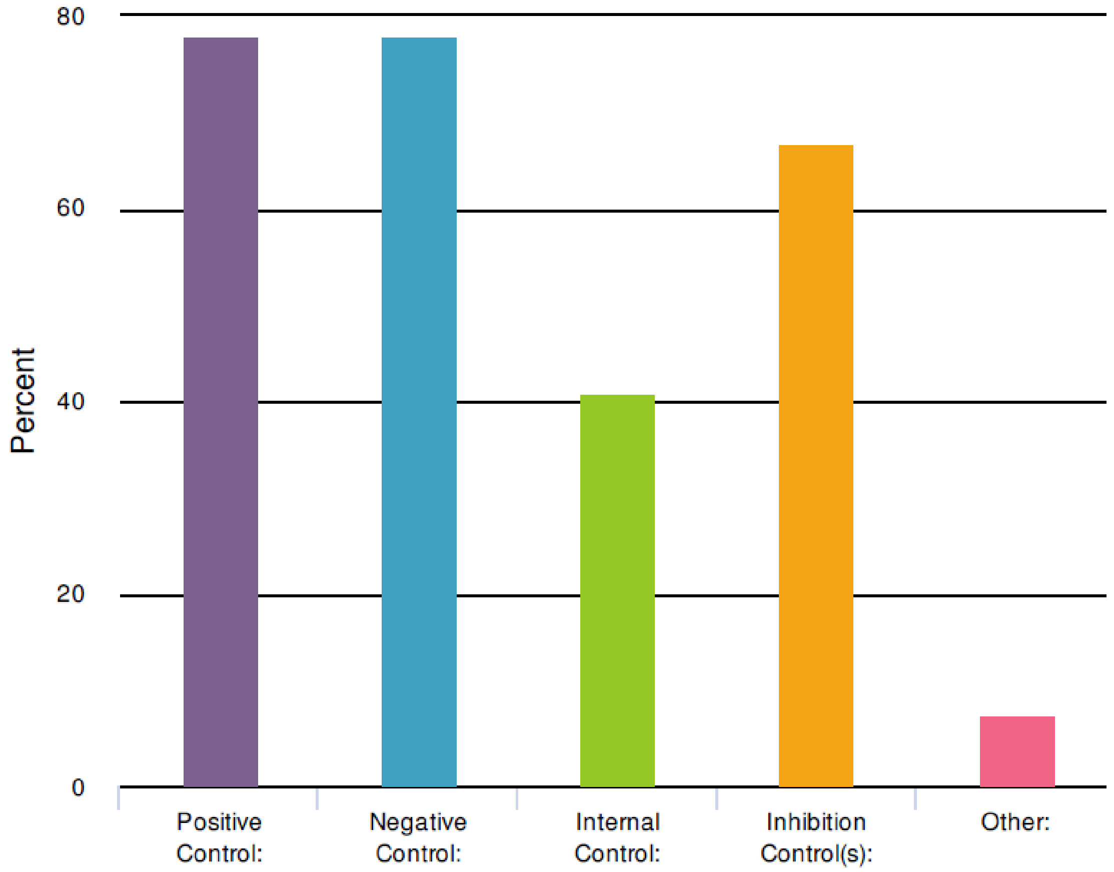
Other Potential Methods – Metagenomics

- **WG2 raised concerns over the sensitivity of metagenomic analyses to provide useful data at this time for SARS-CoV-2**
- Previous untargeted metagenomics studies have generally found bacteriophage populations to dominate the viruses detected
- However, previous metagenomics studies of the virome of wastewater samples have detected a wide range of human virus types, including human Coronaviruses
- A recent study reports greater viral diversity with deep amplicon sequencing relative to untargeted and even target enrichment sequencing

QA/QC Checklist

- **Minimally acceptable QA/QC standards** include:
  - Positive control
  - Negative control
  - Estimate of the limit of detection
  - Reporting of the equivalent volume of sample analyzed
- **Optimally, additional method validation controls would include**:
  - Inhibition control
  - Initial Precision Recovery controls
  - Ongoing Precision Recovery controls
  - Matrix spike
- **The primary source of error in qPCR occurs when the standard curve is generated. Each standard curve should be checked for validity**

Assay Controls



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Surrogate Organisms

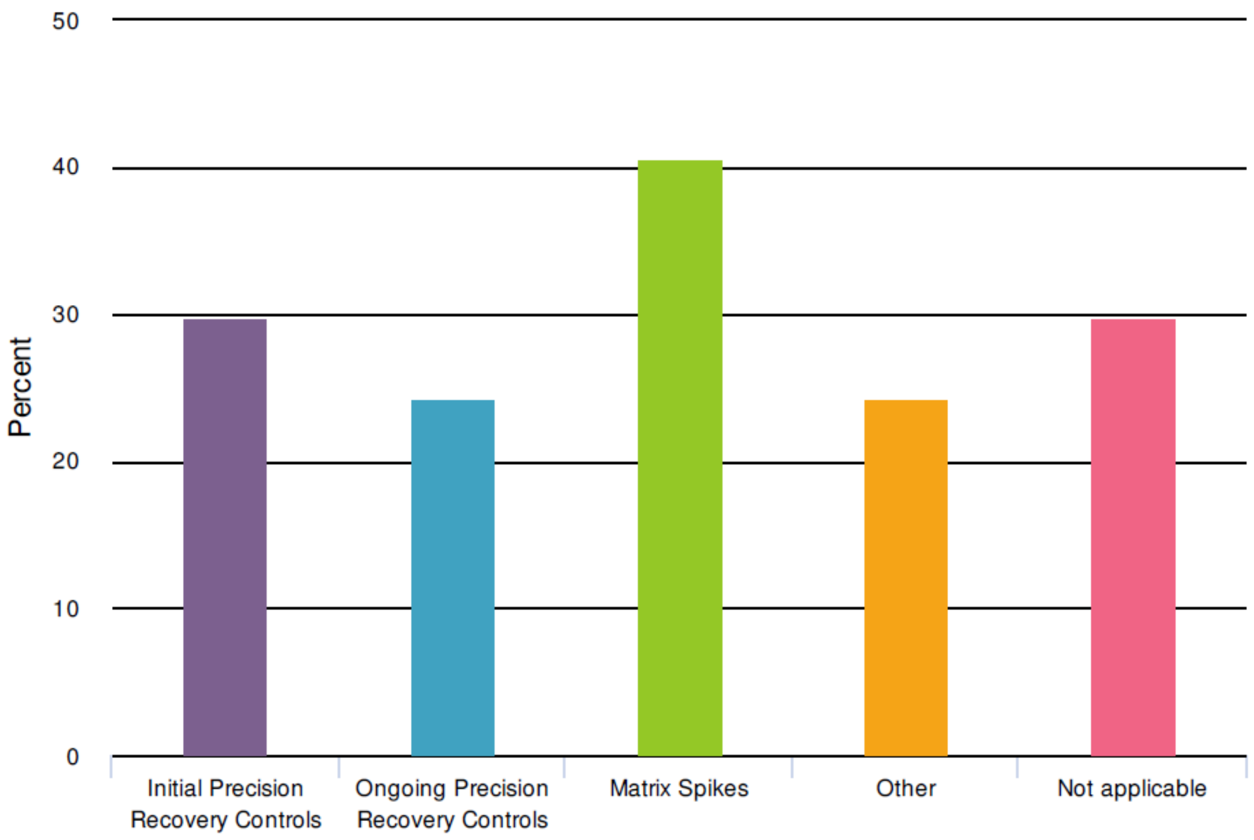
- Surrogate organisms can be used as internal controls to estimate recovery efficiency and as alternative for experimental evaluation of fate and persistence

- Preferred surrogates to represent SARS CoV-2:
  - A non-human infectious Coronavirus strain
  - An attenuated Coronavirus (e.g., Bovine Coronavirus)
  - An alternate enveloped virus (e.g., Pseudomonas φ6)
  - Armored RNA ($$$ and not an enveloped virus)
  - Another indicator virus that is easily culturable
  - Use of a “wild type” spike of native virus

- Survey results show a wide variety of control organisms are currently being used as process controls including:
  - F-specific phages
  - MS2 phage
  - Hepatitis G
  - MHV
  - Murine Norovirus
  - Pepper Mild Mottle Virus
  - Coliphages
  - Wastewater indicator virus
  - Coronavirus 229E
  - Attenuated SARS CoV-2

Method Validation Controls

The most common controls are **matrix spikes (40%)** and **initial precision recovery controls (30%)**



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Guiding Principles for Analysis

- Ultimately, methods need to achieve **reproducible, high quality and preferably quantitative information**
  - Evaluation and validation of methods is important
  - Controls need to be included in each step during initial validation, so that the impact on subsequent steps are understood
  - For routine evaluation, overall recovery controls can be used to streamline costs and efficiency
- QA/QC check-list is essential
- **Respect the matrix** - wastewater is a complex matrix (and quite different from clinical samples)
  - The limit of detection/quantification needs to be established for your assay and the sample matrix
- Metadata needs to be collected for each sample to ensure that the appropriate context can be given to subsequent interpretation of results
  - It is important to report on all of the factors in the study that impact the result (detection data need to be related to water quality and other meta data)
- **Without following these principles data will be of limited comparative value and conclusions drawn will be highly uncertain**

Krista Wigginton, PhD

Associate Professor
Department of Civil and Environmental Engineering
University of Michigan

Analyzing for Indicators of COVID-19

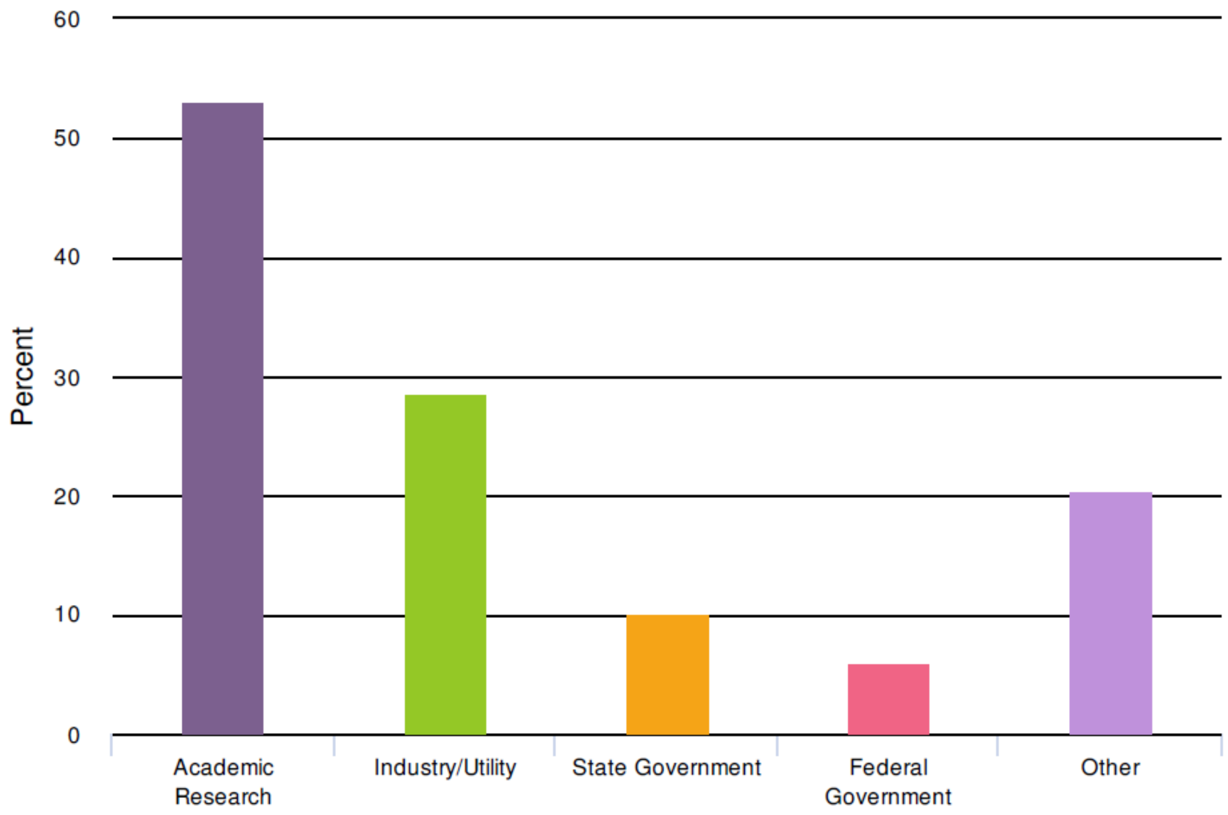
The Water sector is interested in supporting efforts to understand the spread and impact of COVID-19

Environmental surveillance of wastewater can potentially provide an additional source of information

The WRF survey indicated that 49 respondents are already collecting samples and monitoring for indicators of SARS CoV-2

Who is Analyzing for Indicators of COVID-19?

Active collaboration between organizations represents a unique opportunity to shape and refine methods for the environmental surveillance of wastewater as they develop.



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Research Needs – Gene Recovery From Wastewater

Best recovery methods for enveloped viruses: How well do PEG precipitation, ultrafiltration, charged membranes, skim milk flocculation methods recover SARS-CoV-2 genomes?

Which recovery method for which detection: Do we need different recovery methods for qPCR, culturing, metagenomics?

Enveloped vs. non-enveloped viruses: How do recovery methods developed for nonenveloped viruses work for enveloped viruses?

Recovery consistency: Once a method is selected, how consistent is recovery over time?

What concentration factor? Should we concentrate 10x, 100x, 1000x?

Research Needs – Gene Detection and Quantification

Persistence of genetic signal (RNA) in wastewater over time at ambient temps and through storage, shipment

Wastewater matrix effects: Is genetic signal impacted by pH, ammonia, TSS, presence of chemicals/industrial discharge, dilution, salt water ingress, etc.?

Meta data needs: What meta data are required to make interpretation of genetic signal info useful to answer specific questions?

PCR primers: How do primer sets compare in their detection of SARS-CoV-2?

ddPCR vs. qPCR

qPCR standards: How do different standards for qPCR compare? Which result in more accurate results?

Inhibition control: What are the best practices for PCR inhibition control with specific concentration/detection approaches?

Research Needs – Comparison of Platforms

Desktop Review: Conduct systematic literature review and evaluate current methods, experimental comparisons, and pros/cons

MIQE Guidelines: Development of MIQE (minimum information for publication of quantitative real-time experiments) guidelines for environmental surveillance

Inter-lab Comparisons: Round robin testing of methods and analyses, and inter-lab comparisons required for independent validation

Research Needs: Infectious Virus vs. Genetic Signal Strength

Infectious SARS-CoV-2 viruses present in wastewater? Wait for medical research on feces

Gene copy: infective virus ratio: what's the relationship between genetic signal strength (copy number) and the presence of infectious viable virus

Persistence of the viable virus in wastewater and solids over time at ambient temperatures

Major biosafety concerns for culturing viable viruses

Reducing risks: If present, can pasteurizing methods be validated that always maintain genome copies while minimizing risk?

Coordination of Efforts is Essential

- **With medical researchers:**
  - Detection and quantification of infective virus particles in feces
  - Vaccine trials are underway, potentially in small, contained communities. Possibly conduct environmental surveillance research in these studies? (medical researchers)
- **With BSL3 labs:** Coordinate with labs capable of handling environmental samples that could contain higher level risk microbes (e.g., BSL3, PC3)
- **With epidemiologists:** To turn monitoring data into meaningful information on circulation and combine with other methods they currently use for tracking infections
- **With public health departments and public officials:**
  - How might the data and information be used and how does that influence how data should be collected?
- **With each other:**
  - How to collect data so it is comparable?
  - Coordinate/knowledge sharing: preprints, meetings, wait for peer-reviewed research?

Implementation Needs

- **For researchers new to environmental monitoring:** How to provide guidance and/or training to ensure methods and analyses performed to an acceptable standard?
- **Guidance documents for best practice**
  - Sample design
  - Sample collection and storage
  - Sample analysis
  - Data interpretation

- **Video presentations on sample analysis techniques including**
  - Sample collection
  - Concentration
  - Genetic extraction
  - Molecular assays
- **Regional centers of excellence** to mentor local analytical laboratories

Summary

- Environmental surveillance of wastewater has the potential to complement health information in supporting responses to COVID-19
- Researchers, utilities, health labs, and public officials will need to collaborate
- **We invite researchers to share your findings and thoughts with us. We will incorporate what we know so far into methods recommendations for the WRF Summit closing on Thursday**

Doug Yoder, PhD

Deputy Director, Water and Sewer

Miami-Dade County

WORKING GROUP 3

GOAL

To develop recommended approaches for use of indicator concentrations to inform trends and estimates of community prevalence

Working Group 3 - Interpret Results

Co-Chairs

Chuck Haas, Drexel University

Doug Yoder, Miami-Dade Water and Sewer

Gertjan Medema, KWR

Vanessa Speight, University of Sheffield

Participants

Mia Mattioli, Centers for Disease Control and Prevention (CDC)

Jay Garland, Environmental Protection Agency (EPA)

Jeff Soller, Soller Environmental, LLC

John Norton, Great Lakes Water Authority

Jeff Prevatt, Pima County Regional Wastewater Reclamation Dept.

Dimitri Katehis, New York City Dept. of Environment Protection

Steve Rhode, Massachusetts Water Resources Authority

Ken Williamson, Clean Water Services

Paul Kadota, Metro Vancouver

Reynald Bonnard, SUEZ Environmental Research Center

Utilities and Sewershed Monitoring

- Utilities are critical public health institutions
- Utilities are mindful of public and employee safety
- Utilities routinely monitor for operations management and regulatory compliance
- Sewershed monitoring has the potential to provide important data for COVID-19 response in the short-term and other risk data in the future
- Miami-Dade is conducting sewershed and antibody monitoring now

Starting With Why: Miami-Dade, a Case Study

MIAMI-DADE
COUNTY

What Can You Use Sewershed Surveillance Data For?

General Use Cases	Can inform:
Assessment of Community Infection	Tracking disease prevalence in the community. Identification of “hot spots”
Trends/Changes in Infection	Early detection of disease. Tracking the impact of medical and social interventions
Risk Assessment	Risk to utility workers and those exposed to raw sewage
Viral Evolution	Source tracking of the virus

POLL: What is your highest priority use case for sewershed surveillance?

- Assessment of Community Infection
- Trends/Changes in Infection
- Risk Assessment
- Viral Evolution
- Other

Understanding the Potential of Sewershed Surveillance

- Value as an added data source to current surveillance
- Value as an integrated measure of community prevalence
- This area of research rapidly developing, and has the potential to inform our understanding at this early stage
- Data stemming from this type of surveillance can be matched with other clinical data sets for community assessments or decision making

Gertjan Medema, PhD

Principal Microbiologist
KWR Water Research Institute

Use Cases of Sewershed Surveillance for Other Viruses

Poliovirus

- absence of virus circulation in (unvaccinated) population
- early warning outbreaks

Adenovirus, norovirus, rotavirus, parechovirus, enterovirus, astroviruses, hepatitis A and E viruses

- early warning outbreaks
- virus circulation in population
- virus genotypes circulating in population

REVIEW ARTICLE

Role of environmental poliovirus surveillance in global polio eradication and beyond

T. HOVI[1]*, L. M. SHULMAN[2], H. VAN DER AVOORT[3], J. DESHPANDE[4], M. ROIVAINEN[1] AND E. M. DE GOURVILLE[5]

[1] *National Institute for Health and Welfare (THL), Helsinki, Finland*
[2] *Central Virology Laboratory (CVL), Ministry of Health, Sheba Medical Center, Tel-Hashomer, Israel*
[3] *National Institute of Public Health and the Environment (RIVM), Bilthoven, The Netherlands*
[4] *Enterovirus Research Centre (ERC), Mumbai, India*
[5] *Global Poliomyelitis Eradication Initiative, WHO, Geneva, Switzerland*

AEM
Journals.ASM.org

Detection of Pathogenic Viruses in Sewage Provided Early Warnings of Hepatitis A Virus and Norovirus Outbreaks

Maria Hellmér,[a] Nicklas Paxéus,[b] Lars Magnius,[c] Lucica Enache,[b] Birgitta Arnholm,[d] Annette Johansson,[b] Tomas Bergström,[a] Heléne Norder[a,c]

Department of Clinical Microbiology, Sahlgrenska Academy, Gothenburg University, Gothenburg, Sweden[a]; Gryaab AB, Gothenburg, Sweden[b]; MTC, Karolinska Institutet, Stockholm, Sweden[c]; Department of Communicable Disease Control, Västra Götaland Region, Sweden[d]

Eurosurveillance Europe's journal on infectious disease surveillance, epidemiol

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Research article Open Access

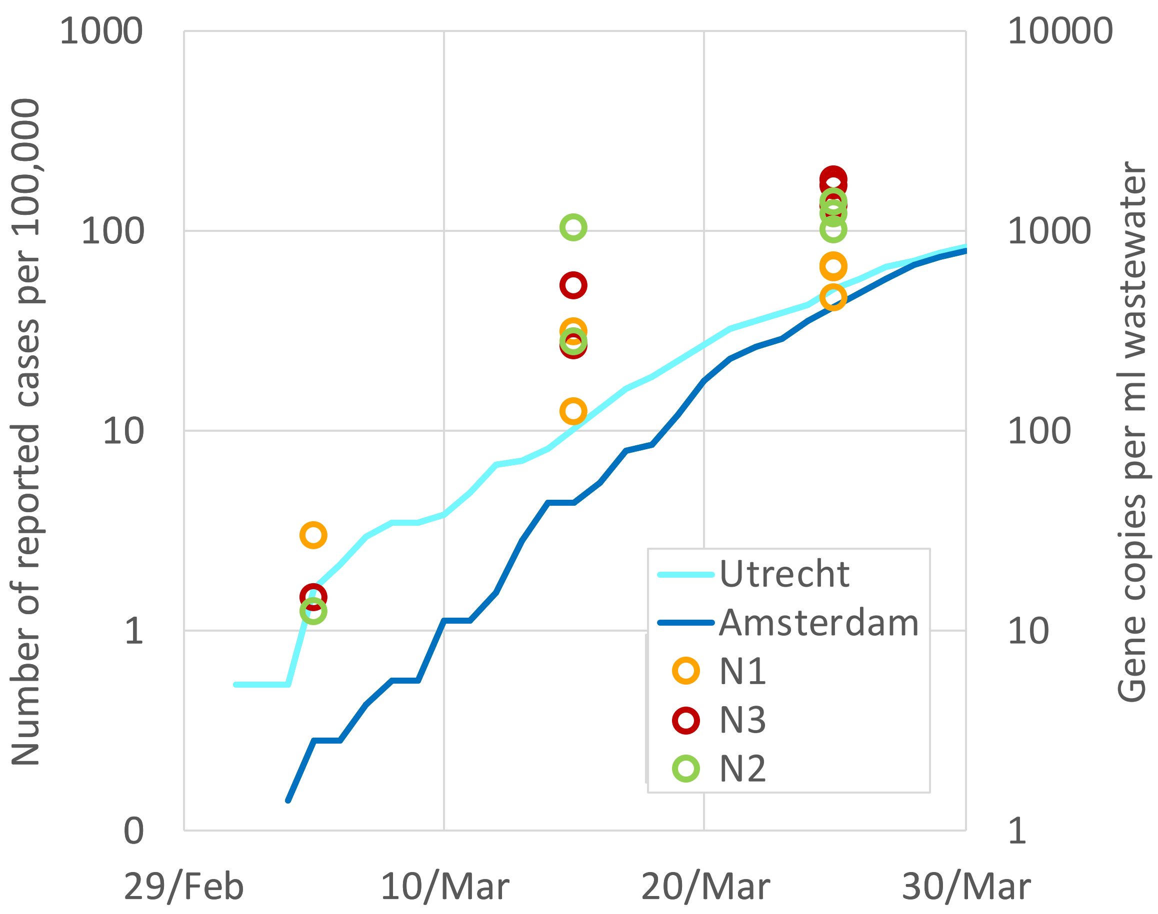
Monitoring human enteric viruses in wastewater and relevance to infections encountered in the clinical setting: a one-year experiment in central France, 2014 to 2015

Like 0 Download

Maxime Bisseux[1,2], Jonathan Colombet[1], Audrey Mirand[1,2], Anne-Marie Roque-Afonso[3], Florence Abravanel[4], Jacques Izopet[4], Christine Archimbaud[1,2], Hélène Peigue-Lafeuille[1,2], Didier Debroas[1], Jean-Luc Bailly[1], Cécile Henquell[1,2]

Sewage surveillance at WWTP in the Netherlands

- Clear increase in reported cases coincides with increase in concentration in wastewater
- Two other WWTP: virus detected in wastewater 6 days before first reported case
- Now national surveillance (RIVM)



Data Assessments

START WITH THE PURPOSE OF THE STUDY (USE CASES)

WHAT CAN WE CURRENTLY DO WITH THE DATA?

Data Assessments: What can we currently do with the data?

- Start with the purpose of the study (use cases)
- Virus circulation in communities?
  - Trends/changes: early warning virus circulation starts?
    - Yes: sensitive and fast enough?
  - Trends/changes: early warning virus circulation increases again?
    - Maybe: how far does RNA signal drop as prevalence in community drops?
  - Community prevalence?
    - Probably: information needed for accurate extrapolation from sewershed to community
  - Virus evolution?
    - Maybe: more informative than patient data? Other virus strains if no/mild symptoms?
- Risk?
  - Estimate health risk for workers?
    - Yes: main uncertainty is if there is infectious virus present in stool/sewage

Information and Data Needs

Virus circulation in communities

- Correlation with health surveillance datasets: virus/disease/antibody surveillance of the community
- Trends: What level of RNA signal rise or drop is informative?
- Quantitative assessment of virus in wastewater
  - Methods: recovery efficiency, limit of detection, quantification of the RNA signal, QA/QC (Working Group 2)
  - Sewershed info: proportion of human fecal waste, flows, residence times, demographics, etc.
- Shedding of virus
  - Concentration, duration, proportion of population, shedding by symptomatic and asymptomatic populations
  - Long shedding: How do you distinguish new cases of disease?
- Sequence of wastewater virus matches virus in community?

Risk

- Presence/concentration of infectious virus in stool/wastewater, relation with gene copies
- Outline risk associated with water droplets, aerosols, fomites, hand contact

Surveillance Coordination Required

- **With medical researchers**
- **With Biosafety Level 3 labs**
- **With epidemiologists, public health departments & public officials**
- **Within the research community**

International Water Research Summit

Jim McQuarrie

Director of Strategy and Innovation

Denver Metro Wastewater Reclamation District

WORKING GROUP 4

GOAL

To develop strategies to communicate the implications of environmental surveillance results with the public health community, elected officials, wastewater professionals, and the public

Working Group 4 - Communication

Co-Chairs

Jim McQuarrie, Denver Metro Wastewater Reclamation Dist.

Cathy Bailey, Greater Cincinnati Water Works

Dan Deere, Water Futures

Participants

Jeff Oxenford, Rural Community Assistance Partnership

Vince Hill, Centers for Disease Control and Prevention (CDC)

Claire Waggoner, CA State Water Resources Control Board

Karen Mogus, CA State Water Resources Control Board

Chris Impellitteri, Environmental Protection Agency (EPA)

Diane Taniguchi-Dennis, Clean Water Services

Gabriella Rundblad, King's College London

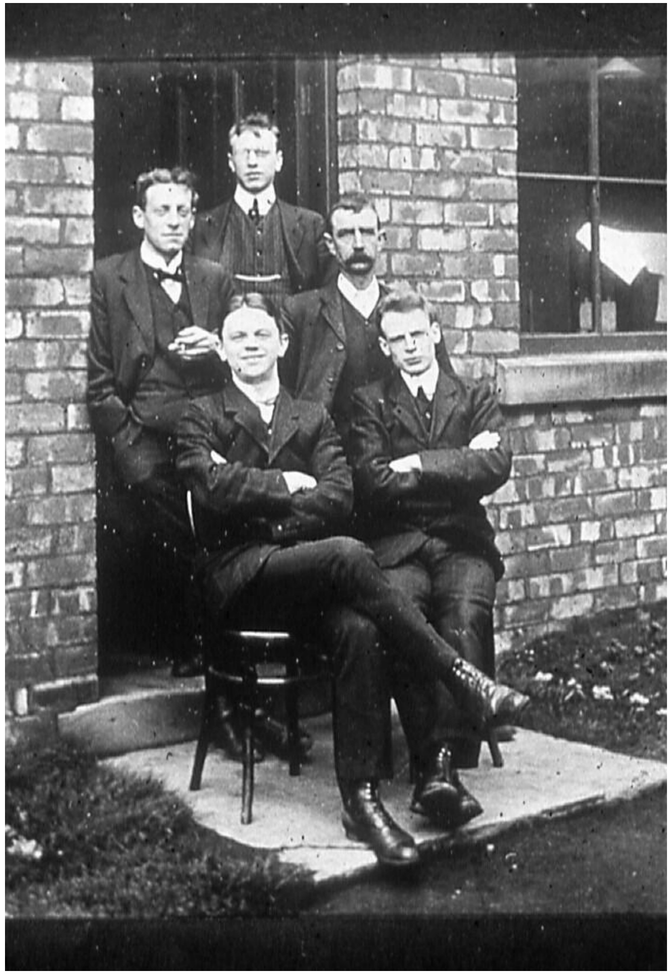
Josef Klinger, Technologiezentrum Wasser (TZW)

Bruno Tisserand, Veolia

Stephanie Rinck-Pfeiffer, Global Water Research Coalition

Yvonne Forrest, Houston Water

From Privy to Sewered Systems to Sewage Treatment



"1914 - E. Ardern & W. T. Lockett discovered the activated sludge process." Historia Sanitaria: Complete Guide Through Sanitation History. https://www.wiki.sanitarc.si/1914-w-t-lockett-discovered-activated-sludge-process/ (accessed April 27, 2020).

BMJ readers choose sanitation as greatest medical advance since 1840

Annabel Ferriman BMJ

More than 11 300 readers of the *BMJ* chose the introduction of clean water and sewage disposal—"the sanitary revolution"—as the most important medical milestone since 1840, when the *BMJ* was first published. Readers were given 10 days to vote on a shortlist of 15 milestones, and sanitation topped the poll, followed closely by the discovery of antibiotics and the development of anaesthesia.

The work of the 19th century lawyer Edwin Chadwick, who pioneered the introduction of piped water to people's homes and sewers rinsed by water, attracted 15.8% of the votes, while antibiotics took 15%, and anaesthesia took 14%. The next two most popular were the introduction of vaccines, with 12%, and the discovery of the structure of DNA (9%).

A total of 11 341 people voted on the shortlist, which was chosen by a panel of experts from a list nominated by readers. Almost a third of the voters were doctors, while a fifth were members of the general public, and one in seven were students. Another tenth were academic researchers. Almost two fifths of the voters were from the United Kingdom, and a fifth were from the United States.

Johan Mackenbach, professor of public health at Erasmus MC Medical Center, Rotterdam, who championed the cause of sanitation, said, "I'm delighted that sanitation is recognised by so many people as such an important milestone. The general lesson which still holds is that passive protection against health hazards is often the best way to improve population health.

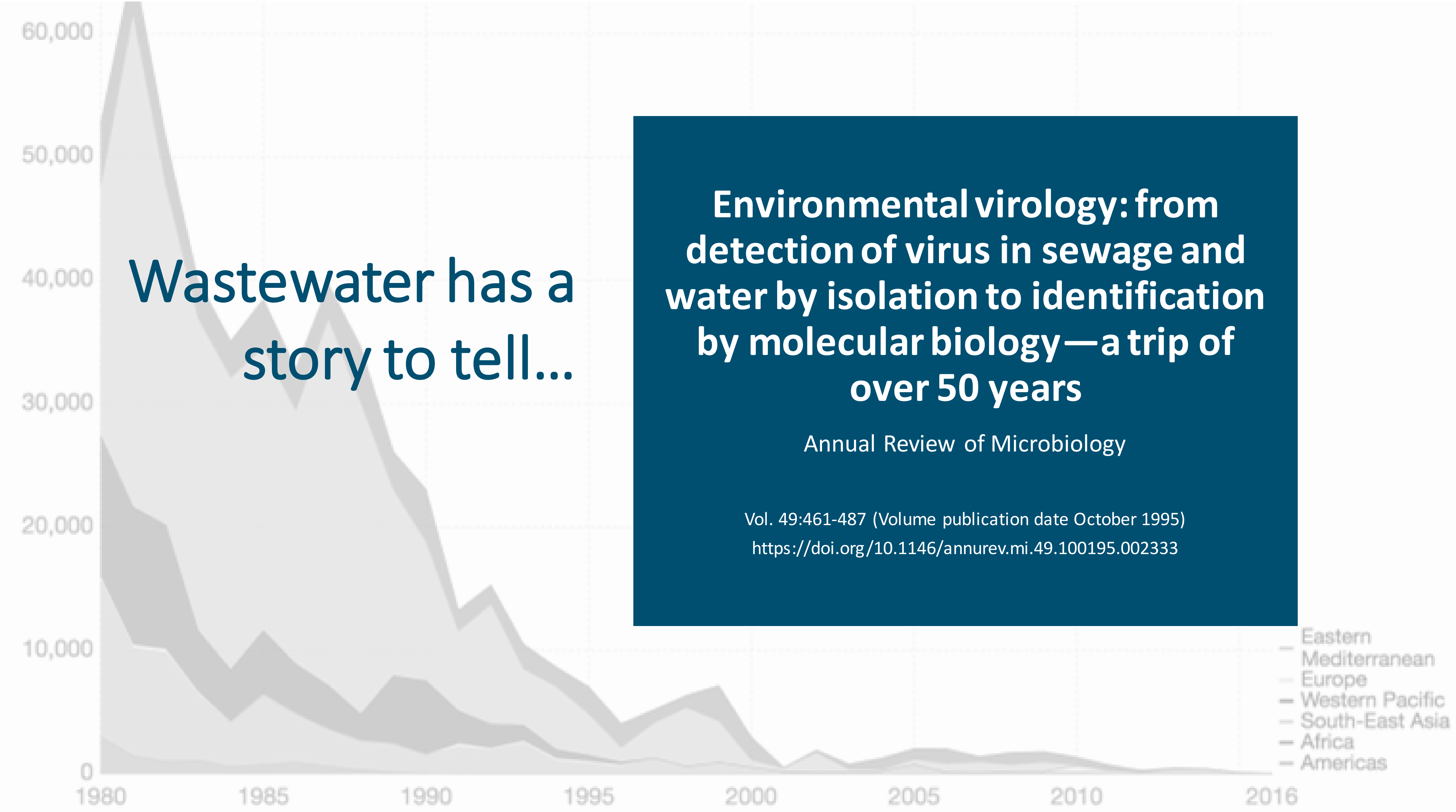
"The original champions of the sanitary revolution were John Snow, who showed that cholera was spread by water, and Edwin Chadwick, who came up with the idea of sewage disposal and piping water into homes.

"Inadequate sanitation is still a major problem in the developing world."

The *Medical Milestones* supplement is distributed with this week's *BMJ*.

BMJ | 20 JANUARY 2007 | VOLUME 334 111

Ferriman, A. "BMJ readers choose the 'sanitary revolution' as greatest medical advance since 1840." BMJ 2007, 334(7585): 111. Publication Date: January 20, 2007. doi: 10.1136/bmj.39097.611806.DB.



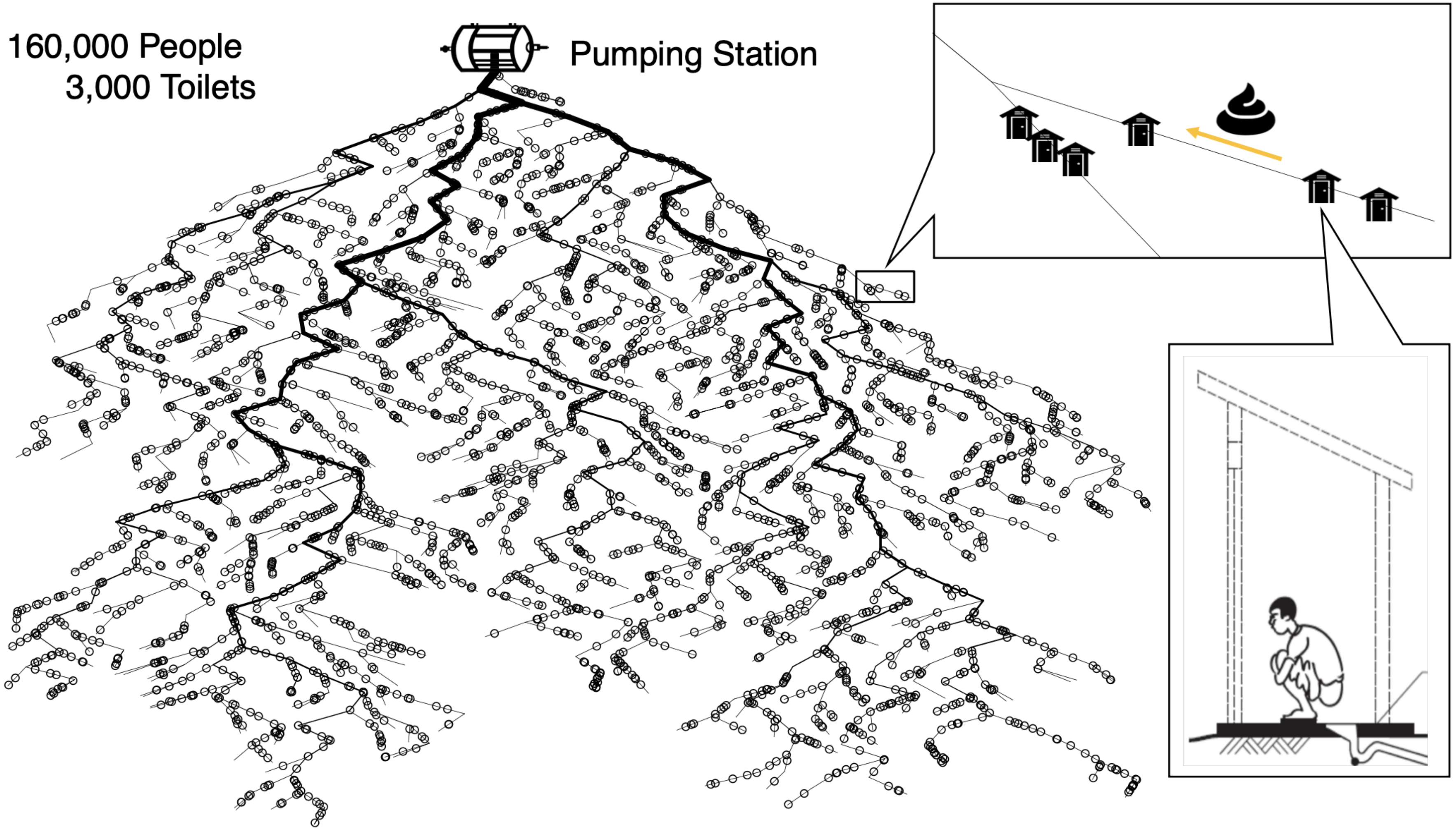
Wastewater has a story to tell…

Environmental virology: from detection of virus in sewage and water by isolation to identification by molecular biology—a trip of over 50 years

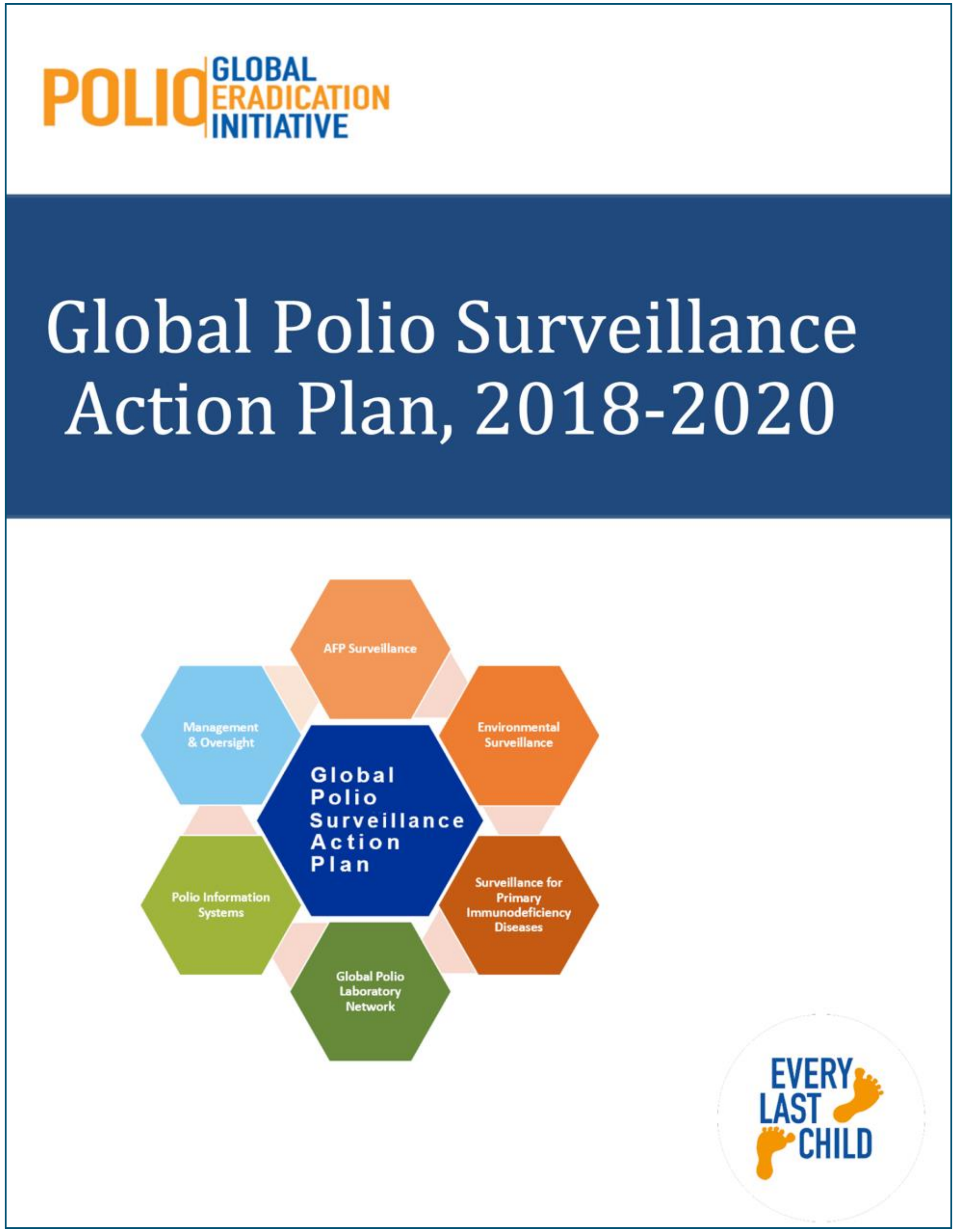
Annual Review of Microbiology

Vol. 49:461-487 (Volume publication date October 1995)
https://doi.org/10.1146/annurev.mi.49.100195.002333

Informing Typhoid Detection and Control

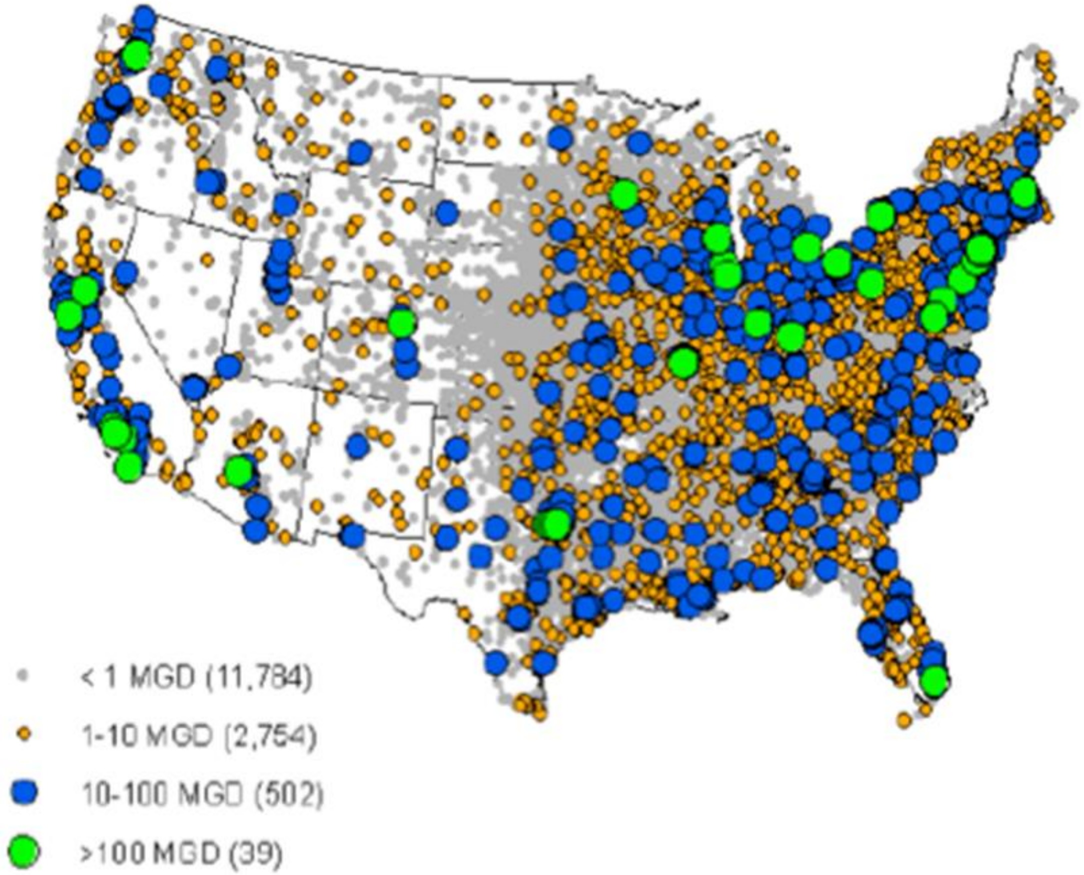


Wang, Y., C. L. Moe, S. Dutta, A. Wadhwa, S. Kanungo, W. Mairinger, Y. Zhao, Y. Jiang, P. F. M. Teunis. "Designing a typhoid environmental surveillance study: a simulation model for optimum sampling site allocation." Epidemics. 100391. 10.1016/j.epidem.2020.100391. (in press). Licensed under CC BY 4.0. https://creativecommons.org/licenses/by/4.0/



Global Polio Surveillance Action Plan, 2018-2020.Geneva: WHO; 2019 (WHO/POLIO/19.10) Licence: CC BY-NC-SA 3.0 IGO Downloaded by Dan Deere, 26April.

From Sewage Treatment to Wastewater Reclamation to Water Resource Recovery Facilities



Infrastructure – 20th century investments set stage for 21st century intelligent cities

Scale – Model concepts for durable and sustainable ES

Stakeholders – Communication key to effective uptake/understanding of ES

Data from Clean Water Needs Survey Database, U.S. Environmental Protection Agency. "Summary of POTW size distribution and U.S. population served (Seiple et al.).

Crisis or Opportunity Communication

- Wastewater was computationally examined as a matric for detection of SARS-CoV-2
- One infected individual theoretically is detectable among 100 to 2,000,000 persons
- Temperature and in-sewer travel time severely impact virus detectability
- 2.1 billion people could be monitored globally in 105,600 sewage treatment plants
- Combined use of WBE followed by clinical testing could save billions of US dollars

ELSEVIER

Science of The Total Environment

Available online 22 April 2020, 138875

In Press, Journal Pre-proof

Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges

Olga E. Hart [a], Rolf U. Halden [a, b, c]

Show more

https://doi.org/10.1016/j.scitotenv.2020.138875

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Hart, O. E., and R. U. Halden. "Computational analysis of SARS-CoV-2/COVID-19 surveillance by wastewater-based epidemiology locally and globally: Feasibility, economy, opportunities and challenges." [published online ahead of print, 2020 Apr 22]. Science of the Total Environment 2020;138875. doi:10.1016/j.scitotenv.2020.138875. Licensed under CC BY NC ND. https://creativecommons.org/licenses/by-nc-nd/4.0/

Dan Deere, PhD

Water Quality Specialist
Water Futures Australia

Communication Deliverables

What needs communicating?

Who are we communicating with?

How are we communicating?

When do we communicate?

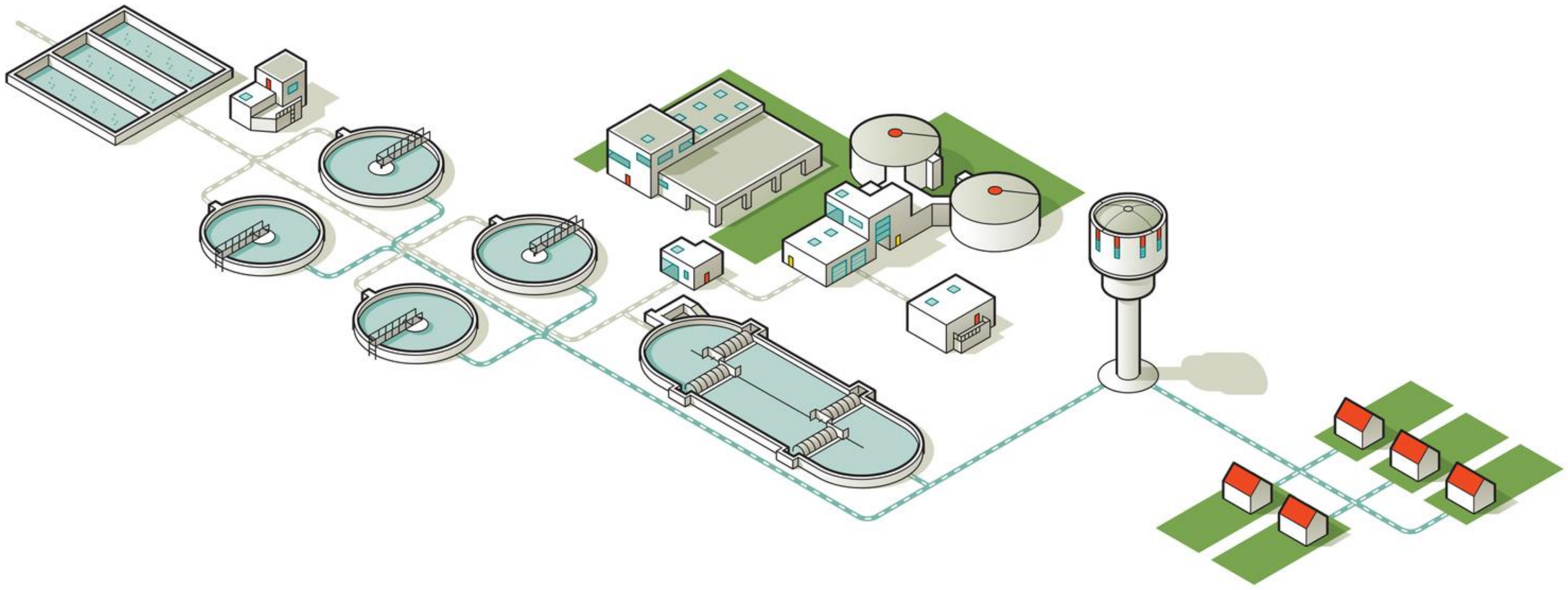
Why do we communicate?

Examples of Upfront and Ongoing Communication

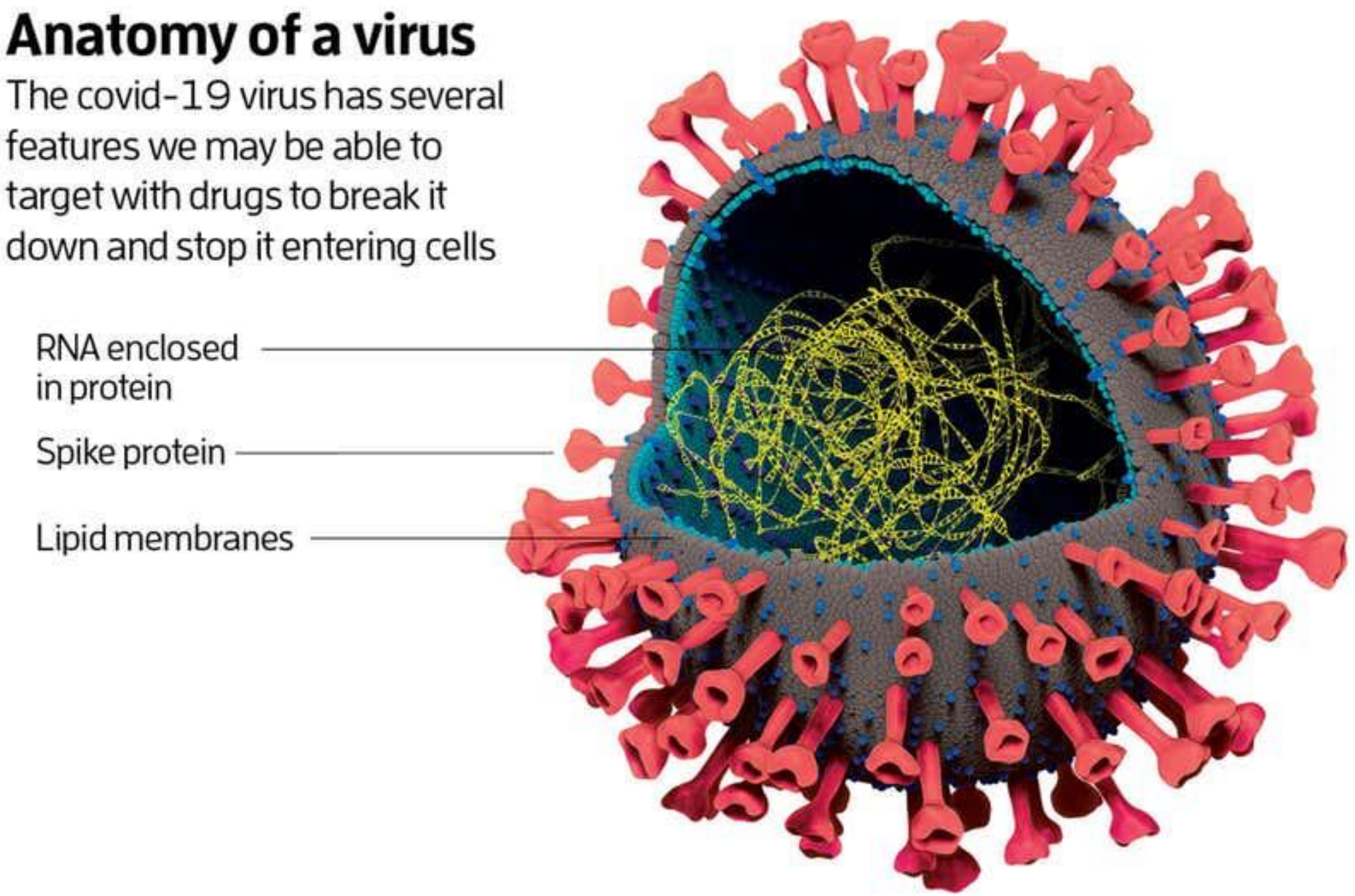
Who	What	How	Why
Public **health** community	**Their needs:** understand how ES can inform them	Review decision-making tools to see where ES fits by ask for feedback	Set **priorities** for design and methods
	Our capability: viral RNA detection and levels of confidence	Clearly document the results and levels of confidence	Ensure decision-makers know what to **expect** and how to **interpret** results
Elected **officials**	**Benefits**, **costs** and **limitations** of the work	Provide plain language summaries via **trusted sources**	Help inform decisions on **funding** ES work and public **messaging**
Wastewater **professionals**	**Their concerns:** fear of the virus in sewage	Direct liaison with workers	To **understand** concerns
	Our evidence: higher risks set standards for safety	Clear advice from **trusted sources**	To **allay** any undue concerns
Public	**Their concerns:** fear being tested and of what's found	Social media and call centre contacts; focus groups	To **understand** concerns
	Our evidence: we're not spying; tight regulation and higher risks set standards for controls	Clear advice from trusted sources	To **allay** any undue concerns

Using Graphics to Illustrate Concept

- Source and gain permission to use existing graphics
- Generate new graphics from this Summit



Example of Genes, RNA, and Remnants of Inactive Virus



Source: Tim Vernon / Science Photo Library.

Glossary of Terms and Alternatives

Jargon	Audience	Alternative
RNA	Public	The genetic code, or genes, carried inside the virus
Sewage	Public	Waste that goes down sinks, toilets, showers, baths and floor drains
Sewershed	Public	The network of sewer pipes that carry water from the buildings that drain to them to the sewage treatment and recycling facility Also: sewer catchment
Disinfection	Public	Inactivates, or 'kills', the virus so it can't infect
Infectious	Public	The virus is active, or 'alive', and in a state where it is able to infect
Non-detect	Epidemiologist	The concentration is less than our limit of detection in the sample collected, adjusted for the method recovery efficiency, and within the limits of the specificity and sensitivity of the method, and reported with a measurement uncertainty, and noting additional uncertainty given the sample number(s) collected
Composite sample	Epidemiologist	A sample collected over a period of time, typically 22 to 24 hours, representing an average for that period for the whole sewershed

Removing Jargon Barriers

Frequently Asked Questions

- If the environmental surveillance (ES) results are negative, is my town free of COVID-19 virus and can we open everything back up?
- If the ES results are positive should I be afraid?
- When will these ES methods be working?
- Can I see the ES results for my community?
- There is sewage in my watershed – Is my water safe ?
- I am a surfer near a sewer discharge – Am I safe?
- I am a plumber and you're finding this virus – Am I safe?

Examples of FAQs and Possible Answers

FAQ	Possible Answer
There is sewage in the water source and in sewers near the water pipes. Is the water safe?	Drinking water is safe since the source water protection, treatment and distribution system protection barriers and sanitary working procedures, as overseen by regulation, protects drinking water from viruses, including the COVID-19 virus. The COVID-19 virus doesn't necessitate a change in these procedures.
I've heard we have environmental surveillance (ES) going on in my town. Can I see the ES results for my town?	The ES happening now is at the methods development stage as we only now learning how to test reliably for this new virus. The ES results will be used as one extra piece of information to help guide decisions on the COVID-19 response. We will provide public advice and feedback on ES results as part of the COVID-19 response advisories.
Are there privacy concerns with ES?	ES typically only tells us something about the presence of COVID-19 in whole towns or suburbs.

Trusted Sources That Provide Useful Resources

- Health agencies
- Wastewater utilities
- Academic institutions
- Trusted journalists and media organizations

Examples of Trusted Sources

What CDC Is Doing to Eradicate Polio

See also the Updates on CDC's Polio Eradication Efforts.

CDC is a strategic partner in the overall effort to eradicate polio worldwide. CDC provides scientific expertise to many polio eradication programs and activities:

- Global Immunization Division (GID) staff members work jointly with WHO and national Ministries of Health to plan and monitor polio surveillance and immunization activities in multiple countries worldwide.
- GID also supports other eradication projects such as conducting epidemiologic and vaccine efficacy studies and performing operational research for supplemental immunization activities.
- The Polio and Picornavirus Laboratory in CDC's Division of Viral Diseases serves as a WHO Global Specialized Laboratory and provides technical and programmatic assistance to the global polio laboratory network overall. CDC's labs provide critical diagnostic services and genomic sequencing of polioviruses to help guide disease control efforts in many countries. In 2014, this included support for implementation of improved laboratory procedures that have increased sensitivity to detect and confirm new polio infection. Other new laboratory procedures are helping countries overcome specific operational challenges, enable more rapid detection of wild poliovirus (WPV), and allow for faster response to importations or spread of virus. Additional efforts include technical assistance to laboratories implementing environmental surveillance for polio detection.
- **Stop Transmission of Polio (STOP)** team members currently participate in 11 month assignments in 42 countries, providing support at the national and sub-national levels. In 2018 alone, the STOP program in collaboration with WHO and UNICEF has deployed 314 public health professionals to improve surveillance for acute flaccid paralysis (AFP, an early sign of possible polio), support planning and implementing polio SIAs, responding to polio outbreaks, and enhancing polio surveillance.
- Country support through deployment of personnel and other resources.

Page last reviewed: January 31, 2013
Content source: Global Health

Polio - Annual Epidemiological Report 2016 [2014 data]

Surveillance report

30 Dec 2016

Publication series: Annual Epidemiological Report on Communicable Diseases in Europe

Time period covered: Reporting on 2014 data retrieved from TESSy* in 18 December 2015

Cite:

Europe has remained polio-free since 2002. The latest assessment by the European RCC of Poliomyelitis Eradication concludes that there was no wild poliovirus or vaccine-derived poliovirus transmission in the WHO European Region in 2014, but the risk of importation and subsequent transmission remains high in some countries.

Download

- Poliomyelitis - Annual Epidemiological Report for 2014 - EN - [PDF-137.21 KB]
- Poliomyelitis - surveillance systems overview, 2014 - EN - [XLSX-24 KB]

Key facts

- The WHO European Region was declared polio-free in 2002. There was neither wild-type nor vaccine-type transmission in the WHO European Region in 2014, but the risk of importation and subsequent transmission remain high in some countries.
- The most recent polio outbreaks in what today constitutes the EU/EEA were in 2001 (three polio cases among Roma children in Bulgaria [1]) and 1992 (outbreak in the Netherlands in a religious community opposed to vaccination [2]).
- Inactivated poliovirus vaccines are used in all EU/EEA countries, except Poland where live oral poliovirus vaccine (OPV) is still used for the fourth dose. Wild-type polioviruses can cause natural disease, while live attenuated polio vaccine viruses may cause vaccine-associated polio paralysis (VAPP), although the risk is very low.
- In 2014, poliomyelitis remained endemic in three countries – Nigeria, Afghanistan and Pakistan [3].
- Imported wild-type and vaccine-type polioviruses still remain a threat to unvaccinated people in the EU/EEA. Maintaining high coverage in all population groups and continued clinical and/or environmental surveillance remain the most important tools for keeping Europe polio-free.

US CDC, 2013, https://www.cdc.gov/polio/what/index.htm, downloaded by Dan Deere 25 April 2020.

ECDPC, 2016, https://www.ecdc.europa.eu/en/publications-data/polio-annual-epidemiological-report-2016-2014-data, downloaded by Dan Deere 25 April 2020.

What Are Your Communication Needs? Send Us Your Questions and Comments!

Who do we need to:

- Hear from?
- Communicate with?

What resources can WRF provide that would help you?

- Graphics, tables and explanatory texts
- Frequently asked questions with model answers
- Jargon and terminology
  - Explanations
  - Alternative terms
- References to trusted and credible resources

POLL: At this point in time, what communication tools do *you* find most useful?

- Scientific papers and research reports
- Technical fact sheets
- Graphics and illustrations
- Frequently Asked Questions
- Interactive web-based information resources
- Other

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International Water Research Summit

Environmental Surveillance of COVID-19 Indicators in Sewersheds

Thank You!