A NIST-Hosted Virtual Workshop on Measuring SARS-CoV-2 in Wastewater and Fecal Material: A Call for Standards

Final Report

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September 2020

U.S. Department of Commerce
Wilbur L. Ross, Jr., Secretary

National Institute of Standards and Technology
Walter Copan, NIST Director and Undersecretary of Commerce for Standards and Technology

The NIST SARS-CoV-2 in Wastewater and Fecal Material Workshop planning committee members included Sandra M. Da Silva, Scott A. Jackson, Christina M. Jones, Nancy J. Lin, Katrice A. Lippa, Paulina K. Piotrowski, and Stephanie L. Servetas of the NIST Material Measurement Laboratory. This report was prepared by the workshop planning committee in conjunction with the presenters of the workshop. The statements recorded here are those of the individual workshop participants and do not necessarily represent the views of all participants, their organizations, the planning committee, or NIST.

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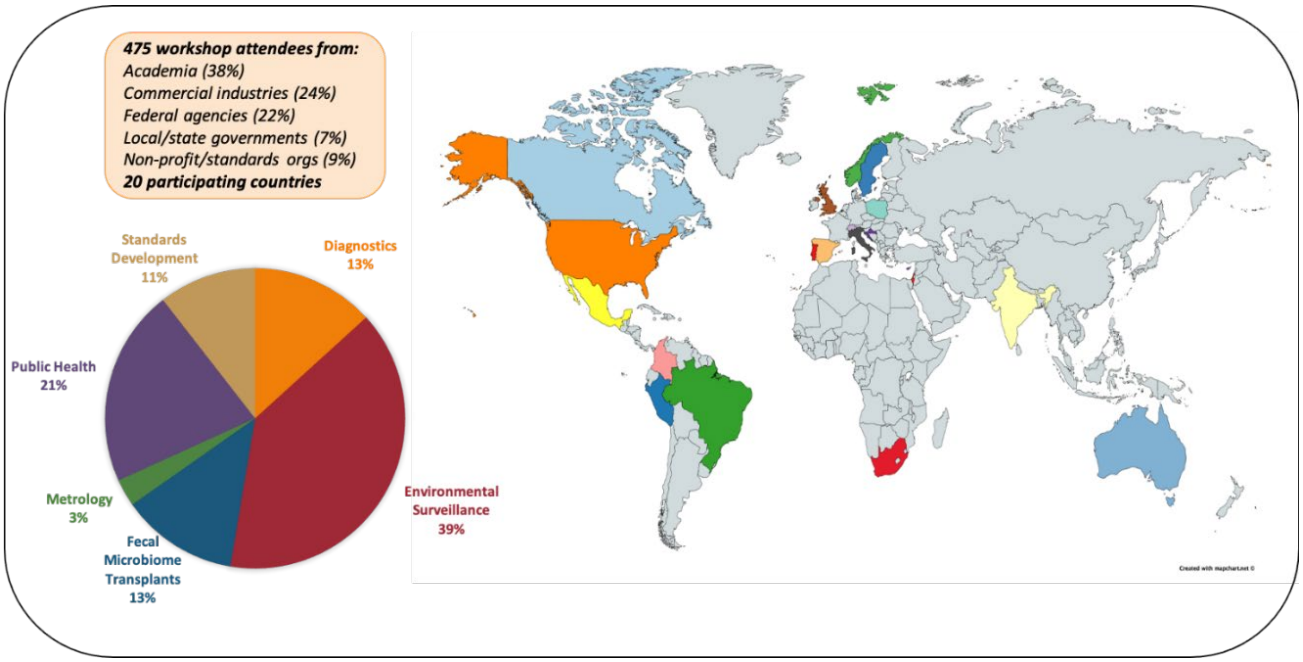
ABSTRACT

Since the coronavirus, now known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first reported in December 2019 to the World Health Organization (WHO), the virus has spread to nearly every continent becoming a serious global health threat. SARS-CoV-2 is a novel coronavirus and has been identified as the causative agent of COVID-19. Scientists throughout the globe have been working tirelessly to find a vaccine but there are still many questions regarding surveillance, detection, viral mutation rate, immunity, and vaccine effectiveness. According to the Centers for Disease Control and Prevention (CDC), in the USA alone over 200,000 lives have been lost to COVID-19 during the ongoing pandemic (CDC.gov). In support of the growing interest in surveillance of SARS-CoV-2 in wastewater and fecal samples, The National Institute of Standards and Technology (NIST) has leveraged its expertise in chemical and biological metrology and standards development as well as its strong relationships with stakeholders to host a virtual workshop on June 16, 2020 that explored the challenges associated with the measurement of SARS-CoV-2 in human stool and wastewater. The workshop included a diverse range of speakers who are international experts in fecal microbiota transplantation, infectious disease diagnostics, and wastewater surveillance, together with a general attendance of over 450 U.S. and international participants from 20 countries. Attendees represented academia; commercial industry; local, state and federal government; and non-profit and standards organizations. The main output of the workshop was a summary of the current needs and possible standards and measurement science solutions for detecting SARS-CoV-2 in the areas of fecal microbiota transplantation, infectious disease diagnostics, and wastewater surveillance. This report includes documentation of key insights from workshop presentations, stakeholder needs assessment based on the panel question and answer session and discussion as well as in-workshop and post-workshop participant polls, and a proposed plan for NIST to develop community-relevant standards. As was requested by the participants, NIST is planning to host a follow-up webinar in late 2020.



Keywords: SARS-CoV-2; COVID-19; coronavirus; wastewater surveillance; stool shedding; fecal microbiota transplant; gut microbiome; clinical diagnostics; standards; reference materials; wastewater-based epidemiology.

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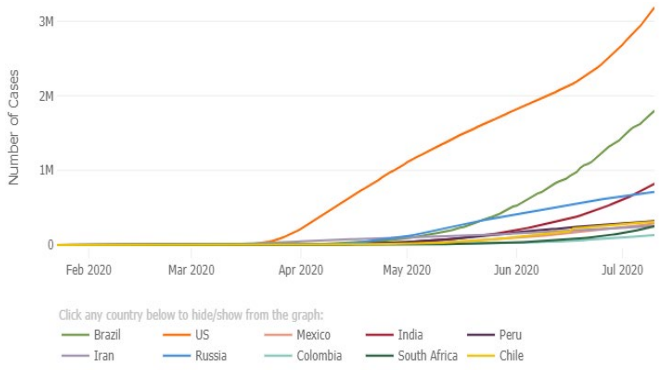
LIST OF ACRONYMS

BCoV	Bovine Coronavirus
CDC	Centers for Disease Control and Prevention
cDNA	Complementary DNA
COVID-19	Coronavirus disease 2019
ESV	Equivalent sample volume
EUA	Emergency Use Authorization
FDA	U.S. Food and Drug Administration
FMT	Fecal Microbiota Transplant
GI	Gastrointestinal
HFUF	Hollow fiber ultrafiltration
IDT	Integrated DNA Technologies
ILS	Interlaboratory study
LOD	Limit of detection
LOQ	Limit of quantification
MERS-CoV	Middle East respiratory syndrome coronavirus
NGS	Next-generation sequencing
NMI	National Metrology Institute
NIST	National Institute of Standards and Technology
PCR	Polymerase chain reaction
QA	Quality Assurance
QAP	Quality Assurance Program
QC	Quality Control
qPCR	Quantitative polymerase chain reaction
RM	Reference Material
RNA	Ribonucleic acid
RT-PCR	Reverse transcription-polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SNWA	Southern Nevada Water Authority
WBE	Wastewater-based epidemiology
WWTP	Wastewater treatment plant

INTRODUCTION

The ongoing coronavirus disease 2019 (COVID-19) pandemic is caused by a novel coronavirus (severe acute respiratory syndrome coronavirus 2, SARS-CoV-2) that results in severe respiratory infection. Recent reports have described the presence of SARS-CoV-2 ribonucleic acid (RNA) in feces of symptomatic and asymptomatic patients suggesting that gastrointestinal (GI) and fecal-oral transmission might be another potential route for the spread of the virus.[1] Understanding the clinical significance of this finding is part of ongoing global research efforts, resulting in a pressing need to detect SARS-CoV-2 in feces and wastewater.



Confirmed COVID-19 cases (millions) per country to date.

Source: Johns Hopkins (accessed on 7/11/2020)

Cautionary measures in clinical practice have been implemented to protect the safety of patients who may be exposed to fecal materials that may contain SARS-CoV-2. For instance, U.S. Food and Drug Administration (FDA) has released guidelines for SARS-CoV-2 including testing the stool donation or stool donor for the virus or RNA prior to fecal microbiota transplants (FMTs), a microbial therapy for treatment of *Clostridioides difficile* infections and other related GI diseases.[2] Moreover, currently there are over 140 commercially available COVID-19 diagnostic tests[3]. While initially validated using nasal swabs, these same tests have been considered for diagnostic testing in feces, which would likely require additional validation of the testing methods.

Shedding of SARS-CoV-2 in stool also leads to the presence of the virus in the sewage and wastewater in various treatment facilities across the US and internationally[4]. The ability to detect SARS-CoV-2 in wastewater influent can assist public health authorities in tracking the rise and fall of COVID-19 in a community and potentially serve as a leading indicator to predict waves of future outbreaks. Wastewater-based epidemiology (WBE) for SARS-CoV-2 is still in the early stages but has the potential to be employed as a surveillance tool for risk management[5]. Despite the potential for gauging hotspots, critical challenges to

[1] Holshue, M. L., Holshue, M. P. H., DeBolt, C. *et al*. First Case of 2019 Novel Coronavirus in the United States. *N. Engl. J.*, 383, 929-36, 2020.

[2] Information Pertaining to Additional Safety Protections Regarding Use of Fecal Microbiota for Transplantation - Screening Donors for COVID-19 and Exposure to SARS-CoV-2 and Testing for SARS-CoV-2. Issued April 9, 2020. (accessed 06/30/2020)

[3] https://www.360dx.com/coronavirus-test-tracker-launched-covid-19-tests (accessed on 09/05/2020)

[4] Ahmed, W., Angel, N., Edson, J. *et al*. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community. *Science Total Environ*., 728, 138764, 2020. https://doi.org/10.1016/j.scitotenv.2020.138764

[5] Wastewater Surveillance of the COVID-19 Genetic Signal in Sewersheds - Recommendations from Global Experts. The Water Research Foundation, April 27-30, 2020. Summit Report (accessed on 7/7/2020).

improve confidence in the detection, quantitation, and determination of viral loads in wastewater must be addressed. For example, an improved understanding of virus shedding across the population and the fate of the virus and/or its RNA genetic material in the sewage environment is necessary to develop robust WBE models that can be used to assess COVID-19 in a given population.

The National Institute of Standards and Technology (NIST) organized a virtual workshop to gather stakeholders in the fields of gut microbiome therapeutics, clinical diagnostics and wastewater surveillance to gain perspective on the predominate challenges in the measurement of SARS-CoV-2 in human stool and wastewater. The goal of the workshop was to discuss the feasibility of developing standardized testing protocols and reference materials (RMs) to address existing needs in detecting SARS-CoV-2 in wastewater and fecal materials, and whether NIST may be able to support these efforts. This report summarizes measurement challenges expressed by attendees and invited speakers and highlights the current needs for standardization and metrology tools that may improve confidence in both diagnostic measurements for fecal materials and wastewater surveillance applications. The workshop recording is available online[6].

[6] https://www.nist.gov/news-events/events/2020/06/nist-hosted-webinar-measuring-sars-cov-2-wastewater-and-fecal-material

PRESENTATIONS

Welcome and Opening

Scott Jackson, NIST

Wastewater biosurveillance has had an international response in attempting to survey SARS-CoV-2 since the first report came out from Netherlands[7] suggesting the presence of the virus in sewage and triggering its potential use to monitor outbreaks. In addition, the presence of SARS-CoV-2 in stool has raised a safety issue for FMTs. In response, FDA has issued a safety alert describing additional safety precautions for screening donated FMT materials for SARS-CoV-2. The FDA also noted that there is limited information on the availability and performance of direct testing of SARS-CoV-2 in stool. As the National Metrology Institute (NMI) and reference laboratory for biological measurements in the United States, this challenge presents a unique opportunity for NIST to respond with the development of appropriate standards for this community. NIST works close with stakeholders to develop RMs, reference data, definitive methods, instruments, measurement services and calibrations, quality assurance programs (QAPs), and interlaboratory studies (ILSs) that fit the community needs. NIST community engagement is vast and diverse resulting in different types of standards that range from documentary standards to physical standards (e.g. SARS-CoV-2 RNA and human fecal material). Additionally, NIST-hosted workshops are intended to gain direct insight and feedback from the community to help guide the development of these standards.

Experts

Bharat Ramakrishna, OpenBiome

Manoj Dadlani, CosmosID

Katarina Papp, Water Quality R&D, Southern Nevada Water Authority

Kyle Bibby, University of Notre Dame

Aparna Keshaviah, Mathematica

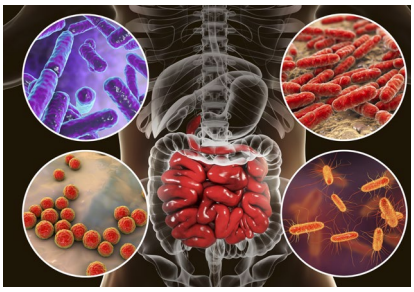
The Biosystems and Biomaterials Division and the Chemical Sciences Division are currently engaged in developing a human fecal RM in partnership with The BioCollective to add measurement assurance in metabolomics and metagenomics analysis of fecal material. SARS-CoV-2 synthetic RNA is a recent NIST output for assay developers and instrument manufacturers to ensure quality control (QC) in viral detection.

For this virtual workshop, NIST is pleased to host a diverse range of speakers that represent international experts in fecal microbiome transplantation, infectious disease diagnostics, and wastewater surveillance to elucidate some of the challenges associated in the measurement of SARS-CoV-2 in human stool and wastewater. We aim to strengthen NIST's relationship with these communities to enable the development of measurement assurance tools and fit-for-purpose standards. Summaries of each of the workshop speaker abstracts and biographic information can be found in Appendix A: Speaker Abstracts and Bios.

[7] Medena, G. Heijinen, L., Elsinga, G. *et al*., Presence of SARS-Coronavirus-2 in Sewage. MedRxiv., March 2020. DOI: https://doi.org/10.1101/2020.03.29.20045880

Fecal Microbiota Transplantation: Responding to the COVID-19 Pandemic

Bharat Ramakrishna, OpenBiome



Fecal microbiome transplant (FMT) is a revolutionary treatment that has proved remarkably effective in treating Clostridioides difficile, a debilitating bacterial infection that strikes 500,000 Americans a year and kills 30,000.

Source: The New York Times

OpenBiome is a nonprofit stool bank that seeks to catalyze research on the human microbiome by providing stool material for clinical application and improving upon best practices for the manufacture of FMT products. As part of this work, OpenBiome applies a rigorous screening of potential stool donors that leads to a 97.5 % exclusion rate[8]. Accounting for SARS-CoV-2, and the emergence of novel pathogens in donor screening in general, is critical to maintaining the safety of FMT to patients. Ultimately there are two key questions to support this function: 1) can the novel pathogen be transmitted through stool and cause subsequent infection? 2) can the novel pathogen be detected with adequate sensitivity in asymptomatic stool donors?

1) Can the pathogen be transmitted via FMT? Unknown. SARS-CoV-2 has been shown to replicate in gut endothelia and is also shed in stool, however the viability and infectivity of these virus particles is unknown[9]. Inoculation of gastrointestinal endothelium via virus in FMT could give rise to systemic infection and COVID-19. Other coronaviruses have also been found in stool and gut endothelium (e.g. MERS and SARS-CoV-1)[10], but the rate of fecal-oral transmission of these viruses is also unknown. COVID-19 patients can develop gastrointestinal symptoms, including nausea, vomiting, and diarrhea though presence or of these symptoms does not appear to correlate with severity of COVID-19 infection, so even asymptomatic individuals may shed viable virus in stool[11].

2) Can the pathogen be detected in donors? Yes. SARS-CoV-2 can be detected in donors through a variety of means. Clinical history and exposure risk factors can be assessed, and testing of non-stool specimens for various pathogens is now part of standard public health practice. The detection of the virus in stool is also possible using molecular nucleic acid amplification techniques, and recently demonstrated via viral culture. One challenge is the incubation period for the virus. SARS-CoV-2 is unique to other betacoronaviruses as it has an incubation period much longer than previously observed in SARS-CoV-1 and MERS. Detection of SARS-CoV-2 virus in stool of some individuals occurred up to 22 days later than detection in respiratory specimens[12]. The amount of virus shed has also not been well defined, and large viral loads may correlate with a higher likelihood of infection and illness severity. Limited data on quantification exists in part due to

[8] Kassam, Z., Dubois, N., Ramakrishna, B. *et al*., Donor Screening for Fecal Microbiota Transplantation. *N. Engl. J. Med*, 381(21), 2019.

[9] Xiao, F., Sun, J., Xu, Y., Li, F. *et al*., Infectious SARS-CoV-2 in Feces of Patient with Severe COVID-19. *Emerg. Infect. Dis*., 26(8), 1920-1922., 2020.

[10] Cimolai, N. Features of enteric disease from human coronaviruses: Implications for COVID-19. *J. Med. Virol.,* 1-11, 2020.

[11] Cheung, K. S., Hung, I. F., Chan, P. P. *et al*., C. Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in fecal samples from the Hong Kong cohort and systematic review and meta-analysis. *Gastroenterology*, 159(1), 81-95, 2020.

[12] Wu, Y., Guo, C., Tang, L., Hong, Z., Zhou, J. *et al*., Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. *The Lancet Gastroenterol. & Hepatol.*, *5*(5), 434-435, 2020.

the lack of standardized methods in performing reverse transcription-polymerase chain reaction (RT-PCR)[13], and inconsistencies in reporting in various clinical studies.

Data from early in the pandemic highlight the challenges in collecting rapid and real-time information about potential SARS-CoV-2 transmission via stool. While studies have been informative, the data does not help inform how best to adequately screen stool donors. Standards in stool specimen collection, specimen preparation and handling, molecular primers, instrumentation, and positive and negative controls are needed to confidently assess the presence of SARS-CoV-2 in stool. Data on the carriage and viability of the virus in asymptomatic individuals is also lacking.

Detection of SARS-CoV-2 in donors is essential for safeguarding FMT in practice. OpenBiome has adopted safety measures to minimize the risk of viral transmission to FMT recipients. Since December 1, 2019 all stool donations have been quarantined to allow time to develop and assess appropriate testing for SARS-CoV-2.[14,15] OpenBiome has since implemented additional diagnostics (via viral testing of nasopharyngeal samples) and clinical assessments to qualify donors. Testing standards and controls developed and supported by organizations like NIST will be important to ensure confidence in these screening methods to mitigate potential risk of transmission to patients receiving FMT into the future.

[13] Han, M. S., Byun, J. H., Cho, Y., & Rim, J. H., RT-PCR for SARS-CoV-2: quantitative versus qualitative. *Lancet Infect. Dis*., 2020. https://doi.org/10.1016/S1473-3099(20)30424-2

[14] Siew, C., Chan, F., Chan, P. Screening FMT donors during the COVID-19 pandemic: a protocol for stool SARS-CoV-2 viral quantification. *The Lancet Gastroenterol. & Hepatol*., 5(7), 642-3, 2020.

[15] Ianiro, G., Mullish, B.H., Kelly, C.R., *et al. Screening of faecal microbiota transplant donors during the COVID-19 outbreak: suggestions for urgent updates from an international expert panel.* Lancet. Published Online March 16, 2020 https://doi.org/10.1016/ S2468-1253(20)30082-0.

Overcoming the Validation Challenges of a Diagnostic for SARS-CoV-2 in Stool

Manoj Dadlani, CosmosID

Microbiome research suffers from a high degree of variability. Variation in methodology crops up everywhere, "from how you extract DNA to how you build your [DNA] library". Two different labs analyzing the same stool sample will often get "very different results." If researchers could agree on a single reference standard, with a known taxonomic composition, they would be able to "understand reproducibility—or the lack thereof—across laboratories."

Source: TheScientist

CosmosID provides next-generation sequencing (NGS) services and bioinformatics solutions focused on microbiome analysis, and rapid identification and characterization of microorganisms. To address the challenges imposed by the current pandemic, the company has also been working on a stool validation protocol for measuring SARS-CoV-2 built on an end-to-end microbiome platform for microbial profiling. It is anticipated this capability can be expanded to future wastewater applications. The predominate rationale for testing SARS-CoV-2 in stool is to ensure patient safety during FMT, which has recently been enforced by FDA safety warning guidelines (March 2020). However, additional drivers such as patient release criteria, the further development of assays targeting stool, and the safety of clinical staff are also of chief importance. Furthermore, the latest motivation is for wastewater surveillance. CosmosID has developed and validated a protocol for the analysis of fresh stool from collection to a bioinformatic analysis. Viral extraction is accomplished using a Qiagen QIAamp viral RNA mini kit on the QIAcube initially utilizing Centers for Disease Control and Prevention (CDC) IDT primers for RT-PCR; more recently, primers were changed to a PerkinElmer kit due to higher efficiency and throughput. Moreover, the kit targets both ORF1ab and N-genes while CDC primers target only N-genes. Method validation controls include SeraCare's AccuPlex SARS-CoV-2 material, which consists of heat-inactivated SARS-CoV-2 virus to challenge the entire extraction step with a whole virus and not only the RNA genetic material. This approach is compatible with assays targeting CDC and WHO consensus sequences in which each sample is spiked with MS2 phage as an internal control, and each extraction batch has a positive and negative control. The Perkin Elmer assay targets both the N-gene located in the nucleocapsid and ORF1ab gene located in the SARS-CoV-2 genome. The limit of detection obtained with these primers seems to be similar to the CDC primers, which is 5,000 copies per mL, but the goal is to achieve lower detection. As of now, the upper limit of detection is 10,000 copies per mL, but modifications are underway to expand the analytical range to higher concentrations of SARS-CoV-2 in the analyte.

This analytical approach has been applied in the analysis of stool for patients that tested positive for COVID-19 by nasopharyngeal tests, thus demonstrating the value of stool testing in practical, real world scenarios. As part of the assay validation, specificity has been addressed by testing SARS-CoV-2 positive-confirmed stools for various pathogens such as norovirus, enterovirus, *Escherichia coli* and *Clostridium difficile* to make sure no cross-reaction was present. In addition, samples have been sequenced to characterize patient flora. Frozen stool samples from The BioCollective have been tested to expand the applicability of the assay. Initial results suggested a potential for polymerase chain reaction (PCR) inhibition exists, and further investigation is required. Different storage conditions including glycerol/phosphate buffered saline, trehalose and lysis buffer have been investigated as well. Stool testing using RT-PCR and sequencing with metagenomics and

meta-transcriptomics is currently available, together with a bioinformatics capability utilizing a microbiome analysis platform that contains SARS-CoV-2 reference data.

Wastewater Surveillance of SARS-CoV-2 in Southern Nevada: Challenges for Sample Collection, Processing, and Analysis

Katerina Papp, Water Quality R&D, Southern Nevada Water Authority

With the ongoing challenges associated with large-scale testing for COVID-19—including test kit shortages, stringent testing criteria and the inherent limitations of current tracking systems on mildly symptomatic and asymptomatic populations, scientists are looking for alternate methods to boost testing in order to predict the spread of the disease.

Source: Global Biodefense

Wastewater biosurveillance is an important tool for public health as it can provide early warning signs for potential outbreaks such as the current SARS-CoV-2 pandemic. It can also aid in assessing hotspots within a community and implementing policy measures. In this respect, the use of wastewater as a biosurveillance tool for public health monitoring needs to ensure that the associated analytical approaches are quantitative with high level of confidence. Currently, there are several sources of uncertainty affecting the performance of commonly used measurement methods including sampling location (e.g. upstream in the sewershed, raw influent), time of collection, and volume of sample. In addition, sample processing is another factor that significantly contributes to the measurement variation. It includes 1) the "equivalent sample volume (ESV)," which is the volume of the original sample that was actually analyzed after accounting for the initial volume of the collected sample, and subsequent concentration/processing factors including ultrafiltration, nucleic acid extraction, complementary DNA (cDNA) synthesis and quantitative PCR (qPCR); 2) the method for sample concentration (e.g. ultrafiltration, polyethylene glycol precipitation or other); and 3) molecular methods and kits/protocols used for each step. Although measurement controls have been set in place (e.g., using bovine or human coronavirus external spike-ins to determine recovery efficiency), inconsistent results with respect to virus recovery have often been observed among different laboratories and their sample processing protocols. For example, hollow fiber ultrafiltration (HFUF) alone and centrifugal concentration using centrifugal filters alone each yielded approximately 50 % recovery of the spiked bovine coronavirus. However, when these two concentration methods were combined, bovine coronavirus (BCoV) recovery decreased to ≈ 2 % only. There is therefore a pressing need for the implementation of quality checks within each different analytical process, including spiked surrogates,[16] to determine virus recovery. These surrogates should be comparable to SARS-CoV-2; surrogates currently used include OC43, 229E (human coronavirus), MS2, phi6 (bacteriophages) and BCoV. Regarding molecular processes, especially with regard to RT-PCR and qPCR assays, implementation of robust calibration standards and positive and negative controls for each qPCR assay is critical for accurate quantification of the viral target.

Another approach to measurement control relies on inter-assay comparisons that comprise calculating assays' efficiencies, assessing cycle threshold (Ct) values for each set of standard dilutions, and determining accurate limits of detection (LODs) and limits of quantification (LOQs). For the wastewater surveillance of SARS-CoV-2, it is recommended to use multiple assays targeting different portions of the SARS-CoV-2 genome

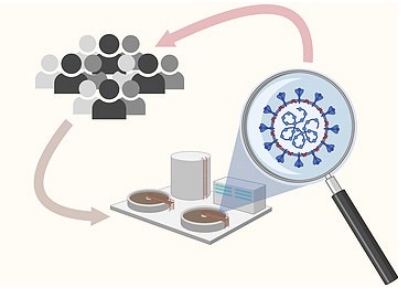
[16] Ahmed, W. et al., Comparison of virus concentration methods for the RT-qPCR-based recovery of murine hepatitis virus, a surrogate for SARS-CoV-2 from untreated wastewater. *Science of Total Environ*., 739, 2020.

and then perform inter-assay comparison to increase the robustness of the data. Assays targeting the nucleocapsid proteins N1 and N2 are common targets for quantifying SARS-CoV-2 in wastewater. However, it is also possible to target the envelope protein using the E_Sarbeco assay as well as a non-structural protein present in the virus genome (*orf1a*). A comparison of these 4 targets has shown substantial differences. Positive results have been observed more often with N1 and N2 (65 % and 48 %) than with *orf1a* and E_Sarbeco (8 % and 20 %, respectively). The LODs among the 4 targets were similar but the LOQs were higher for N1 and N2 (200 copies/µL each) than for *orf1a* and E_Sarbeco (10 copies/µL and 5 copies/µL, respectively). If the SARS-CoV-2 signal is weak, it is possible to observe discrepancies between the assays such that only some will yield a positive signal.

In summary, quality checks are critical to evaluate and understand method performance to accurately characterize the SARS-CoV-2 genetic signal in wastewater. The Water Quality R&D at the Southern Nevada Water Authority will continue to conduct SARS-CoV-2 wastewater surveillance in the local wastewater with an aim to understand any effects of secondary waves of COVID-19.

Wastewater Surveillance for SARS-CoV-2

Kyle Bibby, University of Notre Dame



COVID-19 WBE Collaborative

Welcome to COVID19WBEC.org! The purpose of this site is to empower collaboration on a global scale for wastewater-based epidemiology of SARS-CoV-2, the etiologic agent of COVID-19. We are pleased to partner with the Sewage Analysis CORe group Europe (SCORE) and the Global Water Pathogen Project.

Source: www.COVID19wbec.org

This presentation not only represents research conducted at the University of Notre Dame, but also embodies the work of numerous organizations and academic institutions[17] and represents a true global effort for wastewater surveillance (www.COVID19wbec.org). Two thirds of patients with SARS-CoV-2 excrete SARS-CoV-2 in their stool. However, the variation in viral shedding load among people is quite large and can range over six orders of magnitude. Viral RNA can also be detected in wastewater, and this information can be used to inform our response to potential outbreaks. Several reports in wastewater are based on attempting to detect the viral RNA even though the state of the virus in stool is unclear. According to reports, most of the virus present in wastewater is not in an infectious form. Efforts to investigate the potential for fecal-oral spreading of the disease have been described in the literature[18], thus it is important to remember this distinction when examining published data.

The first report[7] that identified the virus in wastewater came out of Netherlands using endpoint PCR detection. The comparison across different assays suggested lack of comparability and naturally posed questions for the wastewater surveillance community regarding what the most appropriate assay is and whether multiple assays are required given the complexity of wastewater. The same group in Netherlands published a follow-up report[19] and showed that the presence of the virus in wastewater correlated with the cumulative cases observed clinically. Although clinical cases cannot be compared directly with viral shedding in the community due to the low rate of testing, it was interesting to observe a pattern for correlation in this example. The first peer reviewed paper came out of Australia bringing to light several questions regarding appropriate sampling concentration and assay[4]. A study (unpublished), led by Raul Gonzalez (Hampton Roads Sanitation District), has shown the impact of temporal variability on the SARS-CoV-2 signal in influent samples at different wastewater treatment plants (WWTPs). Although a trend is observed, further investigation is required as the stability of the signal and its associated noise level are unclear. A recent study[20] examined sludge as an evaluation material and has shown that the viral concentration precedes clinical diagnostics of COVID-19 cases by approximately a week, and thus suggests that wastewater monitoring could be used as an early warning tool. Nevertheless, before wastewater can be used as public health management tool, questions regarding the methodology (e.g. sample collection and analytical process) need to be addressed to ensure

[17] Bivins, A., North, D., Ahmad, A., *et al.* Wastewater-Based Epidemiology: Global Collaborative to Maximize contributions in the Fight Against COVID-19. *Environ. Sci. Technol., 54, 7754-7, 2020.*

[18] Hamza, I. A and Kyle, B., Critical Issues in Application of Molecular Methods to Environmental Virology. *J. Virolog. Methods*., 266, 11-24, 2019.

[19] Medena, G., Heijnen, L., Elsinga, G., et al., Presence of SARS-Coronavirus-2 RNA in Sewage and Correlation with Reported COVID-19 Prevalence in the Early Stage of the Epidemic in The Netherlands, *Environ. Sci. Technol. Lett*. 7(7), 511-16, 2020.

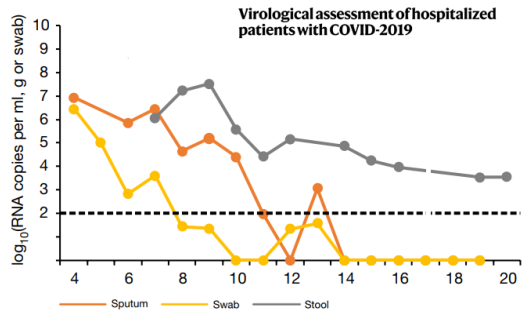
[20] Peccia, J., et al., Zulli, A., Brackeny, D. E. *et al*., SARS-CoV-2 RNA concentrations in primary municipal sewage sludge as a leading indicator of COVID-19 outbreak dynamics. MedRxiv, 2020. https://doi.org/10.1101/2020.05.19.20105999

measurement confidence and data comparability. Additionally, modeling the data as a means of predicting the number of individuals that are infected in a community is a significant challenge. Moreover, the variability of the virus excreted in stool is greater than the variability introduced by the methodologies, thus making it unclear if it is worthwhile to invest in resources to account for the variability in the multiple steps of the measurement workflow.

The wastewater surveillance field has demonstrated successful detection of the virus RNA globally, but the methods are rapidly evolving and there are some important questions regarding confidence in the quantification, interpretation and application of these results as a management tool. Perhaps the most promising application of this approach as a surveillance tool is for vulnerable communities (e.g. hospitals, nursing homes) as a leading indicator of the virus presence. For the case of standards, the matrices of wastewater and sludge, and the associated sewage system infrastructure are highly variable thus any developed standard needs to represent this complexity to be representative and useful for the community.

Aligning Validation of Wastewater Testing for SARS-CoV-2 to Policy Needs

Aparna Keshaviah, Mathematica



Source: Nature

An examination of trends in orthogonal samples (e.g. stool, nasal swab, wastewater) could bring robustness to the wastewater viral RNA assessment. However, for measurement data to be useful to inform a pandemic response, there is a need to develop an informational ecosystem including appropriate validation of analytical methods, sampling and data reporting (Aparna Keshaviah).

Mathematica's mission as a nonpartisan research organization is to improve the health and well-being of the general public. For many years, they have been working with state and local officials to assess the value of wastewater testing as a policymaking tool. Their end goal—to translate the data into actionable insights—was previously focused on the opioid epidemic, and more recently refocused on the COVID-19 pandemic. Engagement of the community (e.g. public health and university officials) is essential to understanding how wastewater testing can inform pandemic management and help balance public health and economic pressures. During method validation, it is important to consider policy needs, recognizing that multiple tiers of evidence, multiple study approaches, and multiple data sources may be required.

Different policy uses of wastewater testing have different informational requirements. A single snapshot of wastewater can help public health officials gauge the presence of the virus in a community and provide an early warning for new waves of infection. But to confidently declare that the virus is *not* circulating in the community, researchers need to characterize LODs and factors that affect such. When wastewater sampling is repeated over time, officials can measure trends in viral loads to assess whether the number of people infected is increasing or decreasing, and accordingly, determine how to adapt policies like social distancing. Here, validation entails understanding how viral RNA loads may vary over time due to fluctuations in environmental conditions, such as temperature or rainfall, and differences in lab protocols. To address the lack of consistency among different laboratories, groups like the Water Research Foundation are leading interlaboratory studies to build towards protocol harmonization. When wastewater sampling is deployed broadly in a region, one can identify hotspots and local epicenters of the virus, which provides a data-driven strategy to shifting resources to where they are needed most. However, with regional comparisons, sampling considerations come into play, to ensure that samples collected in different regions and during different times of day (particularly point-in-time grab samples) are representative of the larger WWTP service population. Further, if wastewater data will inform decisions that affect an entire county, the implications of differences between the WWTP service population and the community at large (with respect to demographics, pre-existing health conditions, and other risk factors) should be considered. Standards are also needed for sampling frequency. Mathematica recently developed an epidemiological model that uses social network analysis to estimate COVID-19 spread. Such a model can inform how quickly a single case will turn into 10 cases or 100 cases, and what resolution of wastewater sampling is required (e.g., daily, weekly) to allow officials to mitigate disease spread. Lastly, an unmet need in the field of wastewater testing is helping officials estimate disease prevalence with wastewater data, which could be used to evaluate the effectiveness of policies to control cases. Translation factors are needed to convert viral RNA concentrations in wastewater into the number of infected individuals in the community.

To estimate these factors, more information on viral shedding in the stool is needed to better characterize the timing, duration, and prevalence of virus in wastewater. It is also unclear how shedding varies according to case severity, demographics, comorbid medical conditions and concomitant medications. An examination of trends of orthogonal samples (e.g. shed from stool, nasal swab, wastewater) could bring robustness to the wastewater viral RNA assessment[21]. Looking forward, such information could help officials assess population coverage of a vaccine, once available, if biosurveillance can detect vaccine-derived virus.

To efficiently generate evidence on the accuracy, precision, and reliability of wastewater data, multiple approaches need to be tapped, including experimental and quasi-experimental (wet and non-wet) lab and non-lab studies. While laboratory measurement validation is critical, there are many other approaches outside the lab that should be pursued simultaneously for the greatest impact. This could include the implementation of an epidemiological approach to translating viral RNA loads in wastewater to estimates of COVID-19 cases in the community. Such an approach could use portable sampling to test the wastewater from a small subpopulation in which there is fairly complete case assessment—such as individuals in a hospital—and correlate RNA levels in the hospital's wastewater with synchronized data on COVID-19 admissions and clinical case presentation. Furthermore, statistical approaches such as meta-analysis and meta-regression could be employed to learn about the relationship between variations in test positivity and confirmed case prevalence, or the influence of sample preparation features on viral detection. In early studies from the Netherlands, test results varied across different assays, and a similar lack of consistency for a different set of assays was observed in studies from Australia.

Ultimately, for wastewater surveillance to inform pandemic response, there is a need to develop an informational ecosystem that brings together wastewater data with complementary community health data and builds validation of sampling, testing, and data reporting into the process. Taking lessons from Mathematica's analysis of wastewater data alongside local pharmacy, law enforcement, and emergency medical services logs on opioid and illicit drug use[22], synthesizing multiple data sources can help fill knowledge gaps and generate a more complete informational picture for epidemic management.

[21] Wölfel, R., Corman, V.M., Guggemos, W. *et al.* Virological assessment of hospitalized patients with COVID-2019. *Nature* 581**,** 465–9, 2020.

[22] Margetts, M., Keshaviah, A., Hu, X.C. *et al*. Using wastewater-based epidemiology with local indicators of opioid and illicit drug use to overcome data gaps in Montana. MedRxiv, April 2020. https://www.medrxiv.org/content/10.1101/2020.04.18.20064113v2

PANEL DISCUSSION/Q&A

Q: This workflow could work for an important but ordinary problem like opioid consumption, but these are not ordinary times. What could be done differently to accelerate this process?

A (Aparna Keshaviah): Collaboration is a key to accelerate the process and includes method harmonization studies and clinical trials involving different researchers and study subjects. Data sharing and looking across studies is the best way to quickly ramp up knowledge to understand the most promising directions to go.

Q: Will wastewater detection tell anything that we wouldn't already know from an increase in non-wastewater testing for COVID-19 US?

A (Kyle Bibby): There are couple of advantages that might help to fill in blind spots of clinical testing. For instance, it could be a leading indicator for asymptomatic people or with limited symptoms. In this situation, a clinical diagnosis might not be involved. Thus, wastewater surveillance could possibly identify locations for those individuals. In addition, this approach costs significantly less than clinically testing an entire community. Wastewater surveillance could be another piece of information that could be used to make decisions.

Q: Has any of the wastewater data been used to estimate a possible surge? The data is still in early stage but has anyone used for management decision?

A (Kyle Bibby): Not yet, except Biobot. The company has been working with several different municipalities that are possibly interested. However, the science is not there yet and should be taken with caution. To use the tool to make management decisions is a bit early but certainly could be used as a surveillance.

Wastewater surveillance is not a novel idea. It has been used for poliovirus monitoring since the 1940s and recently applied in 2013-2014 to monitor silent polio virus outbreak in Israel where they were able to ramp up vaccination approaches.

A (Aparna Keshaviah): Even though the current information is limited and not perfect, we need to provide something to guide officials, who need to make decisions with or without fully validated information.

Q: What do you think would be a standard that would benefit the community considered the challenges in different steps of the process such as sample collection, data etc. Where should NIST start?

A (Aparna Keshaviah): Characterization of viral shedding is a place to start—how it varies across different patients and how to improve measurement precision. Inclusion of the patient metadata would be important to understand the impact of pre-existing conditions on shedding.

A (Kyle Bibby): It is important to have guidelines to ensure details around the methodologies in place, protocol harmonization before we go toward standards. Standards that can address quickly the change in the field.

In terms of wastewater heterogeneity, the standard would have to address day-to-day variation and other factors that contribute for the complexity of the wastewater.

A (Katarina Papp): A standard with one size fits for all, would not be feasible for the wastewater field. Standardization of protocols according to the application would be helpful for the community.

Q: Would developing standard guidance to assist the community help to begin the discussion on harmonizing, method, data, workflow and metadata?

A (Kyle Bibby): The wastewater surveillance field still learning about factors that are important to assure reliable data

Q: Which standards would be good to move forward with standardization for FMT?

A (Manoj Dadlani): Fecal material with known concentration of SARS-CoV-2, standards to address different matrices and sample preparation would be useful. At this point, labs are producing their own reference material.

A (Bharat Ramakrishna) Standards addressing LOD and measurement reproducibility as well as assessing viral viability and copy number would be helpful for the FMT community.

POTENTIAL STANDARDS/NEXT STEPS

Attendees indicated that protocol harmonization, including sample processing, would benefit data comparability within and across laboratories. Guidelines standardizing sampling location, sample volume and temporal sampling, for instance, would help in decreasing technical variability. Standards should account for the heterogeneity and factors that contribute to the complexity of the matrices (e.g. wastewater and human fecal microbiome). The participants indicated that data sharing and use of patient metadata and orthogonal sampling data (e.g. stool, nasal) would improve robustness of statistical data from clinical studies and wastewater surveillance efforts, and thus allow for improved confidence in decision-making.

With respect to a physical or "artifact" standard such as a RM, one option would be a relevant matrix, such as fecal material or wastewater, with a known concentration of SARS-CoV-2. This type of material would challenge sample processing and support LOD, LOQ, and copy number determinations for RT-PCR based assays; interest in a combined matrix-virus standard was expressed by both communities. As a first step, NIST plans to assess the impact of human stool and wastewater matrices on the stability of the SARS-CoV-2 measurement, assay inhibition, copy number, and overall method performance. The development of a "gold standard" protocol is a necessary precursor for value assignment of candidate RMs but could also represent an initial standardization tool for measurement communities and standards organizations to ensure comparability among longitudinal studies and associated data assessments. NIST could also develop a complementary guidance document to provide instructions on how to develop and utilize various field (on-site) control materials, both positive and negative, to ensure the results of the test are valid.

Based on the challenges in the analytical workflow (e.g. qPCR) for detecting and quantifying SARS-CoV-2, NIST aims to develop a SARS-CoV-2 surrogate-based RM that can be easily produced and broadly distributed, while still being effective for quality control through measurement harmonization across field methods and techniques. Candidates for a virus surrogate include BCoV, OC43 and 229E (human coronaviruses), and MS2 and phi6 (bacteriophages) and will be selected based on the genetic similarity with the SARS-CoV-2 and overall stability in the presence of wastewater and fecal material matrices. Employing a whole virus as opposed to the naked genetic material may be preferred as a surrogate material. In such a case, the entire analytical process (including RNA extraction) can be challenged while the virus surrogate will be less subject to degradation in the presence of the fecal and wastewater matrices. However, the design of the surrogate-based RM will be refined through initial feasibility and stability evaluations, and with input from experts in both the wastewater and fecal material communities.

Moreover, any candidate RM that is produced could be included in interlaboratory studies to assist in development of guidelines for protocol standardization, including steps regarding sample collection, sample processing and data analysis. Results of the interlaboratory study will also inform the feasibility of a candidate RM for routine testing methods and will include the most promising results already observed by expert participating laboratories.

Concluding Thoughts

Overall, the webinar was successful in launching an exchange of knowledge between NIST and various stakeholders on the needs and challenges faced in the detection and quantification of SARS-CoV-2 in fecal microbiome and wastewater matrices. NIST staff will carefully consider the feedback provided by the participants and test few approaches to improve measurement confidence in the analytical processes used by these communities. As was highly supported by the participants in the in-webinar poll, NIST will plan to host a follow up webinar by the end of calendar year 2020.

APPENDIX A: SPEAKER ABSTRACTS AND BIOS

Bharat Ramakrishna, OpenBiome. *Fecal Microbiota Transplantation: Responding to the COVID-19 Pandemic*

Abstract: OpenBiome is a non-profit stool bank providing access to fecal microbiota transplantation (FMT) for the treatment of recurrent *Clostridioides difficile* infection. OpenBiome's donor program has routinely screened for a variety of pathogens known to be shed in stool. Shedding of SARS-CoV-2 in stool has been documented and several FMT groups have published recommendations and protocols to minimize the potential transmission of SARS-CoV-2 via FMT. The key considerations for implementing direct stool testing for FMT donor screening will be discussed, including; interpretation of currently available data on stool shedding; potential screening methods for identifying asymptomatic stool donors (including orthogonal tests such as serology or nasopharyngeal viral RNA); and operational considerations for maintaining donor programs during the COVID-19 pandemic. Future areas of research to better inform best practices in donor screening will also be discussed.

Bio: Dr. Bharat Ramakrishna is a Medical Director at OpenBiome, a nonprofit stool bank which manufactures FMT for use in *C. difficile* and in support of microbiome-based research. He has worked in microbial therapeutics and the FMT space for several years with interest areas in infectious disease public health surveillance, microbial virulence, transmission, and the microbiome. Prior to this he spent several years training in Public Health, Infectious Diseases and Clinical Microbiology in Melbourne, Australia.

Manoj Dadlani, CosmosID. *Overcoming the Validation Challenges of a Diagnostic for SARS-CoV-2 in Stool*

Abstract: In addition to fever and pulmonary symptoms, SARS-CoV-2 infection frequently induces severe enteric symptoms, such as diarrhea and nausea, which can be more grave than enteric symptoms from SARS-CoV-1 and Middle East respiratory syndrome coronavirus (MERS-CoV). Recently, SARS-CoV-2 RNA has been detected in stool samples from hospitalized patients testing positive for the virus, both with and without gastrointestinal manifestations. Current literature on COVID-19 indicates that the duration of SARS-CoV-2 viral shedding from feces after negative conversion in pharyngeal swabs can range from 6 to 9.5 days, and possibly longer in some studies. However, as a sample type, stool is more complicated than a standard pharyngeal swab. To address this challenge, CosmosID has developed a real-time reverse transcriptase polymerase chain reaction (RT-PCR) assay to detect SARS-CoV-2 in stool targeting the ORF1ab and N genes. In this presentation, we will describe the methods for creating and validating the test, the controls we used, and the challenges we faced.

Bio: Mr. Manoj Dadlani serves as Chief Executive Officer at CosmosID, Inc., the Maryland based provider of industry-leading solutions for unlocking the microbiome. Previously, Mr. Dadlani served as a partner at Applied Value Group, a management consulting and investment firm, and was co-founder and CEO at Rasa Industries, Ltd., a leading beverage manufacturing company. Mr. Dadlani has substantial experience in strategy, M&A, supply chain management, product development, marketing and business development.

Mr. Dadlani received his bachelor's and master's degrees in Biological Engineering from Cornell University. Services offered by CosmosID's CLIA certified and GLP laboratory cover the entire workflow from study design to sample collection extraction, library preparation, sequencing, data analysis and publication support. CosmosID's cloud-based metagenomics application offers user-friendly access to the largest curated databases for microbial genomics, antimicrobial resistance and virulence data and has been independently validated to return metagenomic analyses at strain level resolution with industry-leading sensitivity and precision.

Katerina Papp, Water Quality R&D, Southern Nevada Water Authority. *Wastewater Surveillance of SARS-CoV-2 in Southern Nevada: Challenges for Sample Collection, Processing, and Analysis*

Abstract: Wastewater surveillance involves monitoring various chemical or microbiological targets to gain insight into the behaviors or characteristics of a community. This tool has previously been used to identify 'hot spots' for opioid abuse and also to aid in eradicating polio. Wastewater surveillance can provide an 'early warning' signal for disease outbreaks in a community and subsequent trend analysis to understand the state of the epidemiological curve (e.g., spiking vs. waning) and/or the effects of mitigation measures (e.g., social distancing). In fact, wastewater is currently being monitored across the globe for the genetic signal of SARS-CoV-2, the RNA virus responsible for the COVID-19 global pandemic. Although primarily respiratory in nature, SARS-CoV-2 is shed in the feces of infected individuals, ultimately winding up in raw sewage. Despite the tremendous potential for wastewater surveillance of SARS-CoV-2, there are multiple challenges associated with detection of its RNA in complex wastewater matrices, including the presumed fragility of the viral particles and co-concentration of inhibitory substances. This presentation will address a number of ongoing method development efforts at Southern Nevada Water Authority (SNWA), including those related to (1) sample collection and concentration, (2) nucleic acid extraction and processing, and (3) identification of appropriate molecular assays and quality assurance/quality control (QA/QC) measures.

Bio: Dr. Katerina Papp is a Postdoctoral Researcher in Research Microbiology at the Southern Nevada Water Authority. She earned her B.S. and Ph.D. in Environmental Microbiology at Northern Arizona University, where she studied soil microbial activity through quantitative stable isotope probing. She also studied human health microbiology at the Translational Genomic Research Institute. Now at SNWA, her research focuses on microbial source tracking and virus occurrence and removal in the natural and engineered environment. That includes monitoring for the genetic signal of SARS-CoV-2 in Southern Nevada wastewater.

Kyle Bibby, University of Notre Dame. *Wastewater Surveillance for SARS-CoV-2*

Abstract: The talk will briefly cover recent case studies in wastewater surveillance for SARS-CoV-2, as well as necessary future technical developments to enable application as a surveillance method.

Bio: Dr. Kyle Bibby is an Associate Professor and the Wanzek Collegiate Chair at the University of Notre Dame in the Department of Civil and Environmental Engineering and Earth Sciences. He completed his BS in Civil Engineering from the University of Notre Dame and PhD in Environmental Engineering from Yale University. He has previously won multiple professional awards, including the 2017 NSF CAREER award. Dr. Bibby

currently leads multiple research projects centered around understanding microbiology relevant to protecting and improving human health and environmental quality and is an Associate Editor for the journal Microbiome.

Aparna Keshaviah, Mathematica. *Aligning Validation of Wastewater Testing for SARS-CoV-2 to Policy Needs*

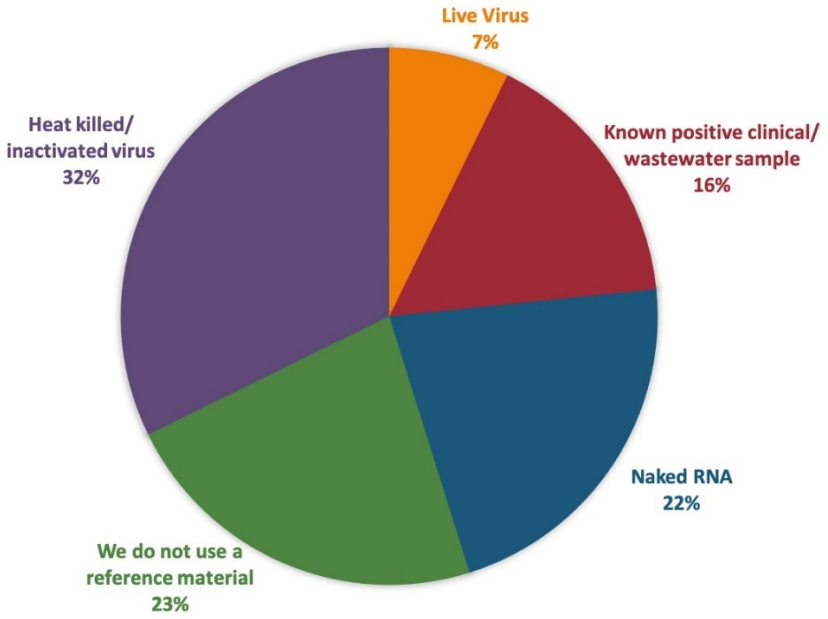
Abstract: With the COVID-19 pandemic, test kit shortages have created large blind spots with respect to disease transmission. Wastewater testing provides a new way to rapidly measure the viral exposure of thousands of people in a community. But given the novelty of approach, public health and public policy officials need to be confident that the method yields reliable information. Different validation approaches provide different insights into the robustness of wastewater data for policymaking. Further, different pandemic management decisions require different levels of methodologic validation. Accordingly, researchers refining SARS-CoV-2 testing methods must consider how the data will be used. Beyond intra-lab validation (to improve viral detection and quantification), approaches to explore include inter-lab validation (such as split-sample analyses), meta-regression (to identify sample processing features associated with improved detection/quantification), and data triangulation (to contextualize and assess alignment between wastewater data and conventional community data sources).

Bio**:** Aparna Keshaviah is a senior statistician at Mathematica who works at the developmental cusp of emerging public health topics. She has directed research on urgent drug policy issues—spanning the opioid epidemic, marijuana legalization, and pharmaceutical drug development and safety—and her work has been widely published in leading journals such as the *New England Journal of Medicine*. At Mathematica, Keshaviah has been at the forefront of bringing innovative data sources, advanced analytics, and visualization tools to health policy research. In 2017, she led a highly visible symposium to explore the real-world potential of wastewater testing to support decision making around the opioid epidemic. Her work to translate the value of wastewater testing for policymakers inspired new federal funding from the National Institute on Drug Abuse, and she has served as an expert reviewer for programs focused on translational research. Keshaviah holds a Master's degree in biostatistics from the Harvard School of Public Health.

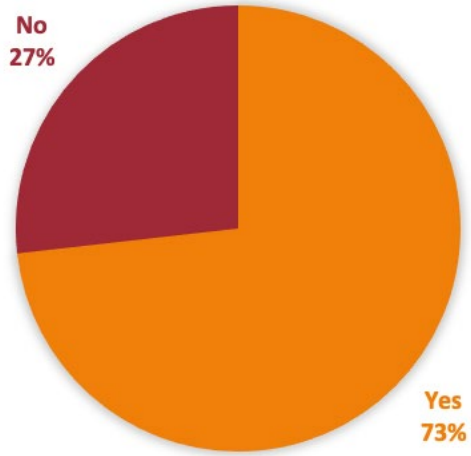
APPENDIX B: IN-WORKSHOP POLLS AND AUDIENCE QUESTIONS

The audience was polled at the conclusion of the speaker presentations, and right before the panel Q&A discussion. The series of four (4) poll questions and the participant responses (with number of responses) are provided below.

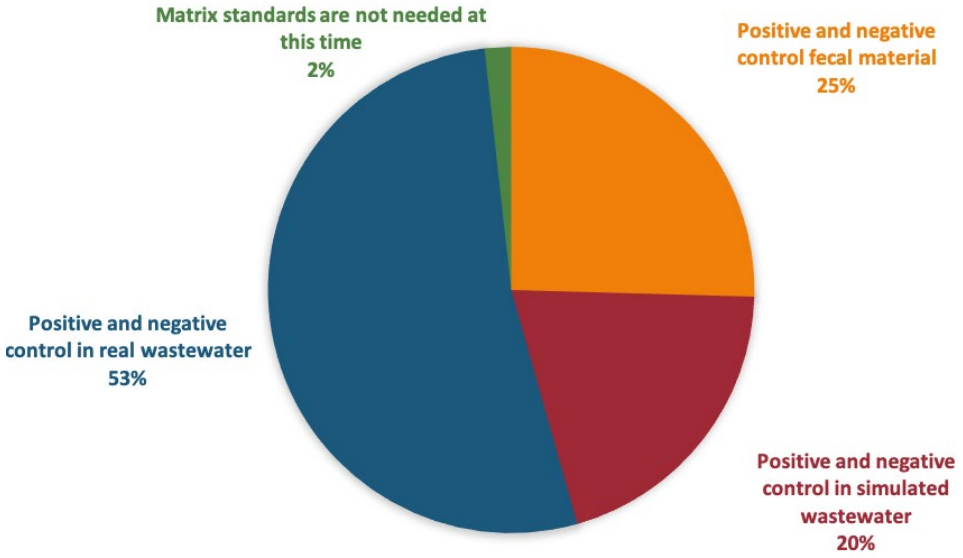
Poll 1: What type of reference material is your group currently using? (124 total responses)



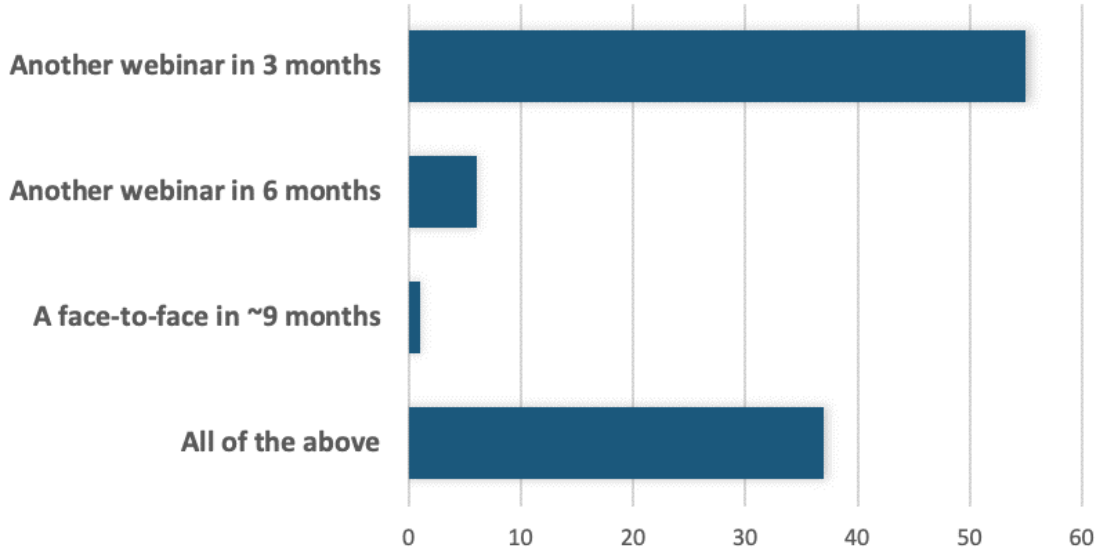
Poll 2: Would standards based on RNA-surrogates for SARS-CoV-2 serve as adequate QA/QC? (112 total responses)



Poll 3: If you had a wish for one standard to be readily available for improving the measurement of SARS-CoV-2, what would that be? (114 total responses)



Poll 4: What are the next steps forward? (99 total responses)



Audience-submitted questions related to Fecal Materials and FMT
Have you confirmed if the RNA you are detecting is virion associated/protected or not?
Are you able to pick up a viral signature for this virus in your metagenomics?
Did you try stool without shield? There is some indication that this sometimes interferes with the PCR.
Can you comment if you've tested of RNA+ vs. intact/infectious virus + stool samples?
Any insight into how freeze thaw impacts the SARS-CoV-2 RNA detection in fecal material?
What is the evidence of F-O [fecal-oral] transmission of SAR-CoV-2 [that is] independent of FMT
Is it not concerning that only 2 samples have been found that are positive for all four SARS-CoV-2 RT-PCR assays?

Audience-submitted questions related to Wastewater Surveillance
Are you using the same RT-PCR kit [for fecal materials] for wastewater as well?
Could sub-genomic RNA derived from human cells skewing target sensitivity in wastewater?
How might standards be different or not between raw sewage from a nursing home or college rather than primary effluent at a treatment plant?
Any advice on schools that want to start monitoring wastewater?
Are we seeing this more as a tool for trending rather than for absolutes?
Is there emerging thinking about the limit of detection within wastewater to make data be useful for decision-making?
What is the expected role of Health Departments in using wastewater testing results?
Have you seen, or do you anticipate, different results in SARS detection using different transport media?
Do you think that the presence of virus E gene in wastewater could indicate the viability of virus in water
The workflow [for wastewater surveillance] could work for an important and 'ordinary' problem like opioid consumption, but these are not ordinary times. What can be done differently to accelerate this process for SARS-CoV-2. [This would] be akin to what FDA is doing through Emergency Use Authorizations (EUAs).
Has targeting the RdRp gene been considered? The WHO recommends first-line screening with an E gene assay followed by a confirmatory assay using the RdRp gene. However, I haven't seen it being used in wastewater epidemiology for SARS-CoV-2 yet, and I'm unsure why not. It sounds like the recovery [in wastewater] was variable, but are you spiking in low concentrations or high concentrations? Can you specify the starting and ending concentrations?
How far away are we from an in-situ sensor that can sample continuously? I am well aware how hostile the environment is inside a sewer - what are the chances of a sensor that can detect COVID-19 (and other pathogens), still be sufficiently rugged, and report out from under the ground?
Composite samples normally have perchloride which is used for order removal. Does it affect test results?

General audience-submitted questions

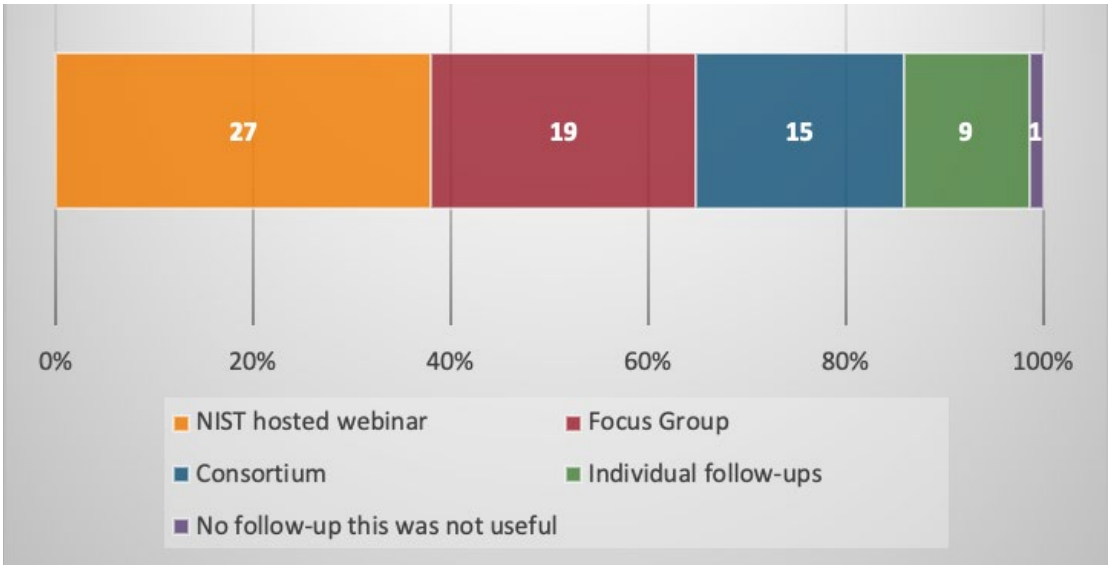
Standard reference material would probably be the most useful. Individual labs could use these common standard references to assess their processes.
After hearing all of this what roles might NIST be playing in standards development?

APPENDIX C: POST-WORKSHOP POLL AND SURVEY

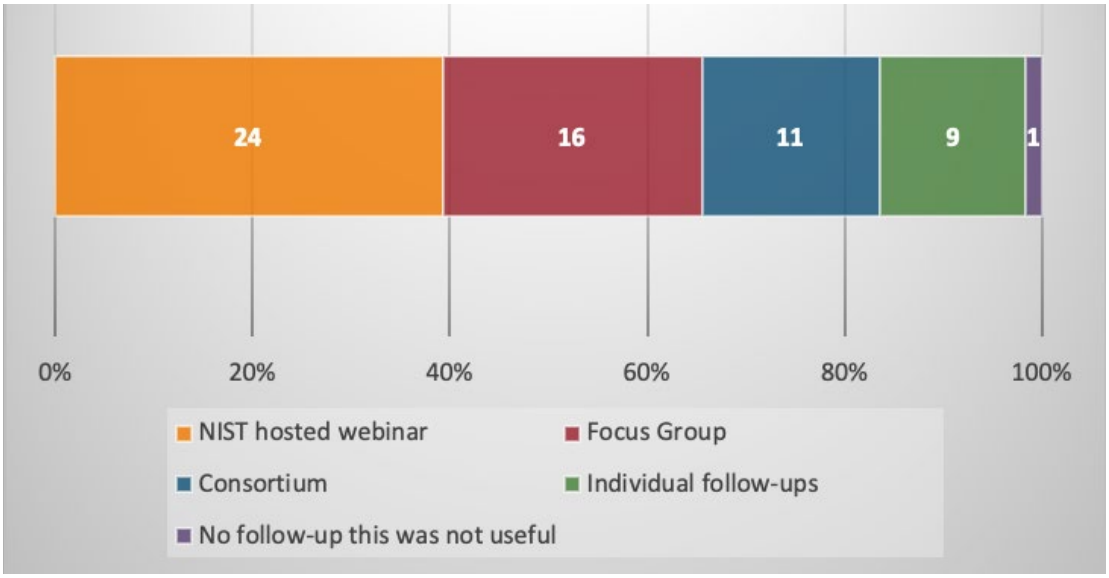
In addition to the in-workshop poll, NIST sent a post-workshop questionnaire to all workshop registrants. The questionnaire comprised 2 poll questions and 7 open-ended survey questions.

To obtain additional insight on how the stakeholders envision future interactions with NIST, the poll questions were focused on how to proceed with respect to wastewater measurements (**Poll 5**) and fecal material measurements (**Poll 6**). Similar responses were reported for each sub-group, with a follow-up webinar receiving the most votes.

Poll 5: Would a follow-up focused specifically on wastewater testing be beneficial? (71 total responses)



Poll 6: Would a follow-up focused specifically on fecal material testing be beneficial? (61 total responses)



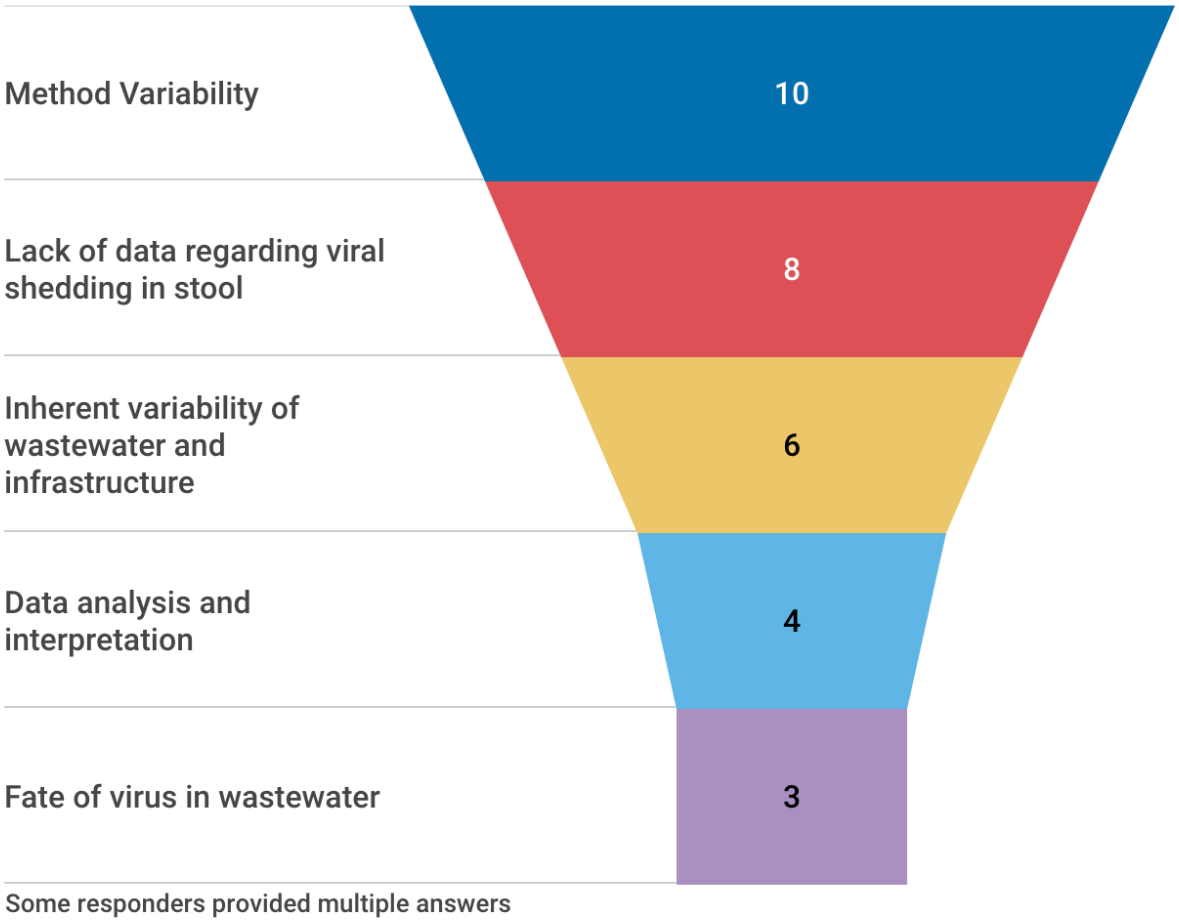
The 7 open-ended questions were answered by a subset of registrants (ranging from 19 to 29 responses). In some cases, participants chose not to answer questions that were not in their field of interest (wastewater vs fecal specimens) or noted that the question was beyond their expertise.

Question 1: If you had a wish for one standard to be readily available for improving the measurement of SARS-CoV-2, what would that be? (29 responders)

A total of 29 registrants responded to this question and the above word cloud was generated (*image made with wordclouds.com*). Once the word list was generated, some words were combined (e.g. feces, fecal, faecal). This question was a follow up to the in-webinar Poll 3. Not surprisingly, a SARS-CoV-2 based standard was the most common request. Notably, while there was a consensus for a viral standard that is quantitative, the type of material requested (e.g. RNA, inactivated virus, surrogate coronavirus, etc.) was more variable. In addition to quantitative viral standards, matrix standards and matched positive/negative control materials were also of interest.

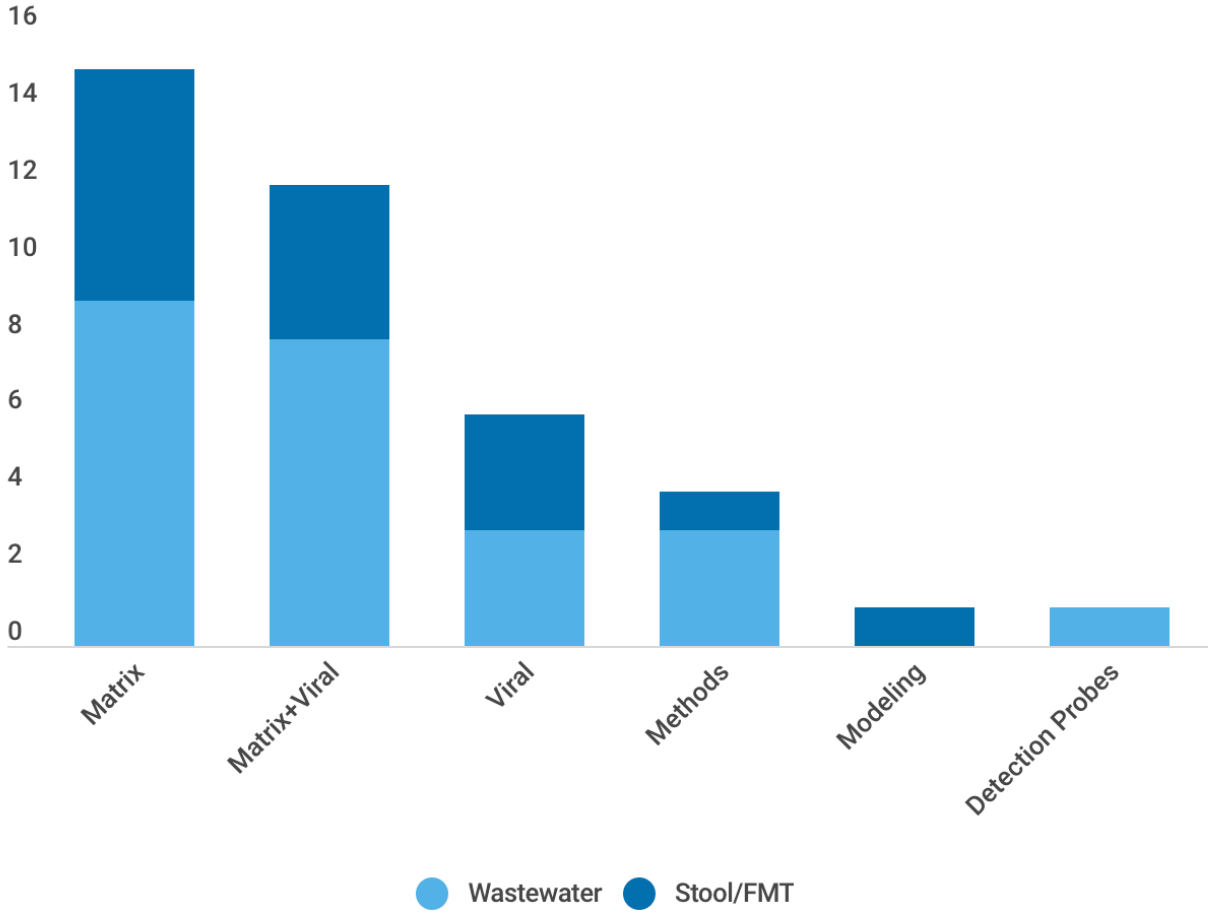
In recognition that wastewater, FMT samples, and clinical stool/fecal specimens are not the same, the remaining 6 questions were directed towards specific sample types. However, after compiling the answers, we found that despite a focus on different matrixes, several areas of overlap emerged in terms of what would be desirable in a standard. The remaining three graphics summarize the responses from survey questions 2-7.

Question 2: What are the largest sources of uncertainty in current wastewater models for SARS-CoV-2 surveillance? (27 Responders) *Image made with Infogram*



The field of WBE has been tasked with rapidly developing methods to track SARS-CoV-2 in the population; however, this requires developing tests for an incredibly complex matrix that produce actionable, reproducible data. To assess the current state of the field, we asked what the largest sources of uncertainty were for SARS-CoV-2 wastewater testing. The responses could be categorized into 5 areas as shown above. Specific sources of concern included methods for sampling and concentrating samples, understanding the effect of extraction efficiency and PCR inhibition, inherent variability of wastewater infrastructure and composition, how to interpret a negative result, and a general lack of knowledge about SARS-CoV-2. As a novel virus, there is still minimal data on viral shedding and the state of the virus in stool and wastewater, making it difficult to extrapolate data from these matrixes to community infections.

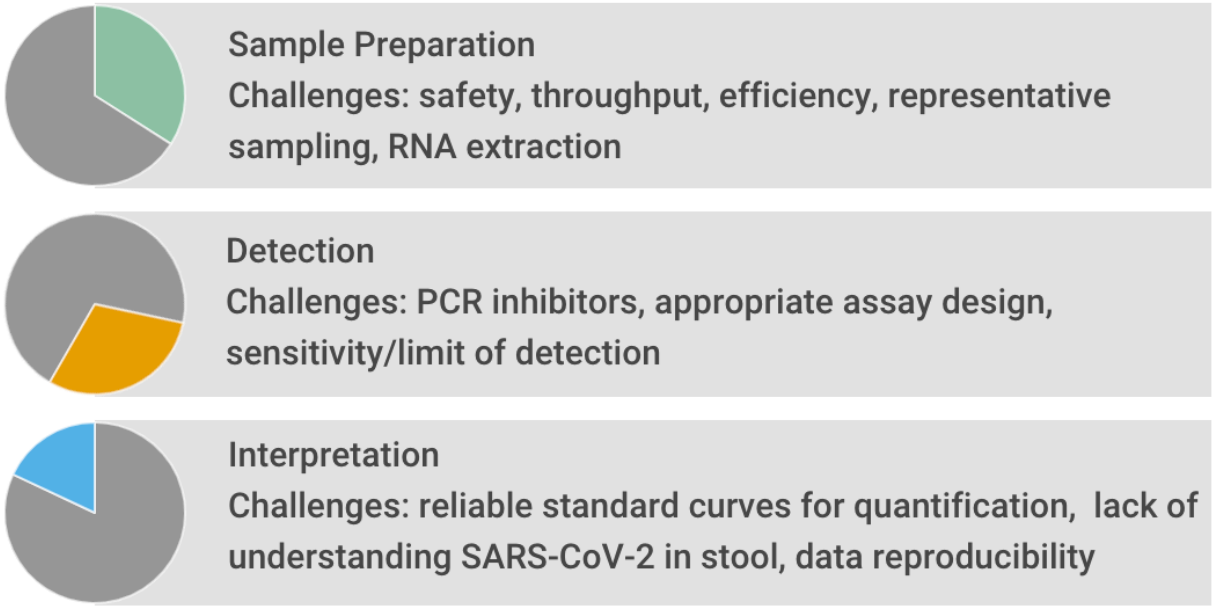
Questions 3-4: What type of matrix-type standards would best serve to provide QA/QC for analytical measurements of SARS-CoV-2 in wastewater or stool/FMT samples? (46 Responders) *Image made with Infogram*



Some responders provided multiple answers

Regardless of the specific matrix of interest, answers regarding a matrix-based standard fell largely into the 4 categories summarized above. “Matrix” can refer to either a real or simulated matrix (in this case wastewater or fecal material); “Viral” could be either SARS-CoV-2 intact virus particles, surrogate virus particles, or nucleic acid from SARS-CoV-2 or a surrogate virus; and “Matrix + Viral” refers to matrix samples with a viral standard spiked in. With respect to wastewater matrix samples, there was support for a standard matrix developed from real wastewater, as well as a simulated wastewater; however, most individuals favored the use of real wastewater. There was also support for paired controls: positive/spiked wastewater and negative wastewater. While the question was directed toward matrix-based standards and material development, there were some responses related to producing standard protocols, including a specific request for a standard method for spiking target material (e.g., virus) into the matrix. On the stool/FMT standard material side, a reference stool sample was the most commonly requested, followed by stool samples with a known SARS-CoV-2 copy number. Similar to the wastewater, a need for positive and negative materials was reported. Notably, there was interest in a standard material developed from a COVID-19 patient as well as a fecal swab inoculated with a known SARS-CoV-2 copy number.

Questions 5-7: What is the greatest measurement challenge for SARS-CoV-2 testing? (62 Responders)



As mentioned above, we recognize that different matrices may present different measurement challenges. In fact, there may even be different challenges when testing a prepared FMT sample versus a clinical fecal sample. Therefore, we asked about the biggest measurement challenge(s) associated with SARS-CoV-2 detection based on the respective matrix (wastewater, FMT, stool/fecal specimen). We noticed numerous overlaps regardless of the matrix being evaluated, and thus chose to provide a summary of all responses. The top responses from the wastewater community included: methods for RNA concentration and extraction, determination of sensitivity and LOD for the assay, and how to translate results to community infection level. Similar to the wastewater community, the FMT community identified sample collection, specifically how to perform representative sampling and how the sample age and volume impact results. They also echoed the need to understand PCR inhibitors and to have a reliable standard curve to estimate concentration. The ability to conduct these assays in a high-throughput manner was identified as a challenge by the FMT community. In addition to the challenges identified by the wastewater and FMT communities, individuals working with fecal specimens (e.g. clinical samples) identified shipping and storage as challenges in the measurement process. Additionally, a lack of understanding of when virus is shed in the stool, how viral shedding changes during the course of infection, and what safety concerns exist was identified as a measurement challenge by the fecal specimen testing community.