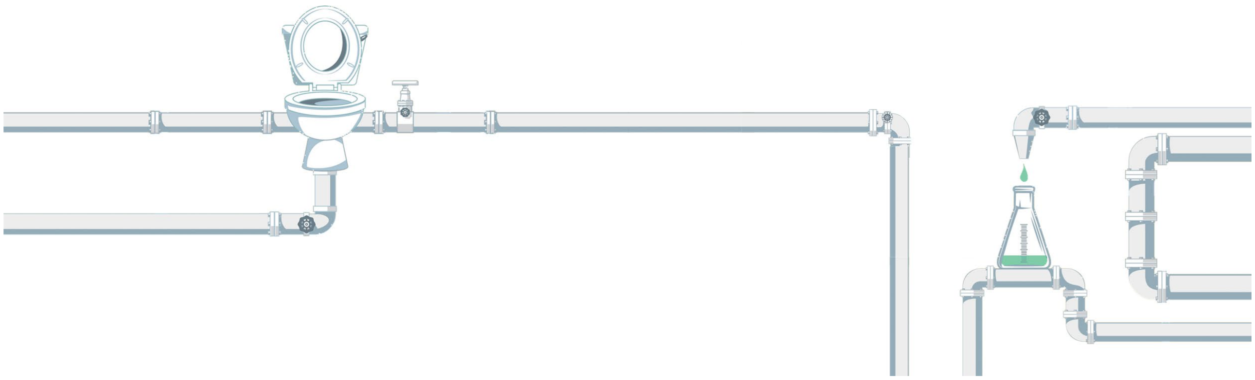
# Capacity Building Program to Develop a Wastewater-Based Epidemiology Framework for Covid-19: Case Studies from Ecuador and Uruguay



The purpose of this global technical guidance note is to share best practices on how to build capacity to establish wastewater-based epidemiology (WBE) in low- and middle-income countries (LMICs) for Covid-19 monitoring. It includes lessons learned on knowledge transfer for sample collection, laboratory analysis, partnership creation, and stakeholder engagement to strengthen existing epidemic preparedness and response systems. WBE can be applied to different types of wastewater samples (e.g., wastewater influent, sludge) and to different targets (e.g., biological pathogens, pharmaceuticals and illicit drugs, environmental contaminants). This global technical guidance note focuses on lessons learned from two capacity building projects conducted in Ecuador and Uruguay in 2020-2021 that used wastewater influent (hereafter, "wastewater") to measure SARS-CoV-2, the virus that causes Covid-19. Challenges associated with applications to other types of wastewater samples and targets are outside the scope of this global technical guidance note.



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## Glossary

Covid-19: Covid-19 is the disease caused by SARS-CoV-2, the novel coronavirus that emerged in December 2019 in Wuhan, China. In March 2020, the World Health Organization declared the Covid-19 outbreak a pandemic.

Ct value: Cycle threshold value. Ct value is an output of a thermocycler, a machine used for PCR technology. Ct value indicates concentration of viruses.

ddPCR: Droplet digital PCR. ddPCR quantifies the amount of DNA or RNA in a sample. ddPCR is a recent technology and generally more sensitive than qPCR.

LMICs: Low- and middle- income countries.

PCR: Polymerase Chain Reaction. PCR is a technique used to detect a target DNA or RNA by amplifying DNAs.

QA/QC: Quality assistance and quality control.

qPCR: Quantitative PCR or real-time PCR. qPCR quantifies the amount of DNA or RNA in a sample.

SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2. SARS-CoV-2 is the virus that causes Covid-19. SARS-CoV-2 can be detected in nasal mucus, saliva, and stool of infected individuals. SOP: Standard Operating Procedure. SOP is a written document that describes step-by-step lab procedures to perform lab analysis consistently.

WBE: Wastewater-based Epidemiology. A field of study that leverages wastewater testing for public health.

# 1. Background

## Introduction to wastewater-based epidemiology (WBE)

Wastewater-based epidemiology (WBE) leverages the existing sewer infrastructure of cities to access aggregated human waste data to inform public health officials and policy makers about population health. Some biological pathogens and drug metabolites are excreted in human feces or urine and can be monitored via WBE. Because wastewater samples represent all members of a community connected to a sewer network independently of their access to healthcare, availability of testing in their community, and health seeking behavior, WBE can provide inclusive data on population health in a timely and cost-effective manner.

In the past couple of decades, WBE has been successfully implemented around the world, demonstrating its public health benefits. For example, routine wastewater monitoring in Israel detected a silent outbreak of polio in 2013 after being declared "polio free" in 1988.[1] In Israel, routine wastewater monitoring for polio has been maintained since 1989. The silent spread of polio detected in wastewater triggered a large vaccination campaign. As a result, the silent outbreak subsided without encountering any clinical cases of acute flaccid paralysis.

The potential of wastewater monitoring extends to a host of biological pathogens, including typhoid,[2] hepatitis A[3], influenza, norovirus,[4] and antimicrobial resistance[5]. WBE can also be used to monitor the use of pharmaceuticals and drugs of abuse,[6] and the presence of environmental contaminants such as pesticides or heavy metals[7]. Most recently, wastewater monitoring was

[1] Brouwer, Andrew F., et al. "Epidemiology of the Silent Polio Outbreak in Rahat, Israel, Based on Modeling of Environmental Surveillance Data." *Proceedings of the National Academy of Sciences - PNAS*, vol. 115, no. 45, National Academy of Sciences, 2018, pp. E10625–E10633, doi:10.1073/pnas.1808798115.

[2] Sikorski, Michael J, and Myron M Levine. "Reviving the 'Moore Swab': a Classic Environmental Surveillance Tool Involving Filtration of Flowing Surface Water and Sewage Water To Recover Typhoidal Salmonella Bacteria." *Applied and environmental microbiology* 86.13 (2020).

[3] Hellmér, Maria, et al. "Detection of Pathogenic Viruses in Sewage Provided Early Warnings of Hepatitis A Virus and Norovirus Outbreaks." *Applied and Environmental Microbiology*, vol. 80, no. 21, American Society for Microbiology, 2014, pp. 6771–81, doi:10.1128/AEM.01981-14.

[4] Hellmér, Maria, et al. "Detection of Pathogenic Viruses in Sewage Provide*d Early Warnings of Hepatitis A Virus and Norovirus Outbreaks." Applied and Environmental Microbiology*, vol. 80, no. 21, American Society for Microbiology, 2014, pp. 6771–81, doi:10.1128/AEM.01981-14.

[5] Heniksen, R. .., et al. "Global Monitoring of Antimicrobial Resistance Based on Metagenomics Analyses of Urban Sewage." *Nature Communications*, vol. 10, no. 1, Robert Koch-Institut, 2019, pp. 1124–12, doi:10.1038/s41467-019-08853-3.

[6] Baz-Lomba, J. .., et al. "Comparison of Pharmaceutical, Illicit Drug, Alcohol, Nicotine and Caffeine Levels in Wastewater with Sale, Seizure and Consumption Data for 8 European Cities." *BMC Public Health*, vol. 16, no. 1, BioMed Central Ltd, 2016, pp. 1035–1035, doi:10.1186/s12889-016-3686-5.

[7] Kinuthia, Geoffrey K., et al. "Levels of Heavy Metals in Wastewater and Soil Samples from Open Drainage Channels in Nairobi, Kenya: Community Health Implication." *Scientific Reports*, vol. 10, no. 1, Nature Publishing Group, 2020, p. 8434–, doi:10.1038/s41598-020-65359-5.

widely adopted across the in the Americas[8] and Europe[9] as a complementary tool to clinical data to monitor the spread of the Covid-19 disease in communities.

In low- and middle-income countries (LMICs), on the other hand, only a few examples of successful application of WBE exist to date. Successful implementation of WBE requires collection of wastewater samples that represent the health of a target population, accurate laboratory analysis of wastewater samples, rigorous data analysis and data interpretation, and coordination with multiple stakeholders. Technical challenges and the need for multi-stakeholder partnerships have been one of several factors that have limited adoption of WBE globally. In LMICs in particular, lack of capacity for sample analysis, lack of financial resources, and sewer infrastructure that serves only a small fraction of the populations are also barriers to the implementation of WBE.

This global technical guidance note aims to share best practices on how to build capacity to WBE in LMICs focusing on the application for Covid-19 monitoring. It first provides key elements in implementing WBE in LMICs. Based on two capacity building projects conducted in Ecuador and Uruguay in 2020-2021, this guidance note also summarizes challenges and solutions, and lessons learned for the successful implementation of WBE.

## Utility of WBE for Covid-19

The utility of WBE depends on the type of pathogens to be monitored, the phase of an epidemic or pandemic, the availability of other data sets of disease activity, and the locations where wastewater testing is conducted. In the case of Covid-19, the infected individuals shed the SARS-CoV-2 virus in feces, as well as in respiratory tract systems, shortly after the beginning of their infection, even before they experience symptoms or even if they remain asymptomatic. Local public health officials can use WBE data to assess community-level infection trends and make informed decisions to help contain the spread of the virus in their communities. The

[8] "National Wastewater Surveillance System (NWSS) – a new public health tool to understand COVID-19 spread in a community." *Waterborne Disease & Outbreak Surveillance Reporting*. CDC, US. https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.html; "Acompanhamento dos Casos de Coronavirus (COVID19) Niterói." Niterói, Brazil. https://geoniteroi.maps.arcgis.com/apps/MapSeries/index.html?appid=1d5a2d1006094558a58be3db42c5475; "COVID-19 wastewater coalition." Canadian Water Network, Canada. https://cwn-rce.ca/covid-19-wastewater-coalition

[9] Amelie Ott. "Monitoring wastewater for COVID-19." *Post*. UK Parliament, UK. Dec. 15, 2020. https://post.parliament.uk/monitoring-wastewater-for-covid-19; "Virus particles in wastewater." *Coronavirus Dashboard*, Government of the Netherlands (Rijksoverheid), Netherland. https://coronadashboard.government.nl/landelijk/rioolwater; "Histórico Resultados y Tendencias EDAR VATar Covid-19." *EDAR evolution dashboard*. Ministry for the Ecological Transition and the Demographic Challenge. Spain. https://miteco.maps.arcgis.com/apps/dashboards/a8c1f281dfc445169a78178b70774a62

World Health Organization (WHO) summarized the major utility of WBE for Covid-19 to be monitoring circulation of SARS-CoV-2, detection of SARS-CoV-2 in locations with limited clinical surveillance, and early warning.[10] This section describes the utility of WBE across five applications: *temporal trend analysis, spatial trend analysis, incidence assessment, screening, and early warning*.

*Temporal trend analysis*: Because wastewater samples provide an inclusive measurement of the spread of Covid-19 among individuals who are connected to a sewer system, a temporal change of the virus concentration in wastewater reflects the true dynamics of the spread of Covid-19 in communities. This information can be used as an independent metric of Covid-19, together with other metrics such as case counts, hospitalization statistics, test positivity rates, to inform public health policies. For example, in the US, the Massachusetts Water Resources Authority, has been monitoring the amount of virus in wastewater in the Greater Boston area since March 2020. Wastewater samples are collected 3-7 times a week, and the testing results are shared on their publicly accessible dashboard.[11]

*Spatial trend analysis*: By collecting wastewater samples from multiple locations in a community, WBE can reveal spatial trends of disease activity. Spatial comparison of disease activity helps identify areas of concern that require targeted interventions. Information from neighboring communities also helps in predicting potential changes in disease burden in a target community. Spatial trend analysis can be applied to any geographic level. In the US, for example, at the city level, the City of Cambridge, MA, collects wastewater samples from four catchment areas across the city.[12] At the county level, the County of New Castle, DE, began wastewater testing in April 2020 and expanded it county-wide.[13] Many states have also started state-wide Covid-19 monitoring via wastewater testing.[14] At the national level, the Centers for Disease Control and Prevention (CDC) and the US Department of Health and Human Services (HHS) launched the National Wastewater Surveillance System (NWSS) across all 50 states in late 2020 in response to the Covid-19 pandemic.[15]

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[10] "Status of environmental surveillance for SARS-CoV-2 virus." *Scientific Brief*. WHO. Aug. 7, 2020. https://www.who.int/news-room/commentaries/detail/status-of-environmental-surveillance-for-sars-cov-2-virus

[11] "Status of environmental surveillance for SARS-CoV-2 virus." Massachusetts Water Resources Authority. https://www.mwra.com/biobot/biobotdata.htm

[12] "Weekly Municipal Wastewater Sampling Data." *Cambridge COVID-19 Data Center*. City of Cambridge, MA, US. https://cityofcambridge.shinyapps.io/COVID19/?tab=wastewater

[13] "New Castle County COVID-19 Wastewater Testing Dashboard." New Castle County, DE, US. https://compassred.shinyapps.io/ncco_wastewater/

[14] "Wyoming COVID-19 Wastewater Monitor." State of Wyoming, US. https://covidwastewatermonitor.wyo.gov; "COVID-19: Wisconsin Coronavirus Wastewater Monitoring Network." State of Wisconsin, US. https://www.dhs.wisconsin.gov/covid-19/wastewater.htm; "Ohio Coronavirus Wastewater Monitoring Network." State of Ohio. US. https://coronavirus.ohio.gov/wps/portal/gov/covid-19/dashboards/other-resources/wastewater

[15] "National Wastewater Surveillance System (NWSS) – a new public health tool to understand COVID-19 spread in a community." *Waterborne Disease & Outbreak Surveillance Reporting*. CDC, US. https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.htm; Jana Parsons and Scott Olesen. "A Review of Biobot's Successful Partnership with HHS and CDC." Biobot

*Incidence assessment*: Efforts to estimate the corresponding number of infected individuals from wastewater virus concentration are underway. Correlations between the virus concentration in wastewater and reported Covid-19 cases have been reported across continents. Accurate estimation of the disease incidence and the universal application of an estimation method are challenging without rigorous understanding of virus shedding dynamics and standardization of wastewater analysis methods. However, if caveated properly, rough estimation of the disease incidence based on wastewater analysis may provide useful information to local public health agencies especially in low-resource settings where public health data is scarce.

*Screening*: Wastewater analysis can be used as a screening method for the presence of a disease in a population inhabiting certain buildings. Schools, university dormitories, correctional facilities, nursing homes, and office buildings have started using wastewater testing as passive group-level Covid-19 testing for their building inhabitants.[16] As the economy opens up and people develop testing fatigue, the role of wastewater testing as a screening method for the presence of Covid-19 becomes more important in order to safely perform their activities. It should be noted, though, that negative wastewater testing results do not guarantee the absence of infections on site because not everybody deposits fecal material while within the confines of the building and because wastewater sampling may miss toilet flush signals if samples are pumped infrequently from sewer pipes.

*Early warning*: WBE can provide an early warning of disease emergence or disease resurgence as SARS-CoV-2 is shed in stool before the onset of Covid-19 symptoms. Testing of individuals for Covid-19 via PCR may take a while to deliver results or may even not be easily accessible, especially in LMICs. Routine wastewater testing can thus detect signals of increasing levels of disease before clinical data becomes available. WBE can also detect the introduction of new variants in a community (e.g., alpha, delta and lambda variants) by deploying genomic sequencing or targeted PCR on wastewater samples.

## Presentation of successful applications of WBE for Covid-19 in the US

The State of Wisconsin

The State of Wisconsin is testing wastewater samples across the state to monitor the spread of Covid-19. Wastewater samples are collected from wastewater treatment facilities from over dozens of large and small cities. The state aims to cover close to 60 percent of its residents via

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Analytics. Aug. 20, 2020. https://biobotanalytics.medium.com/a-review-of-biobots-successful-partnership-with-hhs-and-cdc-8503cf165598

[16] "Targeted Wastewater Surveillance at Facilities, Institutions, and Workplaces." Waterborne Disease & Outbreak Surveillance Reporting. CDC, US. https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/targeted-use-case.html

wastewater testing to understand state-wide trends of the spread of Covid-19 disease more accurately.

This project is led by the state government and is a collaboration between the Wisconsin Department of Health Services, the Wisconsin State Laboratory of Hygiene, and the University of Wisconsin-Milwaukee, as well as many participating wastewater treatment facilities. Successful application of WBE involves a wide range of activities, such as wastewater sample collection, lab analysis, data analysis, and data use. The state of Wisconsin made a concerted effort to coordinate all the relevant activities and developed a dashboard to help understand the spread of Covid-19 in their communities (Figure 1).

Temporal and spatial analysis of wastewater data is important to understand the spread of Covid-19 in communities. Because wastewater testing data is inherently complex, trends of Covid-19 spread need to be analyzed by looking at multiple data points over time or in comparison to data points in other locations. The testing results across time and space can be compared in the state dashboard.

Wastewater testing data is one metric of the spread of Covid-19, and public health decisions are made by looking at multiple indices, such as reported case counts, test positivity rates, and hospitalization statistics. The state's Covid-19 wastewater dashboard shows both SARS-CoV-2 concentrations in wastewater and the number of new cases per 100,000 people in the same area that is represented by the wastewater sample. Contextualizing wastewater data by comparing with other public health data sets helps make public health decisions.

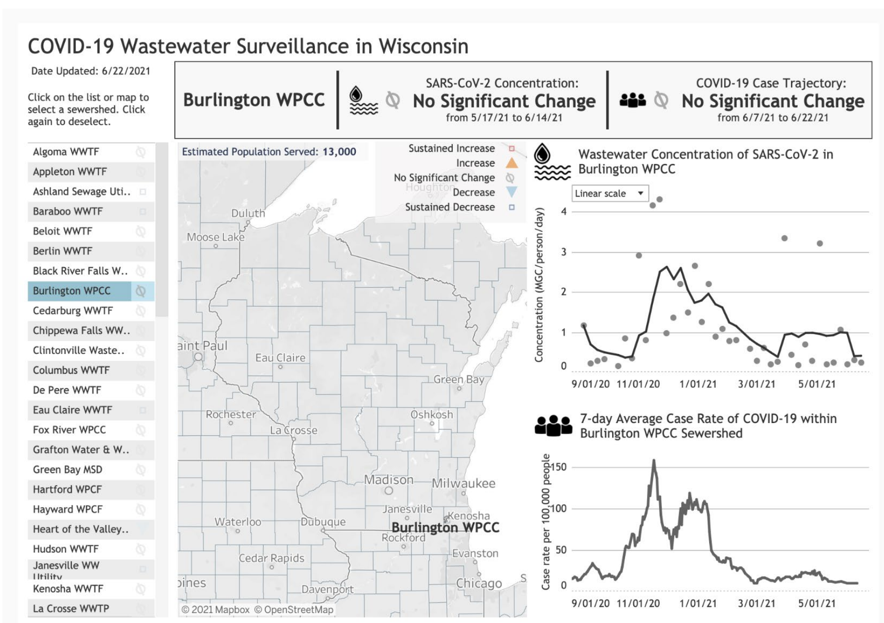

Figure 1. Covid-19 Wastewater Surveillance Dashboard[17] by the State of Wisconsin. The dashboard provides a summary of trends on the spread of Covid-19 as observed in both the change in wastewater virus concentration and in reported clinical cases (top). The list of participating wastewater treatment facilities can be found (left) and their data can be accessed by clicking on the location name. The geographic information of the testing locations is shown on the map (center). Time series of wastewater testing results are presented as the amount of SARS-CoV-2 virus in million gene copies per person per day (middle right). The wastewater testing results are compared against the reported number of clinical cases per 100,000 people in the same area that is represented by the wastewater sample (bottom right). The screenshot of the dashboard was taken on June 24th, 2021.

## The County of New Castle, Delaware

WBE can be applied at a more granular scale to understand the burden of Covid-19 in localized areas and to detect outbreaks in targeted communities. The County of New Castle in the State of Delaware conducts wastewater testing at multiple locations within the county, and different geographic scales including at the neighborhood level and at the building level.[18] Wastewater testing is led by the County Executive and is conducted in partnership with the University of Delaware Center for Environmental and Wastewater-based Epidemiological Research (CEWER). Covid-19 wastewater data from wastewater testing at the neighborhood level is used to strategically deploy mobile testing units and target vaccination efforts in the county. Wastewater testing can be conducted at an even more granular scale, at the building level, e.g., at hospitals and university dorms, to rapidly detect outbreaks of Covid-19 in vulnerable populations.

## The Cambridge Public School System, Massachusetts

In the State of Massachusetts, the Cambridge Public Schools (CPS) system is using wastewater data to help determine whether classes should be run in-person or fully remote.[19] Working with the Cambridge Public Health Department, the CPS system monitors the spread of Covid-19 in their community via various indicators: confirmed Covid-19 cases among students and staff in CPS, staff participation in Covid-19 testing, daily new cases in the community, test positivity rate in the community, and virus concentration in wastewater. Those indicators are updated daily on their dashboard. The CPS system has also worked with a multidisciplinary group of experts from local universities to develop guidelines for school opening. These guidelines evolved as new knowledge on the infectivity of the virus emerged.

[17] "COVID-19: Wisconsin Coronavirus Wastewater Monitoring Network." State of Wisconsin, US. https://www.dhs.wisconsin.gov/covid-19/wastewater.htm
[18] New Castle County COVID-19 Wastewater Testing Dashboard." New Castle County, DE, US. https://compassred.shinyapps.io/ncco_wastewater/
[19] "COVID-19 Data Dashboard." Cambridge Public Schools. https://www.cpsd.us/covid19data

From September through December 2020, the CPS system and a scientific advisory board developed a set of metrics and thresholds to open schools for in-person learning. Among existing indicators that reflect the spread of Covid-19, they chose to follow the following three metrics: daily new cases, positivity rates, and virus concentration in wastewater. Returning to in-person learning required at least two metrics to be below the recommended thresholds—new cases below 25 per day, positivity rate below 5%, and virus concentration in wastewater below 100 copies viral genomes per mL— for at least seven days (Figure 2).

In January 2021, the CPS system updated the guidelines for school opening, reflecting the new scientific evidence of the virus spread and at-risk population, and reconfirming the importance of in-person schooling in children's education.[20] The new scientific evidence supports that the risk of in-school transmission for students, educators, and staff can be brought to acceptably minimal levels under robust infection control strategies. The multidisciplinary group of experts recommended focusing on infection control and prevention to safely open schools even in the context of high community spread. Under the new guidelines, the three metrics, including wastewater data, continue to provide important information of community risks, but the decision of opening schools should not be in a binary yes/no based on the status of the metrics. The CPS system continues monitoring the community risk metrics, but the schools can remain open, provided that they strictly implement infection control strategies.

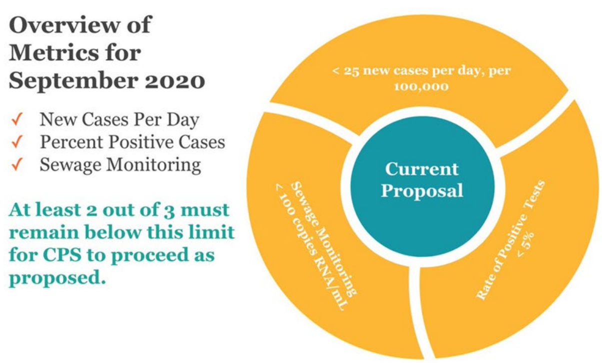


Figure 2. Illustration of Covid-19 community metrics used by the CPS system. From September to December 2020, they used the following three metrics to decide whether schools can be open for in-person learning: daily new cases, positivity rates, and virus concentration in wastewater. At least two out of the three metrics had to remain below recommended thresholds for at least seven days before schools could return to in-person learning. These three metrics are still being monitored as important indicators

[20] "Schools and the Path to Zero: Strategies for Pandemic Resilience in the Face of High Community Spread." Dec. 18, 2020. https://globalepidemics.org/wp-content/uploads/2021/06/SchoolsandthePathtoZero_v3.pdf

of the risk of Covid-19 in the community. However, the threshold values are no longer applied to make decisions about school opening since January 2021.

WBE provides a unique way of monitoring the spread of Covid-19 in communities and supports decision making, such as school opening. The way the CPS system has used wastewater data across different phases of the pandemic illustrates that WBE data needs to be interpreted in each context based on the best scientific knowledge available at the time. Collaborating with the local department of public health and the scientific advisory board, the CPS system has adapted the use of WBE data, considering the updated knowledge on the severity of the disease and at-risk population demographics, availability of interventions, and economic impact of policies. When WBE is implemented globally, the importance of developing appropriate guidelines on the use of WBE that fit each country's context becomes even more important.

## 2. Elements to consider when launching WBE in LMICs

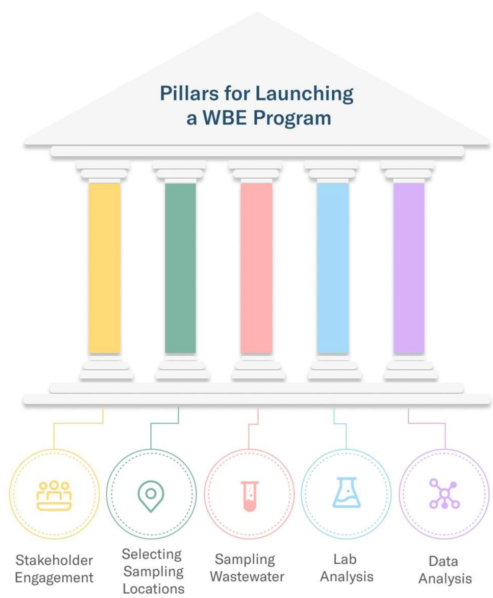


Figure 3. Five pillars for successful implementation of WBE in LMICs

### Stakeholder engagement

Successful implementation of WBE requires multi-stakeholder engagement. Once key stakeholders are identified, roles and responsibilities need to be clearly defined and coordinated at every step of the process (i.e., the collection of wastewater samples, the analysis of lab samples, the interpretation of the data, the decision making based on the data). It is also important to consider action plans upon specific wastewater analysis results or thresholds met (e.g., surge of wastewater virus concentration). Key elements for stakeholder engagement are discussed in this section: *stakeholder identification, transparency in responsibilities and decision making processes, and coordination of activities*.

*Stakeholder identification*: The owner of the project should identify key stakeholders to involve in the project for sample collection, lab analysis, data analysis, and data use. Key stakeholders include diverse groups of individuals and organizations who have technical expertise in WBE, facilities that operate sample collection and analysis, and users of WBE data. Once potential partners are listed, the project owner should communicate with them the goals and expectations of the project, the responsibility of each actor, and the financing arrangements to support the execution of the project before confirming their engagement in the project.

*Transparency in responsibilities and decision making processes*: Since WBE involves multi-stakeholder engagement, responsibilities and decision making processes need to be transparent to all stakeholders involved. It is important to agree at the start on programmatic details, such as a cadence and responsibility of activities involving in wastewater testing (e.g., sample collection, transportation, lab analysis, data analysis), and contingency plans for unforeseen events (e.g., sampling failure due to unfavorable weather events). To adapt to emergent needs for testing, decision making processes need to be established and points of contact for each activity need to be identified for prompt actions.

*Coordination of activities*: For timely analysis of wastewater samples and data use, WBE-related activities need to be coordinated. Coordination of the activities is especially important because sample collection, lab analysis, data analysis, and data use are often done by different stakeholders. Throughout the project, it is recommended that key stakeholders convene regularly to share progress updates and discuss challenges. The meeting frequency can be adapted to the various phases of the project, with more frequent meetings at the beginning of the project to set it up properly.

## Selecting sampling locations

Wastewater sampling locations need to be determined based on the sewage network and with data use in mind. Wastewater samples represent the health of individuals connected to a sewer network. Therefore, selecting wastewater sampling locations has a direct impact on defining the population monitored via wastewater testing. This section describes key elements to take into account when selecting sampling locations: *sampling location and granularity of data, and inclusive testing of populations*.

*Sampling location and granularity of data*: Sampling downstream at municipal wastewater treatment facilities is the most common application of WBE for Covid-19. It can effectively cover a large number of individuals and provides cost-effective monitoring of the spread of SARS-CoV-2 in communities. Sampling more upstream shrinks the population coverage per sample, but it provides spatial granularity of the data, helping to identify hotspots in a finer resolution. For example, sampling at pump stations or manholes offers data on the health status of a group of people that are in a more narrowly defined area than an entire city. Sampling at specific buildings, such as school dorms, hospitals, correctional facilities, office buildings, can provide early warning of disease outbreaks for even more targeted populations.

*Inclusive testing of populations*: In LMICs, vulnerable or remote communities may not be connected to sewer networks and therefore may not be represented in wastewater data. A program manager needs to select sampling locations as inclusive as possible to cover vulnerable populations with limited access to, or those with less developed, sewer systems. At the same time, sampling locations need to be chosen carefully to avoid stigmatizing a population that is represented in wastewater samples.

## Sampling of wastewater

Accurate analysis and interpretation of wastewater data start with an appropriate collection of wastewater samples. The project team should discuss and agree on the following six aspects: *frequency of sample collection, duration and frequency of pumping, sampling device selection, guidelines for safe and standardized sampling collection, reporting of sampling details,* and *responsibility and coordination for wastewater sampling.*

*Frequency of sample collection*: Recommended data collection frequency depends on the application of WBE, the availability of other public health data, and the phase of the pandemic. In general, a minimum of once a week sampling is recommended to monitor trends of Covid-19 in communities when samples are collected from municipal sewer systems. When wastewater testing is conducted at a building level (e.g., school dorms, nursing homes, correctional facilities, office buildings) to provide an early warning of disease outbreak in the facilities, it is recommended to sample wastewater a few times a week. Sampling frequency can be adjusted depending on the phase of the pandemic.[21] Increased sampling frequency is recommended when Covid-19 is spreading rapidly within the community. In practice, the sampling frequency may be constrained by the availability of resources for sample collection (e.g., autosamplers and human resources) and funding, especially in LMICs. However, the sampling schedule should be flexible enough to adapt to emergent needs for testing.

*Duration and frequency of pumping*: The gold standard of wastewater sampling for WBE is 24-hour composite sampling, where wastewater is pumped every hour or more frequently over 24 hours and mixed to compose a sample. Composite sampling is very laborious without an autosampler, though not impossible through collecting and compiling "grab" samples manually.

[21] Keshaviah, Aparna, et al. "Developing a Flexible National Wastewater Surveillance System for COVID-19 and Beyond." *Environmental Health Perspectives,* vol. 129, no. 4, National Institute of Environmental Health Sciences, 2021, p. 45002–, doi:10.1289/EHP8572.

Samples collected over a period shorter than 24 hours or through infrequent pumping may miss signals of SARS-CoV-2 and not be representative of disease activity in a target community. However, depending on the size of the sewer network, the travel time of wastewater, uniformity of the lifestyle of the residents, and prevalence of the disease, a relaxed sampling scheme with sampling duration shorter than 24 hours and pumping frequency less than once an hour may provide sufficient data for wastewater analysis.[22] Resource availability and intended use of data also need to be considered when the duration and frequency of pumping are determined.

*Sampling device selection*: In LMICs, access to autosamplers can be constrained due to limited financial resources and supply chain concerns. Commercial autosamplers cost approximately USD 5,000 - 10,000 when purchased in the US. Sourcing autosamplers in LMICs may cost more due to import and value added tax (VAT) with prolonged transit times to intended sampling sites. Autosampler theft is also a concern when unattended sampling is conducted outdoors. Depending on sampling locations, sample collection frequency, duration and frequency of pumping mentioned above, manual composite or grab samples are an acceptable alternative in resource-limited settings.[23] If the grab sampling method needs to be used, it is recommended to confirm no significant difference is found between 24-hour composite sampling and grab sampling before starting a monitoring program.

*Guidelines for safe and standardized sampling collection*: Although wastewater sampling is not very arduous, it is important to collect samples following pre-established guidelines. The guidelines should define methods for sample collection (e.g., frequency of sampling collection, duration and frequency of pumping), transportation, preservation, and storage with appropriate safety protocols. Clear guidelines help wastewater sampling to be carried out in a safe and consistent manner. It is also recommended to provide training on wastewater collection for the technical staff at the beginning of the project, especially when they are not familiar with wastewater sample collection. When different individuals are involved in sample collection and transportation, responsibilities of each need to be defined to coordinate sampling.

*Reporting of sampling details*: To properly analyze sample results, information of sample collection needs to be reported for each sampling event. Key information includes: sampling location, sampling date and time, sample collection type (e.g., composite sample, grab sample),

[22] "Buenos Aires Water Supply and Sanitation with a Focus on Vulnerable Areas Program. (PROGRAM-FOR-RESULTS) (P172689)." *Final Technical Assessment.* The World Bank. http://documents1.worldbank.org/curated/en/838451612358362292/pdf/Final-Technical-Assessment-BUENOS-AIRES-WATER-SUPPLY-AND-SANITATION-WITH-A-FOCUS-ON-VULNERABLE-AREAS-PROGRAM-P172689.pd

[23] "Buenos Aires Water Supply and Sanitation with a Focus on Vulnerable Areas Program. (PROGRAM-FOR-RESULTS) (P172689)." *Final Technical Assessment.* The World Bank. http://documents1.worldbank.org/curated/en/838451612358362292/pdf/Final-Technical-Assessment-BUENOS-AIRES-WATER-SUPPLY-AND-SANITATION-WITH-A-FOCUS-ON-VULNERABLE-AREAS-PROGRAM-P172689.pd

sample matrix (e.g., raw wastewater, treated wastewater), and flow rate.[24] The sampling details need to be reported systematically and shared with the entities that conduct lab analysis and data analysis in a timely manner.

*Responsibility and coordination for wastewater sampling*: A project manager should communicate the purpose of the program and the responsibility of each person involved in sample collection. Depending on the entity responsible for wastewater sampling and the structure of the contracts, sampling of wastewater can be conducted by an operator of sewer infrastructure (public or private), an external engineering contractor (private), or the same lab responsible for sample analysis (public or private). To facilitate routine sample collection, sampling operations need to be coordinated between a project manager, an operator of sewer systems, and a lab responsible for sample analysis.

## Laboratory analysis of wastewater

Although the techniques used in WBE (e.g., PCR for biological information including the detection of SARS-CoV-2, Mass Spectrometry for chemical information) are not novel in standard labs, WBE requires laboratory methods tailored for its own application. Lab methods need to be developed to enable processing of wastewater samples (e.g., wastewater filtration, concentration), quantification of target concentrations (e.g., qPCR, ddPCR), and correction of data to account for dilution. Biobot Analytics and the World Bank have put together detailed guidelines "Covid-19 WBE Lab Capacity Building Process" for labs in LMICs to build capacity to detect and analyze trends of SARS-CoV-2 in wastewater (Annex 3). This section describes the most important steps for developing lab analysis capacity: *identifying labs for wastewater analysis, selecting lab methods,* and *developing lab methods for trend analysis.* All of these steps need to consider the existing capacity of local labs, the cost of equipment and analysis, existing lab processes, and local supply chains.

*Identifying labs for wastewater analysis*: To conduct wastewater analysis, laboratories must have standard microbiological practices in place that are appropriate for wastewater analysis. Wastewater samples are considered an infectious substance that presents a relatively low risk of causing severe disease. To date, there is no evidence that wastewater samples cause Covid-19 through direct exposure, but wastewater samples need to be treated with caution as they may contain other pathogens. To analyze wastewater safely, it is recommended to conduct all laboratory work in a biosafety Level 2 (BSL2) or equivalent lab, and all laboratory staff should be trained accordingly. Laboratories identified to develop capacity for performing wastewater analysis must have access to some key equipment, such as refrigerated centrifuge machines, qPCR/ddPCR machines, and biosafety cabinets, as well as technical staff with prior experience in

[24] McClary-Gutierrez, Jill S., et al. "Standardizing Data Reporting in the Research Community to Enhance the Utility of Open Data for SARS-CoV-2 Wastewater surveillance." *Environmental Science Water Research & Technology*, vol. 7, no. 9, 2021, pp. 1545–51, doi:10.1039/d1ew00235j.

molecular biology, especially with PCR technology, prior to starting. Sourcing equipment in LMICs can be a costly and lengthy process, and hence selection of partner labs needs to consider availability of these key equipment to minimize project cost and delay. Availability of key equipment also indicates that the labs are familiar with operations using these equipment. In addition to these technical qualifications, a project manager should explain the purpose of the project and clarify financial structure to the analysis labs, and confirm the labs' willingness to participate in the program.

*Selecting lab methods*: Wastewater analysis for SARS-CoV-2 using the qPCR/ddPCR technique typically requires multiple sample preparation processes, such as virus inactivation, filtration, virus concentration, viral RNA extraction, and quantification of the amount of RNA. Various methods and commercial kits exist for each of these steps with varying degrees of efficacy for SARS-CoV-2 analysis in wastewater.[25] Kit-free methods can also be considered as a low throughput but inexpensive option.[26] Lab methods selected for each step of the analysis must be recognized in peer-reviewed studies and possible to implement in the local lab setting taking into account the availability of equipment in their facilities, the experience of the technical staff, the cost of the analysis, and the procurement and supply chain processes associated with the required items. Equipment and reagents required for the analysis need to be identified early in the process because sourcing and transport of these items may be delayed due to supply chain challenges in LMICs.

*Developing lab methods for trend analysis*: Developing reliable lab methods involves establishing quantification methods, implementing a normalization target to enable trend analysis, verifying lab methods, and implementing quality control measures. Once the labs develop methods for virus detection, they should also establish methods to quantify the amount of virus in wastewater samples (i.e., standard curves for qPCR analysis) to analyze trends on the spread of Covid-19 in space and time. In addition to quantifying the target virus of SARS-CoV-2, developing the capacity for quantifying a normalization biomarker is important to correct for dilution of samples. Pepper mild mottle virus (PMMoV) and cross-assembly phage (crAssphage) are the most common normalization biomarkers used for wastewater analysis for SARS-CoV-2. Once the lab methods are developed, they must be validated internally or externally for sensitivity and precision of the analysis before starting to generate data for use. Finally, to continue generating reliable data, it is recommended that QA/QC procedures are implemented in every batch of wastewater analysis.

[25] Ahmed, Warish, 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." *The Science of the Total Environment*, vol. 739, Elsevier B.V, 2020, p. 139960–, doi:10.1016/j.scitotenv.2020.139960.
[26] Whitney, Oscar N., et al. "Sewage, Salt, Silica, and SARS-CoV-2 (4S): An Economical Kit-Free Method for Direct Capture of SARS-CoV-2 RNA from Wastewater." *Environmental Science & Technology*, vol. 55, no. 8, American Chemical Society, 2021, pp. 4880–88, doi:10.1021/acs.est.0c08129.

### Data analysis and data interpretation

For wastewater testing data to be most useful and actionable for decision makers, raw wastewater laboratory data needs to be further analyzed in a way that serves the intended applications. This section describes key factors to consider for analysis and interpretation of wastewater testing data: *conversion and correction of raw laboratory data,* and *understanding sewer networks*.

*Conversion and correction of raw laboratory data*: Recommended standard data analysis includes conversion of raw laboratory data to virus concentration in wastewater, correction for dilution, and correction for different lab methods. Raw laboratory data (i.e., cycle threshold "Ct" values from qPCT thermocycler machines) needs to be converted into a concentration of targets (e.g., viruses, chemical analytes) in wastewater. To assess temporal or spatial trends of the disease properly, the impact of different dilution factors and contributing population size need to be calculated and corrected for. If wastewater testing results are compared across different labs and lab methods, the data needs to be corrected for the difference of the lab processing efficiency. To perform these data correction steps, lab testing results, metadata of samples, and lab protocols need to be documented systematically. Furthermore, additional data analysis and data interpretation may need to be performed in order to achieve the intended goals of data use. Comparison of wastewater data against other public health metrics (e.g., number of clinical cases, test positivity rates, number of hospitalizations), and modeling of disease incidence and prevalence are examples of additional data analysis.

*Understanding sewer network*s: Wastewater testing results need to be interpreted in the context of the corresponding catchment areas. A catchment area, also known as a sewershed, is the area covered by a wastewater sample and it is defined by the sewer network. Therefore, in order to interpret wastewater testing results properly, the information of the sewer network needs to be obtained as accurately as possible. In LMICs, accurate information of functioning sewer networks may not be easily available. However, it is critical to identify which populations are connected to the sewer network and therefore represented in wastewater samples. The percentage of population connected to the sewer network, the presence of large hospitals and industrial complexes in the catchment areas, and the dilution of sewage by rainwater or groundwater also provide useful information when wastewater results are compared across multiple locations.

## 3. Country case studies

### Experience in Ecuador

Background

In March 2020, Ecuador was one of the worst-hit countries in the world by Covid-19, and Guayaquil was the epicenter of the outbreak. In the Guayas Province, where Guayaquil is located, reported case counts reached over 4,300 per week in a population of 3 million.[27] The Covid-19 pandemic subsided in Guayas afterward, but spread nationwide. In August 2021, Ecuador's death toll had reached over 30,000, according to government estimates.[28] Similar to many other countries, reporting delays of Covid-19 cases were evident in Ecuador (Figure 4). The delay in reporting made it difficult for the government to assess the true scale of the Covid-19 pandemic in real time, hindering effective interventions to stay ahead of the virus. The World Bank and Biobot Analytics initiated WBE capacity building activities in Guayaquil in late June 2020. The goals of the activities were (1) to build capacity of local partner labs to perform wastewater testing for Covid-19, and (2) to develop a technical guidance note describing how to support the development of WBE in LMICs.

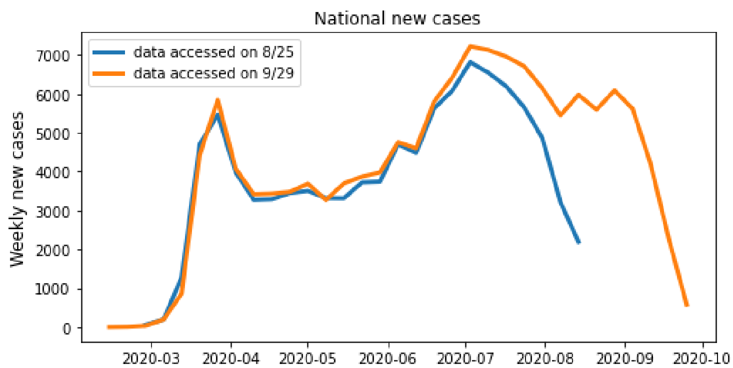


Figure 4. Number of reported new cases in Ecuador. The blue line is based on data accessed on August 25, 2020. The orange line is based on data accessed on September 29, 2020. The deviation between the two lines indicates the significance of reporting delays. The data was obtained from El Servicio Nacional de Gestión de Riesgos y Emergencia[29].

Stakeholders involved

To successfully implement WBE capacity building activities in Ecuador, the World Bank and Biobot Analytics worked with four main stakeholders in Ecuador. First, the Empresa Municipal de Agua Potable y Alcantarillado de Guayaquil (EMAPAG), a municipal water authority in Guayaquil which oversees water and wastewater activities. With our project, EMAPAG was responsible for providing financial support for wastewater analysis and coordinating the activities. Second, Interagua, a private concessionary that operates, administers, and maintains Guayaquil's drinking water, wastewater, and rainwater drainage systems under a 30-year contract with EMAPAG.

[27] "Situation Reports (SITREP) and Infographics - COVID 19 - Start of alert: February 29, 2020." The National Risk and Emergency Management Service (SNGRE). Ecuador, https://www.gestionderiesgos.gob.ec/informes-de-situacion-Covid-19-desde-el-13-de-marzo-del-2020/

[28] "SITUACIÓN NACIONALPOR COVID-19 -INFOGRAFÍA N°512 -23/07/2021Inicio 29/02/2020." The National Risk and Emergency Management Service (SNGRE). Ecuador, https://www.gestionderiesgos.gob.ec/wp-content/uploads/2021/07/INFOGRAFIA-NACIONALCOVID19-COE-NACIONAL-08h00-23072021.pdf

[29] "Situation Reports (SITREP) and Infographics - COVID 19 - Start of alert: February 29, 2020." The National Risk and Emergency Management Service (SNGRE). Ecuador, https://www.gestionderiesgos.gob.ec/informes-de-situacion-Covid-19-desde-el-13-de-marzo-del-2020/

Interagua was responsible for selecting sampling locations, providing information of catchment areas, and collecting wastewater samples. Third, Biobot Analytics partnered with the Escuela Superior Politécnica del Litoral (ESPOL), a local research university that also engages in service contracts. ESPOL joined the project to participate in the laboratory analysis phase of our activities. Lastly, Biobot Analytics worked with the el Comité de Operaciones de Emergencia (COE) Cantonal, the emergency operations committee. Since the start of the Covid-19 pandemic, the main entity responsible for the Covid-19 response in Ecuador has shifted from the national emergency operations committee, to the COE Provincial, and most recently to the COE Cantonal.

Summary of activities

- **Stakeholder identification**: In this program, the following major activities and associated stakeholders were identified:
    - wastewater sample collection (Interagua)
    - wastewater sample analysis (ESPOL)
    - financial support for WBE (EMAPAG)
    - WBE data use (COE Cantonal)

  Biobot Analytics supported the implementation of WBE, including by providing technical guidance for the selection of sampling locations and sampling methods, lab capacity building for sample analysis, data analysis and data interpretation. Within ESPOL, laboratory work was separated into two sections, preprocessing of wastewater and molecular biology work for quantification. A lab to conduct molecular biology work was easily identified because ESPOL already directed a lab that conducts PCR analysis for clinical testing purposes. On the other hand, identifying a lab for sample preprocessing was challenging because no existing labs had the facilities required for wastewater preprocessing. The COE Cantonal was identified as a potential end-point beneficiary of the WBE data. The COE Cantonal requested further demonstration of data to confirm their engagement.

- **Capacity building for sample collection:** Biobot Analytics provided technical guidance for wastewater sample collection. Biobot Analytics and Interagua worked together to select sampling locations and sampling methods. Three sampling locations were selected in Guayaquil to cover distinct locations within the city and to cover a large fraction of the population (Figure 5). The three selected locations covered about 53% of the population. Considering the catchment size and the availability of resources, 24-hr composite samples were collected manually on the hour. Interagua owned autosamplers but had an issue with adequate batteries to conduct automatic sample collection. Biobot Analytics also advised Interagua to log key sampling information, such as sample ID, sampling location, sampling date, sampling methods, and flow rate.

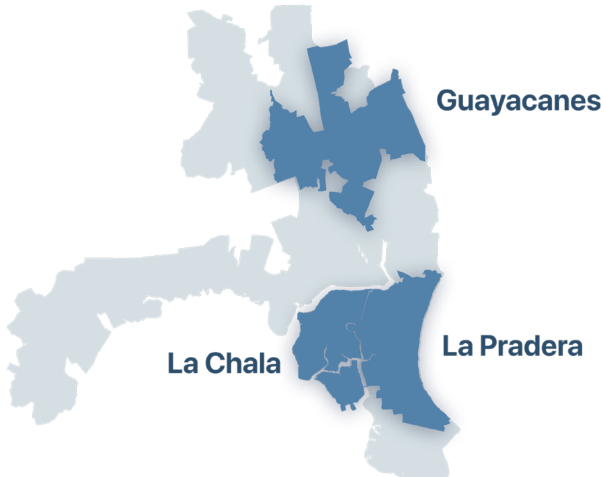


Figure 5. Map of three sampling locations (Guayacanes, La Pradera, La Chala) selected in Guayaquil. The catchment areas for the sampling locations are delineated in blue and the city of Guyaquil is shown in gray.

- **Pilot sample collection and analysis:** A total of 18 wastewater samples were collected from 3 locations in Guayaquil over 6 weeks from July 23, 2020 to August 25, 2020. The samples were collected by Interagua and analyzed by Biobot Analytics for the purpose of baseline data analysis and quality control. SARS-CoV-2 virus in wastewater was quantified in all of the 18 samples collected.

- **Capacity building for sample analysis:** Extensive lab capacity building for sample analysis was conducted with weekly technical guidance meetings. ESPOL and the Biobot Analytics team met virtually once a week to discuss identification of laboratory methods and development of quantification methods of viral titers. A number of experiments were conducted by ESPOL, and the design and the results of the experiments were reviewed jointly with Biobot Analytics.

- **Method Verification:** Once wastewater sample analysis methods were developed, Biobot Analytics and ESPOL conducted the Method Verification test to validate the sensitivity and precision of the methods. Biobot Analytics sent three test samples in duplicates to ESPOL. ESPOL analyzed the samples using the lab methods they established. The test results were evaluated according to the Method Verification criteria described in the "Conduct method verification test" part of the Covid-19 WBE Lab Capacity Building Process document (Annex 3).

For more details, see Phase I & II Executive Review, and Covid-19 WBE Lab Capacity Building Process in the Annex.

Summary of results

- **Capacity building:** Laboratory methods were identified by ESPOL. ESPOL developed virus quantification methods with guidance from Biobot Analytics. The Method Verification test was conducted and ESPOL successfully passed the test. As a result of the project, ESPOL developed the capacity to reliably detect and quantify SARS-CoV-2 virus in wastewater.

- **Data analysis**: SARS-CoV-2 was detected in all of the wastewater samples. The concentration of the virus suggests higher incidence of Covid-19 cases than reported, which suggests insufficient testing, under-reporting of Covid-19 cases, or delays in reporting.

- **Data use**: In September 2020, EMAPAG, the COE Cantonal, Interagua, the World Bank, and Biobot Analytics met for the first time to present the WBE activities performed in Guayaquil. Interagua and Biobot Analytics presented our work to EMAPAG and the COE Cantonal. Both EMAPAG and the COE Cantonal showed interest in the use of wastewater-based Covid-19 data, especially in wastewater-based case estimates. Because WBE is a new tool in public health, using WBE for decision making requires proper understanding of the data characteristics and limitations. EMAPAG and the COE Cantonal requested more examples of use cases and guidelines to leverage WBE data for public health decision making.

- **Other**: At the end of the project, ESPOL signed a one-year contract with EMAPAG to conduct 520 samples analysis in different areas of Guayaquil. This contract is a testimony that the wastewater testing infrastructure in Ecuador possesses sufficient laboratory capacity and local leadership.

For more details, see Phase I & II Executive Review in the Annex.

## Experience in Uruguay

Background

Uruguay had been successful in preventing the spread of Covid-19 since the beginning of the pandemic through September 2020, when reported cases in the whole country were typically under 10 cases per day.[30] Since the mayoral elections in October 2020, cases started to rise and reached nearly 500 cases per day by the end of 2020.[31]

The World Bank and Biobot Analytics initiated WBE capacity building activities in Uruguay in early July 2020. The project start date was met with delays because of the difficulties in establishing

[30] Center for Systems Science and Engineering, Johns Hopkins University
[31] Center for Systems Science and Engineering, Johns Hopkins University

the institutional arrangements between in-country stakeholders. The goals of the activities were (1) to build capacity of local partner labs to perform wastewater testing for Covid-19, and (2) to develop a technical guidance note describing how to support the development of WBE in LMICs.

Stakeholders involved

To successfully implement WBE capacity building activities in Uruguay, the World Bank and Biobot Analytics worked with three main stakeholders. First, Dirección Nacional de Aguas (DINAGUA), the Federal water resource institution responsible for setting up the water and sanitation policy in Uruguay. It was the entity responsible for project coordination. Second, Parque Científico y Tecnológico de Pando (PCTP), a public science and technology park that links the needs of the business, scientific, research, and technology sectors within Uruguay's National Innovation Systems. PCTP led all lab-related aspects of the partnership. Third, the Instituto Polo Tecnológico de Pando (IPTP), an institute of the National Uruguay University's Faculty of Chemistry. PCTP was a subcontractor of IPTP. Wastewater sampling and analysis were conducted by IPTP.

Summary of activities

- **Stakeholder identification**: In this program, the following major activities and associated stakeholders were identified:
    - wastewater sample collection (IPTP)
    - wastewater sample analysis (IPTP)

Biobot Analytics supported the implementation of WBE, including by providing technical guidance for the selection of sampling locations and sampling methods, lab capacity development for sample analysis, data analysis, and data interpretation.

- **Capacity building for sample collection:** Biobot Analytics provided technical advice for wastewater sample collection. Prior to this project, IPTP already had experience collecting wastewater samples for pharmacological analysis. Biobot Analytics and IPTP discussed the frequency of sampling and additional chemical and biological properties of samples to be collected to improve the quality of data analysis (e.g., temperature, pH, salinity, biological oxygen demand, total suspended solid).

- **Pilot sample collection and analysis**: A total of 18 wastewater samples were collected from 6 locations across the country over three time points (August - October 2020). When the project started, Uruguay had very few reported Covid-19 cases, which led to the expectation that wastewater samples were mostly below the limit of detection for lab analysis. Therefore, IPTP selected the six sampling locations that were expected to have higher rates of SARS-CoV-2 positive wastewater samples, both near the capital city and near the border with Brazil (Figure 6). The samples collected from across the country by

IPTP were analyzed by both IPTP and Biobot Analytics for the purpose of baseline data collection and quality assessment.


Figure 6. Map of six cities where samples were collected from (Bella union, Artiga, Rivera, Canelones, Pando, Cuidad de la costa) in Uruguay. The location of those cities is highlighted in blue on the map of Uruguay (gray). The actual catchment areas do not necessarily correspond with the city boundary.

- **Capacity building for sample analysis**: Biobot Analytics and IPTP partnered in lab capacity building activities. Several virtual meetings and collaborative exchanges were conducted to discuss lab sample processing methods, quantification of SARS-CoV-2 in wastewater, and implementation of quality control methods. IPTP's ability to detect SARS-CoV-2 in wastewater was assessed by comparing analysis results by IPTP and Biobot Analytics.

For more details, see Phase I & II Executive Review in the Annex.

<u>Summary of results</u>

- **Capacity building:** Laboratory methods were identified by IPTP with guidance from Biobot Analytics. IPTP's ability to detect SARS-CoV-2 in wastewater was confirmed by comparing analysis results by IPTP and Biobot Analytics: detection status matched with 14 out 18 samples (3 detects and 11 non-detects), while the other 4 samples had inconclusive results.

- **Data analysis:** Increased detection of SARS-CoV-2 in wastewater corresponded with the increase in reported Covid-19 cases in Uruguay. One of the challenges identified in interpreting the data was to obtain accurate population estimates for some sampling sites. Without information on sewage networks, estimating catchment areas and associated population size was challenging.

- **Data use**: IPTP and Biobot Analytics presented our work to DINAGUA and the Ministry of Health – Department of Epidemiology in a virtual meeting. The potential of WBE implementation was also presented and discussed in the meeting.

For more details, see Phase I & II Executive Review in the Annex.

## 4. Challenges to implementing WBE in LMICs and their corresponding solutions

### Technical considerations

- **Lab analysis:** Global implementation of WBE requires guidelines for developing and verifying lab methods for WBE. Although wastewater testing has been used for decades across the world, it has been conducted mainly in localized settings and often by academic labs. Global guidelines for developing lab analysis capacity and standardized lab methods for wastewater testing have yet to be established. Although there are numerous published papers to reference, and commercially available analytical kits for part of the wastewater analysis process, it is difficult to select and develop methods from scratch in a lab that does not have prior experience in WBE. Therefore, it is important to bring in technical expertise to guide the development of laboratory methods. The experts' role is to guide the selection of methods, while understanding the local labs capacity and prior experience, and to design key experiments to establish reliable quantification methods. It is not advised that experts recommend one specific method, regardless of the local labs capacity and prior experience, unless certain lab methods become standardized and accepted globally and associated reagents become widely available in the future. Instead, it is important to prepare a list of key steps in developing lab methods and set performance criteria or milestones to pass in order to evaluate the capacity of the local lab. In this project, Biobot Analytics developed protocols (Annex 3), and these were used to guide capacity building for the partnering labs.

- **Data interpretation:** Raw data from wastewater analysis (i.e., Ct value from qPCR) can have limited value as a standalone number for policy makers. Raw data needs to be converted to wastewater concentration levels, and ideally to an estimated number of new infections. No advanced expertise is required to calculate conversion factors that convert the raw qPCR data to wastewater concentration levels. However, detailed written protocols of the analysis procedures, such as standard operating procedures (SOPs), should exist to calculate the conversion factors accurately. When sample analysis is conducted at multiple lab sites (e.g., sample preprocessing by one lab and qPCR analysis by another lab), cross checking of the analysis procedures are particularly important. Reliable and accurate methods of converting wastewater concentration to a corresponding number of new infections have not yet been established,[32] but research and development efforts are underway.[33] If a case estimate is made based on wastewater data, it needs to be communicated with caveats.

In addition, wastewater data contains inherent variability due to uncertainty in flow rate, population size, amount of virus shedding in infected individuals, and lab processing efficiency. Therefore, it is important not to draw conclusions about "trends" based on a few data points. Instead, it is recommended to look for a consistent trend across multiple data points. Uncertainty of results can be reduced by applying a few techniques such as running samples multiple times and taking an average to reduce lab variability; pumping samples frequently from the wastewater stream (e.g., every 15 minutes instead of every hour); and conducting sample collection frequently (e.g., every day instead of every week). It is also recommended to establish error bars for analysis results in order to interpret the data more accurately.

Wastewater testing data is one metric available to track the spread of Covid-19, and public health decisions are made by looking at multiple metrics. Each metric has its own drawbacks, such as slow data turnaround time, bias of measurement, and variability of data. Wastewater testing data complements public health data sets, but it also has its limitations. For example, wastewater contains an early warning signal of the spread of Covid-19; however, the information is less useful if wastewater testing labs are not set up for quick data turnaround. Wastewater testing provides an inclusive measurement of the spread of Covid-19 in a community because it reflects the health status of all infected individuals, independently of whether they are experiencing symptoms or have access to healthcare; however, the data represents only the individuals who are connected to a sewer system. Wastewater testing also contains data variability as mentioned above, similar to other datasets (e.g., daily variability in reported cases).

- **Understanding of sewer networks**: Wastewater samples represent the health status of individuals that are connected to the sewer network that are upstream of the sample collection sites. Therefore, it is important to understand the sewage networks to accurately appraise the population included and excluded in each sample. In LMICs, a map of sewer systems may not be readily available, or closed sewer systems may not

[32] "National Wastewater Surveillance System (NWSS) – a new public health tool to understand COVID-19 spread in a community." *Waterborne Disease & Outbreak Surveillance Reporting*. CDC, US. https://www.cdc.gov/healthywater/surveillance/wastewater-surveillance/wastewater-surveillance.htm

[33] Noriko Endo. "Mining wastewater data to refine COVID-19 case estimation." Biobot Analytics. Sep. 11, 2020. https://biobotanalytics.medium.com/mining-wastewater-data-to-refine-Covid-19-case-estimation-8dc58d67f706

even exist. Vulnerable populations may not be connected to a sewer system, and as a result may not be represented in wastewater samples. Evaluation of the feasibility of WBE implementation should take into account the coverage of sewer networks and whether the networks are well documented in a database.

## Supply chain

- **Sampling device:** Collection of representative wastewater samples requires frequent pumping of wastewater (e.g., every 15 minutes or every hour) over 24 hours, which is laborious without using an autosampler. Purchasing autosamplers is possible in many countries, but delivery may be delayed due to supply chain challenges. Commercial autosamplers cost USD 5,000 - 10,000 when purchased in the US. Sourcing autosamplers in LMICs may require additional VAT. The use of autosamplers is not limited to WBE testing; autosamplers are useful in collecting wastewater samples for routine testing of operation performance at wastewater treatment facilities. In the US, most wastewater treatment facilities have autosamplers on-site and use them regularly to collect 24-hour composite samples for various types of testing.
- **Lab reagents and consumables:** Key reagents and consumables for wastewater testing for Covid-19 may not be readily available in LMICs. Sourcing some of those items may be arduous and expensive, especially in the middle of a pandemic when supply chains are very constrained. When lab methods are identified, it is important to consider local availability of lab reagents and consumables.
- **Lab equipment**: WBE requires lab analysis to be conducted in a biosafety Level 2 (BSL2) laboratory for safe operations. Additional equipment may need to be purchased for Covid-19 wastewater analysis. These include biosafety cabinets, a refrigerated centrifuge, and thermocyclers. Procurement of such equipment can be a costly and lengthy process, which may delay the project.

## Institutional arrangements

WBE requires the engagement of multiple stakeholders, including analytical labs, wastewater utilities, departments of health, and local municipalities. Depending on a given country's setting, financing can come from wastewater utilities, local municipalities, or national governments, and the primary user of the data may not be the same as these funding agencies. Therefore, it is critical that clear leadership and ownership exist in the country that implements WBE and that the implementation efforts be well-coordinated. Development agencies, such as the World Bank, can help identify key stakeholders and owners of the projects.

# 5. Lessons learned from country experiences in WBE implementation

## Sampling of wastewater

If wastewater autosamplers are not readily available, sampling can be conducted manually every hour over 3 to 24 hours. Manual sampling is laborious, but feasible especially during a pilot study. Once a sustained WBE program is implemented medium- to long-term, it is recommended to source autosamplers to facilitate wastewater sample collection efforts. Not only do autosamplers reduce manual labor but they also allow higher pumping frequency than manual sampling, which improves the data quality.

## International shipping of wastewater samples for quality control

During this program, a few batches of wastewater samples were shipped from Ecuador and Uruguay to the US, and from the US to Ecuador, and international shipping of wastewater samples was met with barriers. At the beginning of the program, Biobot Analytics received wastewater samples from Ecuador and Uruguay for the purpose of baseline data analysis and quality control. Baseline data analysis was conducted to present the utility of wastewater analysis to stakeholders of the program and to start providing training on data use and interpretation. Although these presentations and training could have been done using data from other regions, using local data is generally more powerful in gaining engagement from key stakeholders.[34] Conducting wastewater testing for quality control is also important to ensure the wastewater samples do not have a unique matrix (e.g., high salt content) that affects the lab analysis. Biobot Analytics also shipped a set of samples from the US to Ecuador to conduct a Method Verification test (Annex 3). Shipping wastewater samples across international borders is currently indispensable. However, as the utility of WBE becomes more widely accepted and deployed in LMICs, and lab methods become more standardized, the necessity for international shipping of wastewater samples may become less critical.

## Responsibility and coordination for sampling

Based on the experiences in Ecuador and Uruguay, one key learning is that sample collection needs to be supported by, and coordinated with, wastewater utilities. In Ecuador, the sampling process went smoothly thanks to an initiative spearheaded by Interagua—an operator of wastewater facilities in Guayaquil—and samples were transferred either to Biobot Analytics or to

[34] Jill S. McClary-Gutierrez, et al. "SARS-CoV-2 Wastewater Surveillance for Public Health Action." Emerging Infectious Diseases, vol. 27, no. 9, Centers for Disease Control and Prevention, 2021, pp. 1–8, doi:10.3201/eid2709.210753.

ESPOL relatively quickly. In Uruguay, on the other hand, sampling was the responsibility of IPTP—a research lab—and it was a more laborious process partly because samples were collected from across the country. It is important to partner with wastewater utilities for wastewater collection and to coordinate activities of sample collection and sample analysis effectively. During the project, Biobot Analytics coordinated sample collection and sample analysis activities. Moving forward, it is recommended to establish a routine sampling schedule (e.g., sampling every Monday) and points of contact in both wastewater utilities and research labs.

## Laboratory analysis of wastewater

Developing lab capacity to perform wastewater testing for Covid-19 was one of the main goals of the project, and several important lessons were learned.

Firstly, in choosing partner labs, it is important to locate labs that already own key equipment to the extent possible. Possession of key WBE equipment is an indicator of having performed similar lab analyses. In addition, procurement of necessary equipment can delay implementation of WBE significantly.

Secondly, lab methods for WBE analysis need to be selected considering prior experience of partner labs, availability of equipment, and local supply chains. No single best or standard lab methods exist to date for WBE, and many methods are practically acceptable. Although standardization of methods is important, it is not recommended that partner labs automatically adopt methods that are used by external consultants without taking into account the above considerations because the availability of resources and supply chains are different from country to country, particularly in LMICs.

Thirdly, selected lab methods need to be evaluated for reliability of data based on the performance of the lab analysis. The evaluation criteria can include sensitivity and precision of these methods.

Lastly, establishing recurrent progress update meetings is critical both to track work progress and to motivate achieving meeting milestones and deadlines. In this program, ESPOL, Biobot Analytics, and the World Bank met virtually on a weekly basis to discuss the timeline of methods development, experimental design, and experimental results from the midpoint of the project onwards. When meetings were scheduled on demand at the beginning of the project, the timeline of the work tended to get pushed back. Ideally, the meetings need to include the point of contact of the analysis lab, the external consultant, and an in-country program manager.

### Data interpretation and data use

For Covid-19 wastewater analysis, it is recommended that the data is communicated in terms of the concentration of SARS-CoV-2 virus in wastewater instead of raw data from the lab analysis (i.e., Ct value from qPCR). To this end, standard curves need to be established to convert the raw data to a concentration value. The data interpretation may be difficult especially when the variability of data is large. Frequent pumping using an autosampler, establishing standard operating procedures, and applying normalization methods can reduce data variability.

Wastewater data can provide an inclusive and independent estimate of the scale of infectious diseases at the population-level, in near-real time. In Ecuador, similar to many other LMICs, significant delays in reporting of Covid-19 cases was evident, making it difficult to make optimal public health decisions. Wastewater analysis results can complement public health data sets and provide independent and accurate trends of Covid-19 disease activity in a cost-effective and time efficient manner.

### Stakeholder engagement

Successful implementation of WBE requires engagement of stakeholders from multiple fields. WBE has progressed from a relatively niche technology to a widely-used public health tool since the start of the Covid-19 pandemic, yet the practical use of the technology is not yet well understood by large swaths of decision makers in public health departments and wastewater utilities. To best demonstrate its feasibility and efficacy, it is recommended to start by conducting a pilot study. It is crucial to build up interest, secure buy-in from key stakeholders, and identify a focal point in the public health department and in wastewater utilities at the beginning of the pilot. Once local buy-in has been secured, it is important for the team that is spearheading the WBE project to communicate with the various stakeholders, such as the partners responsible for wastewater sample collection and lab analysis, and to guarantee sufficient financial support and accountability for relevant stakeholders. The goals of the project and the responsibilities of each stakeholder should be articulated and well established from the start.

## 6. Next steps

WBE is an effective tool to monitor outbreaks, epidemics, and pandemics of infectious diseases on a large scale, in a cost-effective manner, and in near-real time. Beyond Covid-19, WBE can be implemented to monitor a host of biological pathogens, use of pharmaceuticals and drugs of abuse, and the presence of environmental contaminants that negatively impact human health. Developing lab capacity for wastewater testing is feasible in national or regional research labs in most LMICs, provided they receive adequate technical support and mentoring. However, sustainable implementation of WBE also requires engagement and leadership of local stakeholders, including financial support. Lab capacity development should not be conducted without sufficient engagement of local stakeholders.

One of the key factors to secure buy-in and engagement of local stakeholders is to demonstrate the application of WBE data to improve public health. Although WBE has been used for nearly two decades around the world, its application has been limited to localized settings, such as academic centers. Since the start of the Covid-19 pandemic, WBE has received renewed attention, and its global adoption has expanded quickly, further developing knowledge on the use of WBE data. Although many applications of WBE are currently established in developed countries, successful implementation of WBE in Ecuador and Uruguay demonstrated the feasibility and utility of WBE in LMICs. The implementation of WBE is expected to encounter challenges specific to each country's context. Learning and sharing these challenges and developing solutions to address them is critical to update and improve global technical guidance notes for WBE implementation.

# Annex

1. Executive summary I
2. Executive summary II
3. Covid-19 WBE Lab Capacity Building Process

# Expertise to provide global technical support to build capacity in wastewater surveillance for COVID-19 *(contract 7196813)*

Contractor: Biobot Analytics, Inc.

Report revised after feedback received on first & second drafts and resubmitted: November 06, 2020

## Phase I Mid-Term Executive Review

*Phase 1: Stakeholder establishment & local lab capacity building*

Summary:

**The overall objectives of Phase I in Ecuador and Uruguay have been achieved.**

In Ecuador, stakeholders working in wastewater collection, laboratory wastewater analysis, and wastewater data use were identified. Interagua successfully collected wastewater samples from three locations in Guayaquil over a period of six weeks. The samples analyzed at Biobot confirmed that all the samples met QA/QC standards and were positive for SARS-CoV-2. Local lab partners, PROTAL and BIOMED, were identified and they confirmed their willingness to participate in Phase II. The next steps include establishing lab capability, validating the lab's analytical methods, and securing financial resources for Phase II. Finally, Guayaquil's Emergency Operations Committee (COE Cantonal) will likely play a role in Phase II as a data user. In Phase II, Biobot will continue to support capacity building for laboratory analysis and data interpretation.

In Uruguay, the start of Phase I was delayed for several weeks. However, major stakeholders were identified and their engagement enabled expedited progress of the project. The Instituto Polo Tecnológico de Pando (IPTP) has successfully collected 12 samples so far, and Biobot confirmed those samples met QA/QC standards. Two samples collected most recently were detected positive for SARS-CoV-2 for the first time, reflecting the COVID-19 surge during the election season. Both IPTP and Biobot were able to confirm the presence of SARS-CoV-2 in those two samples. Detection of SARS-CoV-2 in wastewater and the demonstrated lab analysis capability of the local lab identified, IPTP, will accelerate obtaining buy-in from extended stakeholders in the nation. IPTP has confirmed its willingness to participate in Phase II. The next steps include validating the lab's analytical methods and training wastewater data use. We also aim to engage data users at the municipal and national level to ensure wastewater epidemiology remains a key source of data for decision makers.

**Wastewater data use in Ecuador and Uruguay may differ but both aim to limit spread.**

In Ecuador, in light of the current epidemic trajectory and health systems constraints, the ability of wastewater data to provide near real-time data on COVID-19's scope in communities will be primordial. Current COVID-19 surveillance efforts in Ecuador suffer from significant reporting delays, and the wastewater data already suggests that there is a large number of unreported cases. The quantification of the virus found in wastewater and its translation into a case estimate of new infections will be one of the complementary tools used by epidemiologists and decision makers to properly understand the scope of the COVID-19 epidemic across time and space.

In Uruguay, wastewater data will be primarily used as an early warning system of COVID-19 clusters of cases. Uruguay currently has a low incidence rate of COVID-19; however, there are concerns of COVID-19 spread around the country in light of the recent elections and the porous border with Brazil.

**Biobot's key learnings on global implementation of capacity building in wastewater-based epidemiology from Phase I will be instrumental for a successful execution of Phase II and beyond.**

Key learnings from Phase I stem from challenges identified and insights derived from collaborating with scientific teams, public health officials and development assistance funders. They include:

- **Establishing local lab capability requires sufficient resources.** The following factors are considered essential in establishing a new partnership with a local lab and building its laboratory analysis capability: (1) clear communication of the project goals, project scope, and incentives, (2) engagement of local labs to support the development of lab methods for wastewater analysis, (3) experts' (Biobot's) support on validating the lab methods, (4) initial financial support to source necessary equipment, (5) sustainable financial support and incentives to provide wastewater analysis as a service. In this context, local lab capacity building cannot only be achieved by collaborative efforts between local labs and Biobot; it also requires support from local or national government with respect to financing and commitment to long-term engagement.

- **Access to local COVID-19 clinical data sets optimizes wastewater data use.** Determining the extent to which COVID-19 has spread and monitoring its trends can be challenging given clinical data collection and sharing constraints coupled with clinical testing constraints. However, combining existing clinical and epidemiological datasets with wastewater data can help address those challenges and makes wastewater data even more useful since it helps estimate the gap in measurements between reported cases in the community and infections found through wastewater analysis. To this end, it is important from the start to partner with officials who own these data sets and discuss the complementary aspect of wastewater data. In addition, details on wastewater samples, such as catchment area, contributing population size, and flow rate, help interpreting wastewater analysis results more accurately.

## Global Project Management

Communication and project management between the World Bank and Biobot have overall been positive. The project experienced significant delays in both Ecuador and Uruguay for different reasons, and the World Bank and Biobot agreed to modify the timelines initially proposed. The following sections summarize the progress achieved on proposed activities and highlight the challenges we encountered in conducting capacity building in the two different contexts. Lessons learnt from the pilot projects in the two countries are useful in developing a guidance note for enabling global support in building wastewater-based epidemiology capacities in developing countries and emerging economies.

### Ecuador

I. Stakeholder Identification

Several local stakeholders were identified early on in the process - EMAPAG, a municipal water authority; Interagua, a private concessionary managing wastewater on a 30-year contract with EMAPAG; and ESPOL, a local research university. In our project, Interagua is responsible for collecting wastewater samples; ESPOL for conducting lab wastewater analysis; EMAPAG for providing financial support for wastewater analysis.

Initially, ESPOL expressed reservations about entering this partnership due to a lack of communication on the project aims, laboratory analysis methods, and financial support. Biobot communicated with ESPOL on the project aims and laboratory methods, and their willingness to participate was confirmed. The World Bank separately discussed with EMAPAG and confirmed financial support for lab analysis by ESPOL.

In addition, el Comité de Operaciones de Emergencia (COE) Cantonal was recently identified as a key stakeholder based on their leading role in the COVID-19 response. In Ecuador, the main responsibility for the COVID-19 response has been shifted from COE National to COE Provincial, and then to COE Cantonal. COE Cantonal , the World Bank, Interagua and Biobot had their first meeting on Sep. 30th, 2020. COE Cantonal expressed strong interest in wastewater data, especially wastewater-based case estimates.

II. Local Laboratory Assessment & Gap Analysis

Two labs from ESPOL were identified to conduct laboratory wastewater analysis: PROTAL with Dr. Bajaña for sample inactivation, filtration, and concentration; and BIOMED with Dr. Cárdenas for RNA extraction and quantification.

Biobot has developed a “Laboratory Methods Assessment Sheet”. This assessment sheet lists potential lab analysis methods for each step of wastewater analysis (i.e. inactivation, filtration, concentration, extraction, quantification), from which participating lab partners can select their methods. This assessment sheet also helps identify equipment that needs to be purchased to conduct wastewater lab analysis. Biobot also compiled a list of lab methods references that participating labs can study to choose the methods that are best suited for them. These references were shared along with the assessment sheet.

PROTAL lab has completed the assessment sheet and submitted a list of equipment to be purchased to the World Bank and EMAPAG requesting financial assistance for procurement. Sourcing lab equipment may take time, and it may be the major bottleneck in advancing to Phase II. Although PROTAL confirmed that they will be capable of conducting sample inactivation, filtration, and concentration, developing and testing lab methods can take a while without a proper incentive. The scope of this project alone only allows to process 18 samples during Phase II. It is important that a longer-term service contract, involving a higher number of samples to analyze, is agreed between PROTAL and EMAPAG to ensure that PROTAL is sufficiently incentivized to develop, test and refine their wastewater analysis methods.

BIOMED lab has not completed the assessment sheet yet. However, our communication revealed BIOMED already has the capacity to conduct PCR analysis, and that no major equipment needs to be sourced for wastewater analysis. Development of methods for RNA extraction and quantification is expected to go smoothly at BIOMED.

Biobot is also developing a participation agreement document. This agreement clarifies the roles and responsibilities of Biobot, the World Bank and participating labs. Its main goal is to frame partnerships between the World Bank, Biobot, and local labs for global application of wastewater-based epidemiology.

III. Training and Support for Weekly Sample Collection

Sample collection and shipping protocols were shared with Interagua and sample collection and shipping has gone smoothly for six consecutive weeks with only minor and expected issues. The major shortcoming of the sampling thus far is that it has been done manually. Interagua has autosampling equipment but the batteries are dead. The team has reported that they have ordered a new battery but the process to receive it will take some time. Once the autosamplers are back up and running with a battery, the precision of the data coming from the samples will be improved and sampling can be scaled, not requiring manual sampling.

IV. Weekly Lab Sample Analysis & Data Analysis by Biobot

Sample receipt, lab analysis, and data analysis have gone smoothly for six consecutive weeks at Biobot. All of the samples received passed QA/QC and were positive for SARS-CoV-2. There were a few minor delays from Interagua in completing metadata forms or sampling logs and obtaining spatial data for sampling locations; however, the information has now all been received and the team is confident that data analysis will continue to go smoothly going forward.

V. Meetings on Data Interpretation and Contextualization

Two data interpretation meetings were conducted. The meetings went smoothly and initiated robust conversation between all stakeholders about the methods, process, and use of wastewater surveillance data. The group was very engaged, and Interagua suggested presenting our work to a larger set of stakeholders, such as municipal governments and health officials.

We also held a high-level meeting on September 30th, 2020, with members of the COE Cantonal of Guayas in attendance. The main stakeholders (the World Bank, Interagua, and Biobot) presented during the meeting about the partnership and wastewater sampling results. Importantly, Biobot pointed out the problem of current COVID-19 monitoring in Ecuador--significant delay in case reporting and underreporting. Wastewater data suggests the number of infected individuals is significantly higher than the number of reported cases and wastewater monitoring can provide near real-time data on COVID-19 cases.

## Uruguay

VI. Stakeholder Identification

Local stakeholders were identified early on in the project and include three primary entities: Dirección Nacional de Aguas (DINAGUA) — the Federal water resource institution which manages the wastewater infrastructure and will be overseeing this project — Parque Científico y Tecnológico de Pando (PCTP) — a public science and technology park that links the needs of the business, scientific, research, and technology sectors within Uruguay's National Innovation Systems — and Instituto Polo Tecnológico de Pando (IPTP) — an institute of the Faculty of Chemistry. PCTP makes a service contract with IPTP, and PCTP is leading all laboratory aspects of the partnership.

The project kicked off with significant delays because in-country stakeholders wanted to set up an official launch of the project with the World Bank. This does not mean that in-country stakeholders are not supportive of the project. So far, the key stakeholders have been engaged and responsive, allowing expedited progress of Phase I activities.

VII. Local Laboratory Assessment & Gap Analysis

Labs in IPTP were identified to conduct laboratory wastewater analysis. Dr. Umpiérrez is the point of contact for collecting wastewater samples and conducting laboratory analysis. Laboratory analysis is conducted at Dr. Rufo's lab.

IPTP already has methods for detecting SARS-CoV-2 in wastewater and has started laboratory analysis. Their method, however, has not been validated and has yet to yield any positive result. IPTP has completed the Laboratory Methods Assessment Sheet.

Biobot offers two levels of laboratory methods capacity building. The first is quantitative verification of the lab methods. To do this, Biobot sent an inactivated SARS-CoV-2 positive sample to IPTP in October, to confirm that their methods can yield positive detection of SARS-CoV-2. The second is qualitative verification of the lab methods. The quantitative verification will start when the following conditions are met: (1) the lab methods can detect SARS-CoV-2 qualitatively, (2) standard curves to quantify SARS-CoV-2 are developed, and (3) the lab methods incorporate quantitative analysis for Pepper Mild Mottle Virus (PMMV), a fecal biomarker commonly used as internal control in wastewater analysis.

VIII. Training and Support for Weekly Sample Collection

Given the low incidence of COVID-19 in Uruguay, the sampling strategies were modified to optimize for detection of SARS-CoV-2. Instead of conducting weekly sampling at three fixed locations, biweekly sampling from six high-risk areas was proposed. Sampling collection and shipping protocols were shared with IPTP. Sample collection and shipment for the first two batches of wastewater samples has gone smoothly.

At this moment, Dr. Umpiérrez is responsible for collecting wastewater samples. For the project to be sustainable and widely adopted across Uruguay, concerted support for wastewater collection and sustainable financing will be required.

IX. Weekly Lab Sample Analysis & Data Analysis by Biobot

Sample receipt, lab analysis, and data analysis have gone smoothly for the first two batches of samples at Biobot. All of the samples received passed QA/QC. Both Biobot and IPTP detected SARS-CoV-2 in wastewater for the first time in two samples in the second batch. Detection of SARS-CoV-2 in wastewater corresponds to the COVID-19 surge in the nation following the recent elections.

X. Meetings on Data Interpretation and Contextualization

One meeting was held to report the result for the first batch of wastewater analysis. Although all of the samples in the first batch were negative, we agreed that the importance of wastewater analysis in the context of Uruguay is to achieve early detection of COVID-19 spread.

## Lab capacity building plans

Biobot has developed a first version of a lab capacity building plan (see figure below) combining Biobot's expertise and experiences in developing lab capabilities and the lessons learnt through working with partners in Ecuador and Uruguay. The timeline of the capacity building depends not only on the preparedness of participating labs, but also on the support from local or national governments with respect to financing and commitment to long-term engagement. We continue to learn the needs of local labs and adjust the lab capacity building plan accordingly throughout the project. The final version of the plan will be one of the deliverables of the project.

| PHASE | 1 Stakeholder Identification | 2 Lab Method Validation | 3 Capacity Building for Quality Control and Data Interpretation |
|---|---|---|---|
| DURATION | 2 weeks | 6 weeks | 6 weeks |
| OBJECTIVES | • Identify list of local labs to partner with<br>• Identify and secure buy-in from relevant stakeholders to financially and logistically support local lab analysis (e.g. national/local governments, water and health authorities, national laboratories) | • Select labs to partner with<br>• Support partner labs to develop lab analysis capabilities<br>• Verify lab capability of analyzing wastewater data quantitatively | • Enable labs to conduct quality control<br>• Enable labs interpret raw PCR data<br>• Ensure good quality of laboratory analysis<br>• Ensure smooth and timely data transfer |
| ACTIVITIES | • Organize meetings with national labs, universities, and water treatment experts to collate a list of local labs<br>• Organize meetings with potential participating labs to describe the purpose of the project, lab methods, logistics, and financial support<br>• Confirm partner labs' willingness to participate in the project<br>• Organize a presentation on the use of wastewater-based epidemiology to gain buy-in from relevant stakeholders to financially support local lab analysis | • Share references of lab methods to be used for wastewater analysis<br>• Review information on potential partner labs, including current lab capabilities, methods proposed to be used, and gaps<br>• Select labs to participate based on a set of criteria and series of discussions with stakeholders<br>• Sign participating agreements for labs to partner with Biobot and agree on data sharing protocols<br>• Validate local lab methods through testing with blind samples sent by Biobot | • Help implement quality control protocols<br>• Help converting raw PCR data into wastewater virus concentration data<br>• Conduct QA/QC for samples analyzed at local labs<br>• Work closely with partner labs to help improve lab methods (incl. sharing best practices)<br>• Communicate data sharing protocols to local labs and provide feedback |
| DELIVERABLES | • Shortlist of labs to partner with<br>• List of key stakeholders to support local lab analysis to enable wastewater-based epidemiology | • Participating agreement signed<br>• Lab method and list of lab items to purchase identified<br>• Confirmation of lab method validated | • Data sharing protocols<br>• Method verification protocol<br>• QA/QC results |

The tables below summarize the project progress and anticipated timelines for Ecuador and Uruguay. Progress status is indicated using four milestones: *completed, on track, at risk, delayed*.

**Milestone** Completed On track At risk Delayed

Activities *at risk* or *delayed* are listed below with suggested actions to take:

- **Ecuador:** '*Validate local lab methods through testing with blind samples sent by Biobot'* is delayed.
  The list of lab equipment to be purchased is being put together. The timeline for equipment delivery needs to be obtained to facilitate capacity building planning moving forward.

  In addition, it is critical to incentivize participating labs to finish developing their analysis methods. This can be done by confirming financial support for the analytical service beyond this short pilot. ESPOL showed interest in writing a proposal for a long-term contract with EMAPAG where ESPOL conducts lab analysis, Biobot conducts data and epidemiology analysis for EMAPAG with their financial support.

- **Uruguay:** '*Organize a presentation on the use of wastewater-based epidemiology to gain buy-in from relevant stakeholders to financially support local lab analysis.'* is at risk. IPTP and DINAGUA have been engaged in the project, but we have not connected with data users such as municipal governments and health authorities. So far, IPTP has been conducting both wastewater sample collection and analysis. Clarifying long-term responsibilities and financial support is critical in implementing wastewater-based epidemiology sustainably in Uruguay. Recent detection of SARS-CoV-2 in wastewater and the demonstrated lab analysis capability of the local lab identified, IPTP, will accelerate obtaining buy-in from extended stakeholders in the nation.

## Timeline for Ecuador

| | Activity | Expected timeline to completion | Status |
|---|---|---|---|
| **Phase I - Step 1** | Organize meetings with national labs, universities, and water treatment experts to collate a list of local labs | | **N/A** |
| | Organize meetings with potential participating labs to describe the purpose of the project, lab methods, logistics, and financial support | | ✓ |
| | Confirm partner labs' willingness to participate | | ✓ |
| | Organize a presentation on the use of wastewater-based epidemiology to gain buy-in from relevant stakeholders to financially support local lab analysis | | ✓ |
| **Phase I - Step 2** | Share references of lab methods to be used for wastewater analysis | | ✓ |
| | Review information on potential partner labs, including current lab capabilities, methods proposed to be used, and gaps | | ✓ |
| | Select labs to participate based on a set of criteria and series of discussions with stakeholders | | ✓ |
| | Sign participating agreements for labs to partner with Biobot and agree on data sharing protocols | 2 weeks | … |
| | Validate local lab methods through testing with blind samples sent by Biobot | 6 weeks | ⚠ |
| **Phase II - Step 3** | Help implement quality control protocols | 4 weeks | |
| | Help convert raw PCR data into wastewater virus concentration data | 1 week | |
| | Conduct QA/QC for samples analyzed at local labs | 6 weeks | |
| | Work closely with partner labs to help improve lab methods (incl. sharing best practices) | 6 weeks | |
| | Communicate data sharing protocols to local labs and provide feedback | 4 weeks | |

## Timeline for Uruguay

| | Activity | Expected timeline to completion | Status |
|---|---|---|---|
| **Phase I - Step 1** | Organize meetings with national labs, universities, and water treatment experts to collate a list of local labs | | **N/A** |
| | Organize meetings with potential participating labs to describe the purpose of the project, lab methods, logistics, and financial support | | ✓ |
| | Confirm partner labs' willingness to participate | | ✓ |
| | Organize a presentation on the use of wastewater-based epidemiology to gain buy-in from relevant stakeholders to financially support local lab analysis | | ! |
| **Phase I - Step 2** | Share references of lab methods to be used for wastewater analysis | | ✓ |
| | Review information on potential partner labs, including current lab capabilities, methods proposed to be used, and gaps | | ✓ |
| | Select labs to participate based on a set of criteria and series of discussions with stakeholders | | ✓ |
| | Sign participating agreements for labs to partner with Biobot and agree on data sharing protocols | 2 weeks | … |
| | Validate local lab methods through testing with blind samples sent by Biobot | 6 weeks | … |
| **Phase II - Step 3** | Help implement quality control protocols | 4 weeks | |
| | Help convert raw PCR data into wastewater virus concentration data | 1 week | |
| | Conduct QA/QC for samples analyzed at local labs | 6 weeks | |
| | Work closely with partner labs to help improve lab methods (incl. sharing best practices) | 6 weeks | |
| | Communicate data sharing protocols to local labs and provide feedback | 4 weeks | |

# Expertise to provide global technical support to build capacity in wastewater surveillance for COVID-19 *(contract 7196813)*

Contractor: Biobot Analytics, Inc.
Report submitted: February 3, 2021

## Phase II Executive Review

*Phase II: Building best practices & data integration*

Summary:

**Laboratory capacity building moved slowly but the overall objectives of Phase II in Ecuador and Uruguay were achieved.**

Laboratory capacity building activities moved slower than originally planned. In Ecuador, finalizing the selection of lab partners and sourcing equipment took time and slowed down the process of capacity building. After finalizing the selection of lab partners, Escuela Superior Politécnica del Litoral (ESPOL), the World Bank, and Biobot started to hold weekly meetings, which greatly facilitated a steady progress in capacity building and technical knowledge transfer. In Uruguay, capacity building activities went relatively smoothly but a lack of strong leadership at Dirección Nacional de Aguas (DINAGUA) and of regular communication between local partners, Biobot and the World Bank remain a concern. The Instituti Polo Tecnológico de Pando (IPTP) established their own laboratory methods and they tested some samples using these methods; Biobot confirmed their analysis results qualitatively. One of the main lessons learned from this experience is to be realistic when setting a timeline. This is due to the delays associated with procuring the necessary equipment, conveying the right stakeholders and aligning them on an action plan. In addition, scheduling regular check-in meetings will ensure progress and maximize knowledge transfer.

**To implement wastewater surveillance in Ecuador and Uruguay sustainably, engagement and financial support from local governments will be critical.**

The benefit of wastewater surveillance can be fully observed when the data is used. For wastewater data to be trusted, the quality of data needs to be assured. In addition, to conduct wastewater surveillance sustainably, local leadership needs to be at the helm and financial support needs to be in place. In Ecuador, Empresa Municipal de Agua Potable y Alcantarillado de Guayaquil (EMAPAG) agreed to commit financially to the project, at least in the short term. In Uruguay, the local leadership at DINAGUA is weak and there is no system for sustainable implementation of Wastewater-based Epidemiology (WBE) established yet. Local leadership and financial support was important in empowering local laboratory partners to get engaged in developing laboratory capacity for WBE; however, those will be even more important as WBE becomes sustainably implemented as a local routine surveillance system. Thus, moving forward and taking a long term view, in order for WBE to be a permanent element of the public health surveillance infrastructure, it would be recommended to look beyond capacity building of lab analysis. Indeed, it should also include engagement of data users, e.g., Public Health experts and local authorities, and establishing sustainable partnerships including financial support.

## Global Project Management

Overall, capacity building for laboratory analysis took longer than anticipated, but the objectives of Phase II in Ecuador and Uruguay were achieved. The delay was due to the extended time period it took to finalize the selection of partner labs, supply chains, and securing financial support. With guidance from Biobot, the local partner labs now have laboratory methods for the detection of SARS-CoV-2 virus in wastewater. Using these methods, the partner labs have successfully detected SARS-CoV-2 virus in wastewater. Setting up regular meetings was key in providing frequent feedback and ensuring steady progress.

### Ecuador

I. Lab Partner Selection Finalization
Dr. Granda stepped up and oversaw sample analysis, as well as communication with Interaga/EMAPAG. Due to some internal workflow, sample preparation work was handed off from Dr. Bajaña's lab in ESPOL to Dr. Granda's lab. Dr. Cardenas's lab remained as a partner to conduct Polymerase Chain Reaction (PCR) analysis.

II. Capacity Building for Laboratory Analysis
Equipment sourcing. Due to resource and budget constraints, the initial method identification and testing was conducted borrowing equipment available temporarily at other labs in ESPOL. Purchasing large equipment dedicated for wastewater analysis will be approved after obtaining further proof of concept. Delivery of large equipment will take a few months.
A few items (e.g., filters, RNA kits) were not readily available locally. Those items were purchased in the US by Biobot upon request from ESPOL and shipped to ESPOL to facilitate their method identification and testing. This expense was outside the project budget and ESPOL still needs to reimburse this expense to Biobot. In addition, a few more items necessary for the lab analysis were identified and sourced locally. Those purchases were made with financial support from Interagua.

Method identification. A few laboratory analysis methods to be used were identified based on the prior experiences of the local labs, literature reviews, and technical guidance from Biobot.

Method testing. The local labs shortlisted a few of their own methods to conduct the laboratory analyses required for wastewater testing. Biobot provided guidance and technical recommendations on which experiments to prioritize in finalizing laboratory methods to be used and answered any question throughout the process. The local labs successfully detected SARS-CoV-2 virus in wastewater using their methods identified.

Communication. In November 2020, the World Bank and Biobot agreed to set up weekly meetings with Dr. Granda. These meetings were really useful and facilitated providing technical guidance and ensuring steady progress.

Next steps. Once the methods to detect viruses in wastewater are established, data quantification processes and quality control processes need to be introduced to make the data reliable and informative. Data quantification processes include establishment of standard curves with synthetic RNAs. Quality control processes include running positive, negative, and internal controls along with test samples. Accessing the precision of the analysis is also important for quality assurance.

III. Sustainable Partnership and Stakeholder Engagement for Data Use

EMAPAG, Interagua, ESPOL and the World Bank convened frequently to finalize financial support arrangements for continued wastewater surveillance in Guayaquil. EMAPAG agreed to contract the wastewater surveillance work to Interagua, and then Interagua subcontracts to ESPOL the lab analysis work. Interagua also agreed to commit US$10,000 to source necessary equipment and chemical reagents to perform lab analyses. In addition, EMAPAG is finalizing a contract with ESPOL for US$138,000 to perform 520 analysis in the following 12 months under the existing loan with the World Bank. This contract will support routine wastewater surveillance in Guayaquil and to build sustainable infrastructure for WBE in Ecuador.

The Comité de Operaciones de Emergencia (COE) Cantonal is the main actor of the Covid-19 response in Ecuador as of late 2020. The COE Cantonal, the World Bank, Interagua, and Biobot had their first meeting on Sep. 30th, 2020. The COE Cantonal expressed strong interest in wastewater data, especially wastewater-based case estimates. A second meeting with them has not been scheduled yet.

## Uruguay

IV. Lab Partner Selection Finalization

Dr. Umpiérrez and Dr. Rufo from the IPTP remain the selected local lab partners to conduct wastewater analysis.

V. Capacity Building for Laboratory Analysis

Method identification. IPTP has developed lab methods in place for detecting SARS-CoV-2 in wastewater and has started to perform laboratory analysis on wastewater samples.

Method testing. Biobot compared the Lab analysis results by IPTP to those from Biobot. Biobot found general agreement between their results and ours with respect to detection status. This result suggests the validity of IPTP's laboratory methods. See the comparison results below.

**Table 1. Comparison of wastewater analysis results by IPTP and Biobot**
Detected: >2 primers detected with Ct<40; Inconclusive: only 1 primer detected with Ct<40; Not detected: 0 primer detected with Ct<40

| **Sampling location** | **1st batch** | | **2nd batch** | | **3rd batch** | |
|---|---|---|---|---|---|---|
| | **IPTP** | **Biobot** | **IPTP** | **Biobot** | **IPTP** | **Biobot** |
| **Rivera** | Not detected | Inconclusive | Detected | Detected | Detected | Detected |
| **Artigas** | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected |
| **Bella Union** | Not detected | Not detected | Not detected | Not detected | Not detected | Not detected |
| **Canelones** | Not detected | Not detected | Not detected | Not detected | Detected | Inconclusive |
| **Cd de la Costa** | Not detected | Inconclusive | Not detected | Not detected | Detected | Inconclusive |
| **Pando** | Not detected | Not detected | Detected | Detected | Not detected | Not detected |

Communication. No regular meetings with IPTP were set up. Capacity building activities have been on pause since Dec. 2020.

Next steps. Next steps for method development include data quantification, quality assurance, and quality control. Data quantification processes include the establishment of standard curves with synthetic RNAs. Quality assurance can be done by assessing the precision of the methods. Quality control processes can be done by running positive, negative, and internal controls along with test samples. Biobot has shared with IPTP an example of the use of internal controls.

VI. Sustainable Partnership and Stakeholder Engagement for Data Use
The leadership at DINAGUA seems to be weak, and it has been difficult to reach out to the MInistry of Health. Although Uruguay has been initially successful in containing Covid-19 (average reported cases in the country was around 10 cases per day until October 2020), the country is experiencing an increase in cases since November 2020 (average reported cases in January 2021 is over 500 cases per day). This surge of cases may increase interest in WEB in the near future. With the wastewater lab analysis capacity established in the country and demonstration of the utility of WBE, it is expected that stakeholder engagement can become stronger in the future.

## Lab capacity building timeline

The lab capacity building timeline originally developed was reviewed and updated (see figure 1 below). The capacity building timeline reflects Biobot's expertise and experiences in developing lab capabilities and the lessons learnt through working with partners in Ecuador and Uruguay. The timeline below if figure 1 was updated since its first version and Phase I increased from 2 weeks to 4 weeks; Phase II increased from 6 weeks to 12 weeks; and Phase 3 increased from 6 weeks to 8 weeks. Modification to the timeline was made on the expected duration of each of the three phases. The timeline of the capacity building activities depends not only on the preparedness of participating labs, but also on the support from local or national governments with respect to financing and commitment to long-term engagement. The content and the timeline developed in this project will be instrumental in applying WEB in other emerging economies.

| PHASE | 1 Stakeholder Identification | 2 Lab Method Validation | 3 Capacity Building for Quality Control and Data Interpretation |
|---|---|---|---|
| DURATION | 4 weeks | 12 weeks | 8 weeks |
| OBJECTIVES | • Identify list of local labs to partner with<br>• Identify and secure buy-in from relevant stakeholders to financially and logistically support local lab analysis (e.g. national/local governments, water and health authorities, national laboratories) | • Select labs to partner with<br>• Support partner labs to develop lab analysis capabilities<br>• Verify lab capability of analyzing wastewater data quantitatively | • Enable labs to conduct quality control<br>• Enable labs interpret raw PCR data<br>• Ensure good quality of laboratory analysis<br>• Ensure smooth and timely data transfer |
| ACTIVITIES | • Organize meetings with national labs, universities, and water treatment experts to collate a list of local labs<br>• Organize meetings with potential participating labs to describe the purpose of the project, lab methods, logistics, and financial support<br>• Confirm partner labs' willingness to participate in the project<br>• Organize a presentation on the use of wastewater-based epidemiology to gain buy-in from relevant stakeholders to financially support local lab analysis | • Share references of lab methods to be used for wastewater analysis<br>• Review information on potential partner labs, including current lab capabilities, methods proposed to be used, and gaps<br>• Select labs to participate based on a set of criteria and series of discussions with stakeholders<br>• Sign participating agreements for labs to partner with Biobot and agree on data sharing protocols<br>• Validate local lab methods through testing with blind samples sent by Biobot | • Help implement quality control protocols<br>• Help converting raw PCR data into wastewater virus concentration data<br>• Conduct QA/QC for samples analyzed at local labs<br>• Work closely with partner labs to help improve lab methods (incl. sharing best practices)<br>• Communicate data sharing protocols to local labs and provide feedback |
| DELIVERABLES | • Shortlist of labs to partner with<br>• List of key stakeholders to support local lab analysis to enable wastewater-based epidemiology | • Participating agreement signed<br>• Lab method and list of lab items to purchase identified<br>• Confirmation of lab method validated | • Data sharing protocols<br>• Method verification protocol<br>• QA/QC results |

**Figure 1. Lab capacity building timeline**

## Abbreviations

WBE: Wastewater-based Epidemiology
PCR: Polymerase Chain Reaction
EMAPAG: Empresa Municipal de Agua Potable y Alcantarillado de Guayaquil
ESPOL: Escuela Superior Politécnica del Litoral
COE: el Comité de Operaciones de Emergencia
IPTP: Parque Científico y Tecnológico de Pando
DINAGUA: Dirección Nacional de Aguas

## Covid-19 Wastewater-based Epidemiology Lab Capacity Building Process

As a part of the contract named *"Expertise to provide global technical support to build capacity in wastewater surveillance for Covid-19. Phase 2 (contract #: 7200257)"*, Biobot Analytics and the World Bank Group have developed a Covid-19 Wastewater-based Epidemiology (WBE) Lab Capacity Building Process to guide labs in low- and middle-income countries (LMICs) to build capacity to detect and analyze trends of SARS-CoV-2 in wastewater. The Covid-19 WBE Lab Capacity Building Process lists key activities to perform in a step-by-step format to establish reliable laboratory methods for testing and analyzing wastewater for SARS-CoV-2 using quantitative Polymerase Chain Reaction (qPCR). To date, qPCR is the most commonly used technique to quantify the amount of SARS-CoV-2 in wastewater. Droplet digital PCR (ddPCR) is another technique that is commonly used. In general, the ddPCR technique is more sensitive than the qPCR technique, but the ddPCR machine is more expensive. This document is developed assuming the use of qPCR. If ddPCR is used, different quantification methods need to be applied (i.e., "Develop standard curves for quantification" step needs to be tailored for ddPCR). The Covid-19 WBE Lab Capacity Building Process is designed by a "verifier/consultant" and followed by "participating labs". Participating labs are local laboratories that aim to develop the capacity to conduct wastewater analysis. A verifier/consultant is an expert in WBE with experience in developing laboratory methods for wastewater testing and analysis. The guidelines and acceptance criteria described in this document are designed by Biobot Analytics, and they can be refined as the knowledge in the field evolves.

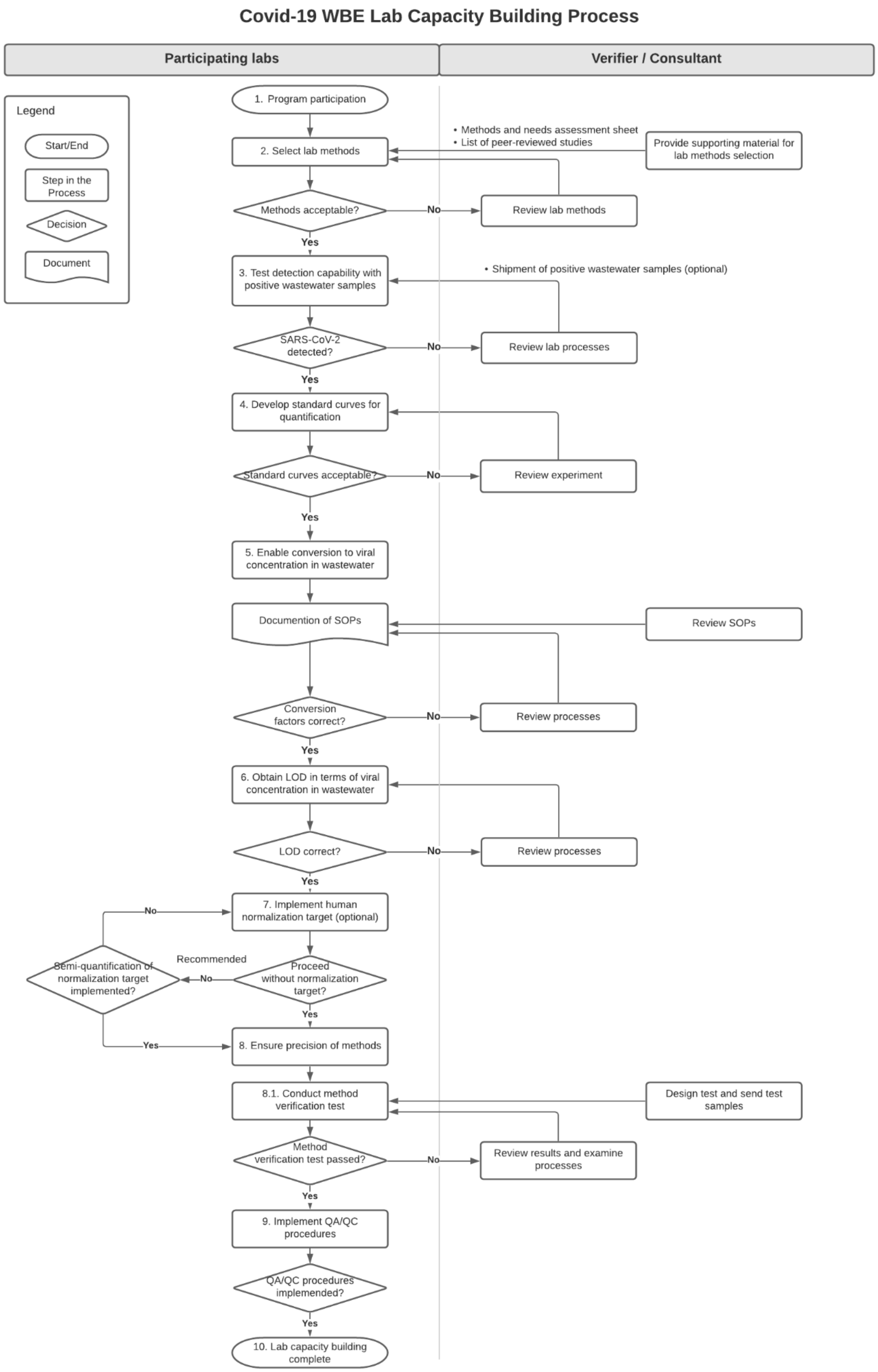


Figure 1. Flow chart of the Covid-19 WBE capacity building process

## 1. Beginning: Program participation

At the beginning of the capacity building program, a program manager identifies a few candidate laboratories to participate in the capacity building program based on their prior experience in environmental testing and molecular biology work. The program manager further examines the eligibility of the labs for the program and confirms the labs' willingness to participate in the program. Once the program manager identifies participation of labs in the capacity building program, the participating labs go through the various steps of the Covid-19 WBE Lab Capacity Building Process to establish reliable laboratory methods in order to detect and analyze the trends of SARS-CoV-2 in wastewater.

Prerequisites for program participation include:

- Biosafety level 2 (BSL2) laboratory. For self-assessment, the United States Centers for Disease Control and Prevention (CDC) has put together a checklist
- Access to some of the key equipment, such as refrigerated centrifuge machines, qPCR/ddPCR machines, biosafety cabinets, etc.
- Technical staff with prior experience in molecular biology, especially with PCR technology

## 2. Select lab methods

The participating labs select the lab methods to be used for the Covid-19 WBE Lab Capacity Building Process and submit them to the verifier to complete this step. The labs select lab methods they use to detect and quantify SARS-CoV-2 in wastewater for the following key steps:

- Virus inactivation
- Filtration
- Virus concentration
- Viral RNA extraction
- Quantification

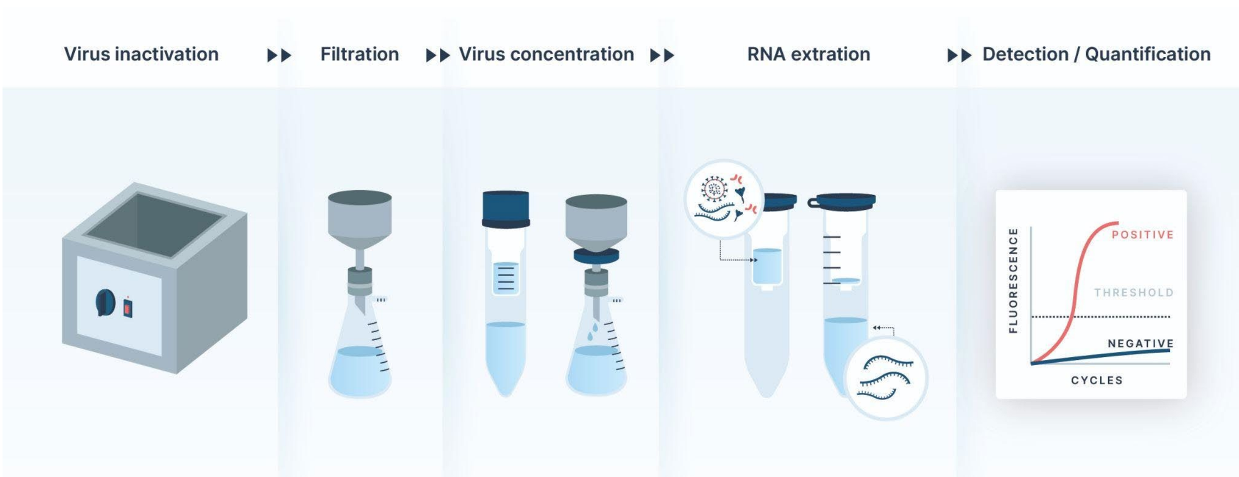

Figure 2. Key steps for detection and quantification of SARS-CoV-2 in wastewater

*Input from Verifier/Consultant:* The verifier provides supporting material for lab methods selection, such as a lab methods and needs assessment sheet and a list of peer-reviewed studies prior to labs submitting their chosen methods.

*Decision:* The verifier reviews the lab methods submitted by the participating labs. To successfully pass this step, the selected lab methods need to meet the following acceptance criteria:

- The methods are used in peer-reviewed studies and proven to be effective in detecting SARS-CoV-2 in wastewater
- The participating labs can implement the methods relatively easily into their lab settings considering the availability of equipment at their facility
- The participating labs have technical staff who have similar, or comparable, experience to ensure that they can implement the lab methods well
- The items required for the selected lab methods are available in the local setting within a reasonable timeline considering local procurement and supply chain processes, and the cost of those items is inexpensive for the program manager and participating labs

The participating labs can proceed to the next step (step 3) if the verifier concludes that the lab methods meet the pre-established criteria. If not, the participating labs must repeat this step of the process with assistance from the verifier.

*Input from Verifier/Consultant:* If the methods selected by the participating labs do not meet the acceptance criteria, the verifier shall review the lab methods and the procurement and supply chain processes.

## 3. Test detection capability with positive wastewater samples

In this step, the participating labs test if their methods can detect SARS-CoV-2 in wastewater.

*Decision:* To pass this step, the labs need to run wastewater samples that are positive for SARS-CoV-2 virus and detect the virus qualitatively, i.e., detection vs non-detection. The participating labs can proceed to the next step (step 4) if the methods can detect SARS-CoV-2 in wastewater samples. If not, the participating labs must repeat the testing process with assistance from the verifier.

*Input from Verifier/Consultant:* If the lab methods implemented by the participating labs fail to detect the presence of SARS-CoV-2 in positive wastewater samples, the verifier shall review the lab processes of the participating labs, providing counsel on the lab methods and the experimental design. If the viral concentration of the wastewater samples is suspected to be too low to detect SARS-CoV-2 with the selected methods, the verifier shall ship SARS-CoV-2 positive samples that have relatively high concentrations to the participating labs.

## 4. Develop standard curves for quantification

In this step, the participating labs develop standard curves to quantify the amount of SARS-CoV-2 in assays. A standard curve is an empirical relationship between Ct (cycle threshold) values from a thermo cycler and the concentrations of a target viral DNA/RNA in PCR wells. Ideally, a standard curve is built for each primer set (e.g., N1 and N2 for SARS-CoV-2).

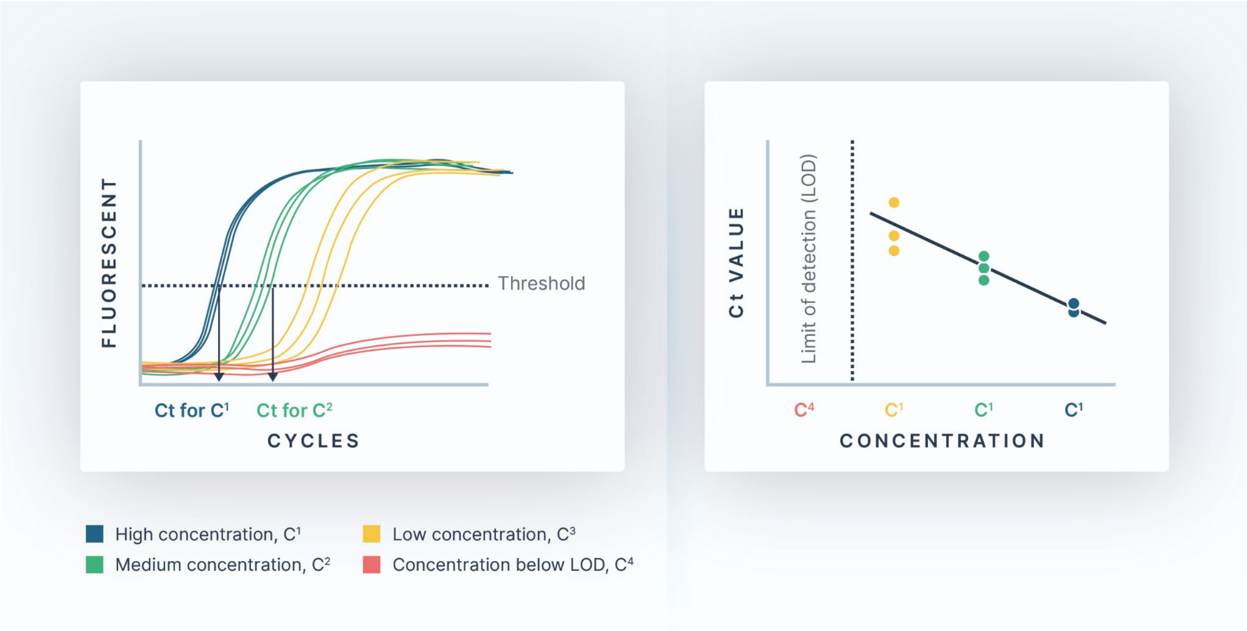


Figure 3. Illustration of how to develop a standard curve.
A relationship between virus concentrations and Cycle threshold (Ct) values is established experimentally. In a thermocycler, fluorescent signals increase more rapidly in high concentration samples than in low concentration samples. Hence, Ct values are smaller in higher concentration samples than in low concentration values. A few different ways of calculating Ct values exist. The empirical relationship between the virus concentrations and the Ct values is called a standard curve.

*Decision:* The standard curves need to meet the following acceptance criteria:

- Standard curves are linear
- Slopes of standard curves are close to the theoretical value
- Variability of the measurements is small
- Lower limit of detection (LOD) is low
- X-axis values of the standard curves (concentration or total copies) are acceptable

The verifier shall define the exact threshold values of the acceptance criteria, considering available technologies, biology of virus shedding, prevalence of Covid-19 in the community, and how the data is used. The participating labs can proceed to the next step (step 5) if the standard curves meet the acceptance criteria. If not, the participating labs must repeat the standard curves development process with assistance from the verifier.

*Input from Verifier/Consultant*—If the standard curves do not meet the acceptance criteria, the verifier shall review the experiment to help find flaws or shortcomings of the experimental design or calculations.

## 5. Enable conversion to viral concentration in wastewater

The participating labs calculate conversion factors to translate the concentrations of a target viral RNA (i.e., SARS-CoV-2 RNA) in PCR wells to the concentrations in wastewater samples. Calculation of the conversion factors requires understanding of the entire analysis steps in detail.

*Documents:* The participating labs need to develop detailed standard operating procedures (SOPs) of the lab methods. SOPs should include analysis steps, equipment used including catalogue numbers, volume of reagents, etc.

*Input from Verifier/Consultant*—The verifier reviews the SOPs to confirm the SOPs describe each step of the lab processes in detail so that the processes are performed in a consistent manner.

*Decision:* Both the participating labs and the verifier examine if the conversion factors are correct based on the SOPs. The participating labs can proceed to the next step (step 6) if correct conversion factors are obtained. If not, the participating labs must repeat the documentation and calculation processes with assistance from the verifier.

*Input from Verifier/Consultant*—The verifier examines if the conversion factors are correct based on the SOPs. If not, the verifier reviews the lab processes and assists in obtaining the accurate conversion factors.

## 6. Obtain LOD in terms of viral concentration in wastewater

The participating labs can now obtain the limit of detection (LOD) in terms of viral concentration in wastewater using the standard curves and the conversion factors obtained in the previous steps.

*Decision:* Both the participating labs and the verifier examine if the calculation of the LOD is correct based on the standard curves and the conversion factors. The participating labs can proceed to the next step (step 7) if a correct LOD is obtained. If not, repeat the calculation process with assistance from the verifier.

*Input from Verifier/Consultant*—The verifier examines if the LOD is correct based on the standard curves and the conversion factors. If not, the verifier reviews the lab processes and assists in obtaining the right LOD.

## 7. Implement human normalization target (optional)

In this step, the participating labs implement a human normalization target as an optional but a recommended step. Because SARS-CoV-2 is mainly excreted in feces, a human normalization target needs to be a human *fecal* biomarker. Pepper mild mottle virus (PMMoV) is the most common human fecal *RNA* biomarker and cross-assembly phage (crAssphage) is the most common human fecal *DNA* biomarker used for wastewater testing. Although both PMMoV and crAssphase can be used as a human normalization target for SARS-CoV-2 analysis, PMMoV is easier to use because both SARS-CoV-2 and PMMoV are RNA viruses.

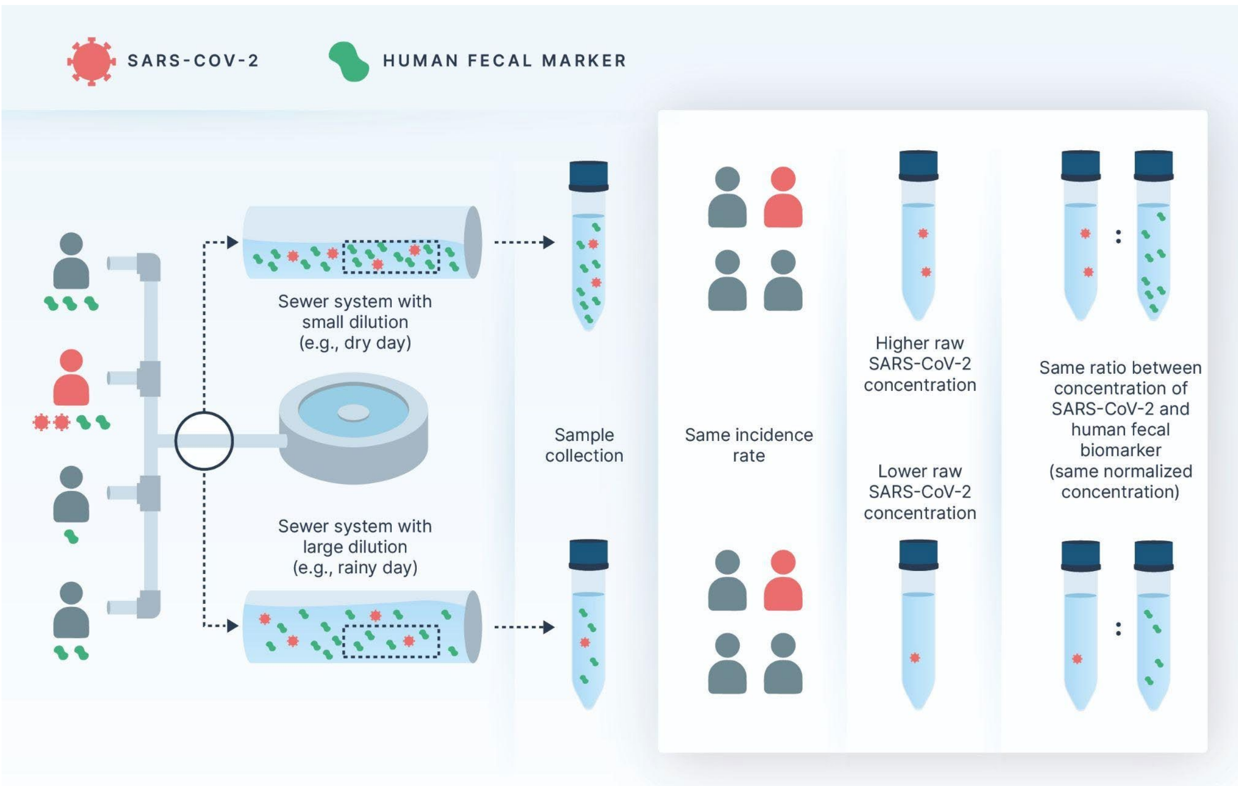


Figure 4. Concept of normalization with a human fecal biomarker
The concentration of SARS-CoV-2 in wastewater is affected by dilution in a sewer system. The amount of rain, industrial water use, different water consumption patterns are the examples that determine dilution in a sewer system. In a diluted sewer system (bottom), raw SARS-CoV-2 concentration is lower than that in a non-diluted sewer system (top), even though the incidence rate in the underlying population is the same. The impact of dilution can be corrected by calculating the ratio between the concentration of SARS-CoV-2 and the concentration of a human fecal biomarker (the SARS-CoV-2 concentration normalized to the concentration of a human fecal biomarker). The normalized concentration is the same for a diluted and non-diluted sewer system. A human fecal biomarker is a biomarker that is shed by a majority of individuals in feces, while the amount of shedding may vary person to person to some extent.

A human normalization target is not required to quantify the amount of SARS-CoV-2 in wastewater; however, it is very useful to account for dilution (rainfall, industrial water use, difference in per capita water consumption, etc.), allowing spatial and temporal trend analysis.

Because the primary purpose of implementing a human normalization target is to account for dilution, but not to quantify the absolute amount of the target, standard curves do not need to be developed explicitly for the human normalization target. The standard curves developed for SARS-CoV-2 can be used to semi-quantify the amount of a human normalization target.

*Decision:* The participating labs decide whether they want to implement a human normalization target in their analysis. The participating labs can proceed to the next step (step 8) if the participating labs decide not to implement a human normalization target. If the participating labs decide to implement a human normalization target:

> *Decision:* Test if the selected lab methods can detect the target in wastewater samples. Quantification of the target can be done either by developing standard curves specifically for the target (quantification) or by applying the same standard curves developed for SARS-CoV-2 (semi-quantification). The participating labs can proceed to the next step (step 8) if methods to quantify a human normalization target are implemented (quantification or semi-quantification). If not, the participating labs must repeat the step for implementing a human normalization target.

## 8. Ensure precision of methods

Once the methods for virus detection and quantification are developed, the participating labs test if the methods are precise enough. Precision of the lab methods is important in order to provide reliable and reproducible results. Precision of the lab methods can be tested via a method verification test.

### 8.1. Conduct method verification test

A method verification test provides verification of lab methods in terms of sensitivity and precision of the methods. Testing methods, verification criteria, and test samples need to be defined and prepared by the verifier/consultant. The acceptance criteria for precision shall be defined by the verifier/consultant considering available technologies and how the data is used.

For example, Biobot Analytics (verifier/consultant) developed a method verification test where:

- Biobot Analytics prepares and sends three test samples at different concentrations in duplicate (total of six sample tubes)
- Participating labs analyze the sample using the lab methods developed in the program
- Biobot Analytics evaluates the test result to verify the lab methods based on the following criteria:
    - Sensitivity of analysis: The labs need to be able to detect both SARS-CoV-2 and a selected human normalization target in all of the six sample tubes.

- Precision of analysis: The labs need to obtain a fold change of the virus concentration between the duplicate sample tubes within 3.0 for each of the three test samples, and within 2.5 for at least two out of three test samples for both SASR-CoV-2 and a selected human normalization target.

*Input from Verifier/Consultant:* The verifier designs a method verification test and sets verification criteria. The verifier also prepares and sends test samples to the participating labs to be used for the test.

*Decision:* The participating labs conduct the method verification test, and the verifier evaluates the results based on predefined testing criteria. The participating labs can proceed to the next step (step 9) if the participating labs successfully pass the method verification test. If not, the verifier reviews the results and provides consultation for the improvement of lab processes. The participating labs must repeat this process until they pass the method verification test.

*Input from Verifier/Consultant:* If the participating labs fail the method verification test, the verifier reviews the results and discusses with the participating labs how to improve their lab processes for a successful result the next time.

## 9. Implement QA/QC procedures

In this step, the participating labs implement QA/QC procedures to ensure the quality of lab analysis. At the minimum, a positive control and a negative control (no-template control) need to be run with each PCR plate. To further improve the quality of the analyses, the CDC also recommends using the following laboratory controls: matrix recovery controls, inhibition assessment, and extraction blanks.

*Decision:* The participating labs need to implement at least a positive control and a negative control to pass this step.

## 10. Lab capacity building complete

The participating labs complete the Covid-19 WBE Lab Capacity Building Process when all of the steps above are achieved.