WASTEWATER SAMPLING FOR EPIDEMIOLOGICAL SURVEILLANCE

ENVIRONMENT AND CLIMATE CHANGE CANADA
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BACKGROUND

The COVID-19 pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has demonstrated the value of wastewater sampling for surveillance of epidemiological priorities.

Environmental surveillance of wastewater (WW) is not a novel concept and has been successfully employed in the past to detect viral outbreaks of measles and poliovirus,[1–3] the presence of a wide range of pharmaceuticals including legal and illicit substances (e.g. cannabis, opioids, cocaine, methamphetamine), psychoactive substances, pesticides, and heavy metal exposure[4–6]. Additionally, this approach has been used to monitor (i) endogenous biomarkers of health (e.g. oxidative stress markers), (ii) chronic conditions such as *diabetes mellitus* (through detection of metformin), (iii) the impact of lifestyle choices (tobacco, alcoholism), and (iv) the presence of antibiotic resistant bacteria[4,7].

Routine surveillance of wastewater provides public health practitioners with a tool for mass surveillance in instances where clinical testing is unavailable or cost prohibitive[4,8,9]. Consequently, this strategy offers a high-impact, low cost option to obtain highly representative, non-invasive biological samples from targeted populations serviced by wastewater collection systems, as a scalable and viable compliment to clinical surveillance in controlling an epidemic[4,9,10].

Implemented properly, wastewater epidemiological surveillance has the potential to[11–13]:

1. Act as an early warning signal[14–19] for introduction of an infectious agent into a population.
2. In the state of endemicity, demonstrate resurgence in community transmission, enabling timely deployment of public health response and conservation of scarce healthcare resources.
3. Provide reliable markers denoting progress towards control, independent from clinical testing regimes.
4. Provide data that are both temporally and spatially related to the emergence of clusters of infection.

5. Allocate resources to high risk populations to prevent and mitigate burden of disease.
6. Foster public trust and compliance with public health recommendations informed by disease tracking metrics.

Notable characteristics of wastewater-based surveillance include:

- **Efficient use of resources**[14–19] - Pooled population sample, highly representative of large urban areas
- **Timeliness** - Results can be derived within 24-48 hours, depending on time between specimen collection and processing;
- **Early warning system** - identify rapid increases in case prevalence by up to a week in advance of traditional clinical methods;
- **Reliability and reproducibility** - Technology and expertise readily available within nearly all levels of government;
- **Expandability to many public health issues at minimal cost**[4,6,9,10] - Opioid use, antimicrobial resistance, and emerging pathogens.

WASTEWATER-BASED SURVEILLANCE

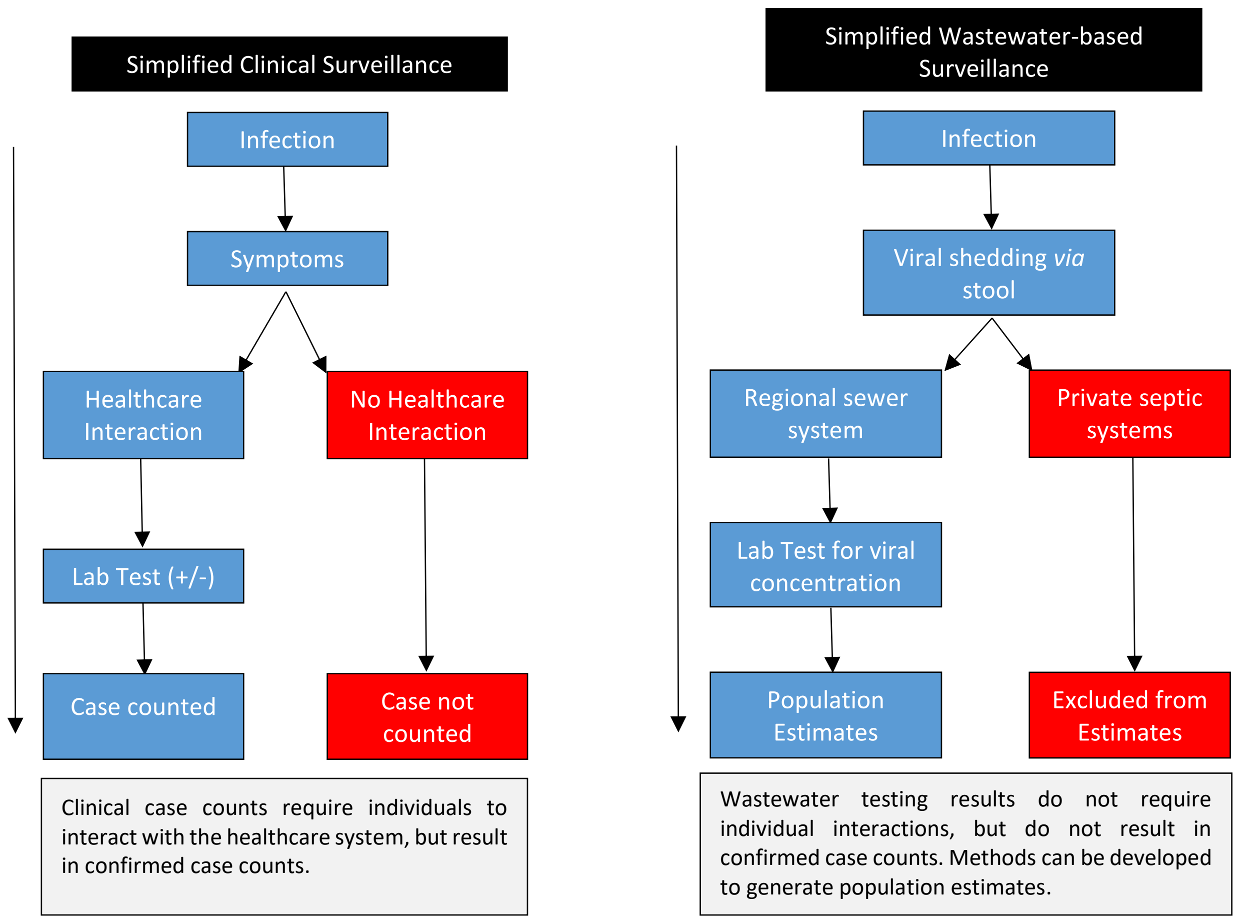


Figure 1: Comparative Illustration of clinical and wastewater-based surveillance

Clinical surveillance of an infection outbreak is highly dependent on individuals with symptoms interacting with the healthcare system. In contrast to the classical clinical communicable disease surveillance, wastewater-based surveillance results are independent of individuals seeking medical help and bypasses the medical system (Figure 1). This approach relies on testing a non-invasive, pooled, biological sample from an aliquot of wastewater collected at either a centralized wastewater treatment plant servicing a community (downstream) or sentinel sites (upstream) (e.g. sewer line manholes/pump stations) in a neighbourhood, and effluent access points for congregate living/working facilities (e.g. long-term care homes, university residences). This method also provides data that are both temporally and spatially representative of infection trends at the community, neighbourhood and institutional level[20–22]. However, there are limitations to this approach, which include the following methodological and procedural factors:

- development of reliable, standardized quantification protocols,
- sensitivity and limits of detection of molecular methods used to monitor the agent in wastewater,
- variability in shedding rates between people and within people at different stages of infection and required adjustments to account for potential differences in concentration,
- variability in duration or persistence of shedding by previously infected/asymptomatic individuals,
- unknown stability of the indicator in wastewater,
- physical and logistical limitations in access to appropriate sampling points in the wastewater collection system,
- variable flow conditions and wastewater travel times in sewer systems,
- attribution of wastewater source and mixing that occurs in sewer lines with subsequent dilution effects, and signal degradation which limits temporal and spatial resolution based on prevalence of infection status,
- identification and determination of reliable biomarkers for sample normalization to control for systematic variances in wastewater recovery processes,
- determination and standardization of sampling protocols, design and assays to optimize sensitivity, frequency, periodicity and speed of testing,
- capacity to provide accurate estimates of the overall prevalence of infection at the community, neighbourhood or institution level (e.g. accounting for commuting and population transience),
- detection of the agent in populations not connected to a sewage network, and
- addressing potential risk of exposure to infectious particles for workers collecting wastewater samples[20,21,23–32].

The methodological and procedural issues related to environmental surveillance of infectious agents do not preclude the utility of wastewater monitoring as a semi-quantitative early detection system for trends in presence, absence and re-emergence[16]. Wastewater surveillance can provide public health officials and decision makers with advance warning to decide where diagnostic testing should begin, and when to introduce measures such as lockdowns to contain infection hotspots. This approach can also minimize the imposition of restrictive measures in areas where it is not required, provide data to inform when these measures can be scaled back to balance human and economic health, and maintain public trust[20,21].

Occupational Health and Safety Considerations

There are general occupational health and safety considerations to follow when working with wastewater. All considerations align with recommendations by the United States Centre for Disease Control[33]. Recognizing hazards and assessing risks is the first step to identifying and controlling these risks in a workplace. Identifying and controlling workplace hazards is required of all employers; as such a risk assessment to determine the most appropriate controls and actions for a particular workplace for which a task, i.e. collecting samples of wastewater, for the purposes of epidemiological analysis must be conducted. It is unlikely that the sampling collection point (e.g. treatment ponds, wet wells, clarifiers or channels), method of collection, environmental conditions (icy, wet), or ease of access, etc., will be identical for each scenario. Thus, a case-by-case risk assessment will be required. Once the risk of the task has been determined, then appropriate mitigating controls can be implemented. Other hazards which could lead to injuries such as, but not exclusively, chemicals, confined spaces, slipping, tripping, falling etc., may be present at the collection site. The risk assessment/task hazard analysis should identify where and how these hazards may exist. Standard Operating Procedures (SOPs) with mitigating controls must be developed to prevent injuries or illness.

The Hierarchy of Controls

Under most Canadian Occupational Safety and Health (OSH) legislation, a hierarchy of methods to protect workers from job hazards exists. Typically, the preferential control is the elimination of the hazards. However, this may not be possible because direct sample collection of the matrix containing the hazard is required. Therefore, apply:

(i) engineering methods to prevent or minimize exposure to the hazard; i.e.

- Ensuring ventilation systems are functioning properly when working around areas where wastewater may be aerosolized and chemicals are used.

(ii) administrative management of hazard exposure or work methods;

- Conduct a risk assessment/hazard analysis of the site prior to commencing work to identify potential hazards which can lead to injury and implement mitigating measures;
- follow all safety procedures, whether pictorial, written or verbal, while on site;
- persons obtaining samplers (i.e., samplers) must receive training on disease prevention including information on basic hygiene practices; use and disposal of personal protective equipment; and proper handling of wastewater;
- sample containers should be cleaned and disinfected after sample collection to avoid any possible transmission of potential hazardous materials during subsequent handling/analyses.
- Sampling personnel receive vaccinations that are in line with recommendations for workers exposed to wastewater in consultation with local health authorities (e.g., in cases where Hepatitis A is endemic in the population). Vaccines such as Tetanus should be up to date.
- Samplers should promptly seek medical attention if displaying any signs or symptoms of diarrhea, such as vomiting, stomach cramps and watery diarrhea.
- Avoid aerosolizing wastewater or minimizing exposure time in areas where aerosolizing is occurring;
- wash hands with soap and water immediately after handling wastewater;
- avoid touching face, mouth, eyes, nose, or open sores and cuts while handling wastewater;
- after handling wastewater, wash your hands with soap and water before eating, drinking or using the toilet;
- do not smoke eat, or drink while handling wastewater;
- keep open sores, cuts, and wounds covered with clean, dry, waterproof bandages and further protected with PPE;
- if suffering from an ailment, which could be exacerbated by contact with wastewater or other hazards present on site, consult a physician before engaging in the task.

(iii) Appropriate Personal Protective Equipment (PPE) for the sampler and proper training in its use [donning (putting on) and doffing (removing) it] to prevent injury and avoid self-contamination. The following equipment is recommended:

- splash-proof face shield to protect eyes, nose and mouth from splashes;
- protective face mask to prevent inhalation of aerosols;
- liquid-repellent coveralls to protect against contamination of clothing and body;
- waterproof gloves to protect hands; and
- rubber safety boots.

Additionally, it is recommended to:

- wash hands with soap and water immediately after doffing PPE,
- bag contaminated, reusable PPE in an impermeable bag for washing with an appropriate soap and disinfected if required.

N.B.:

(i) Federal/Provincial/Territorial (F/P/T/), Municipal jurisdictional authorities and facility Occupational Health and Safety requirements may have supplemental, or alternative, requirements for personnel/visitors working on their site.

(ii) The OHS recommendations listed above are based on current best practices and procedures. Worker health and safety risks are likely to vary among specific locations, therefore a trained health and safety professional should be consulted to create a site-specific worker health, and safety plans.

Wastewater Treatment Terminology

Descriptions of sampling locations must use clear and consistent terminology. There are variations in terminology between different wastewater treatment plants (WWTPs) and different regions of the world. The schematic in Figure 2 represents typical North American treatment processes and terminology.

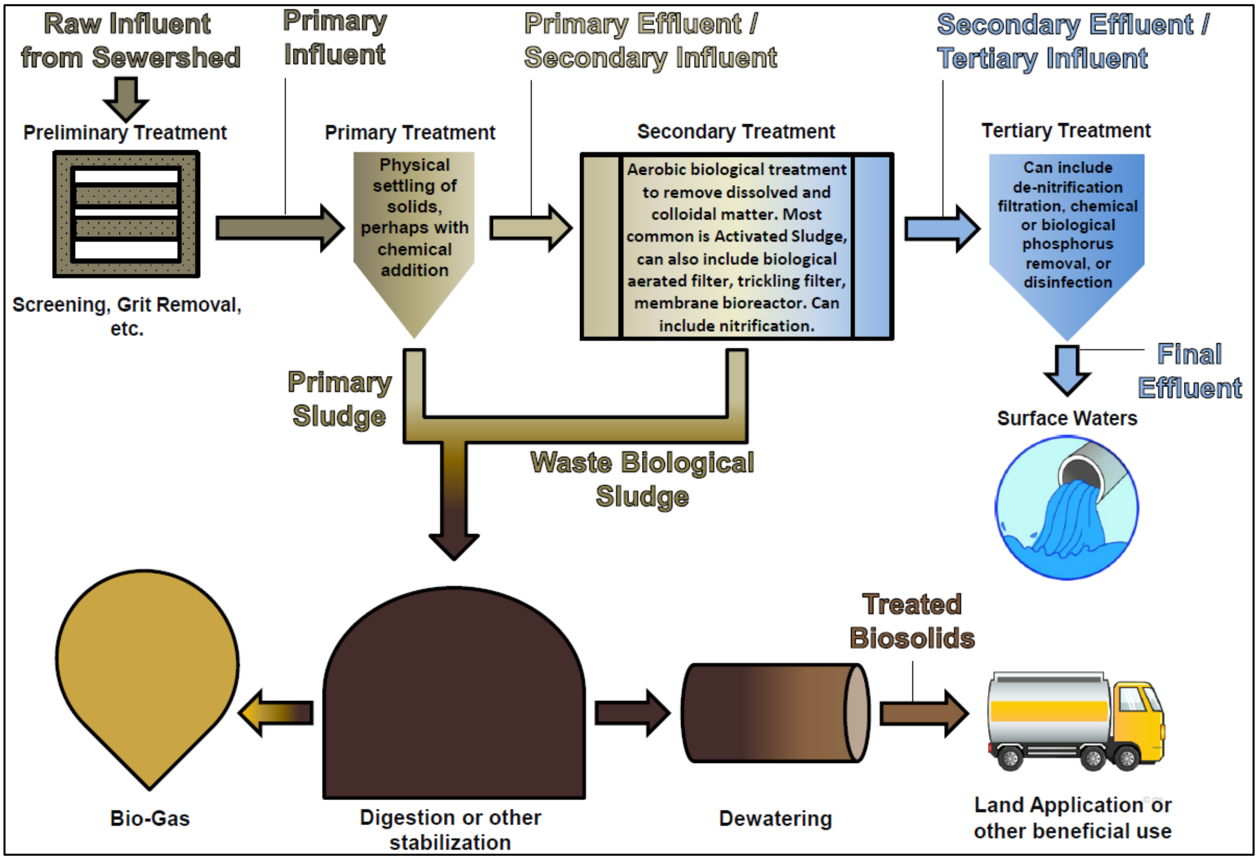


Figure 2: Typical wastewater treatment schematic and sampling point terminology

Sewershed and Treatment System Characteristics

Although most wastewater treatment systems include most of the unit operations and processes illustrated in Figure 2, there can be variations, e.g. absence of a primary clarifier in an extended aeration activated sludge system, or unusually long hydraulic retention times in a WWTP that was designed for higher future flows. Furthermore, every sewershed is unique, containing different proportions of residential, industrial, commercial and institutional (ICI) inputs. Table 1 lists the general information that should be collected when assessing a sewershed or WWTP for sampling.

Other sewershed and WWTP information that may be relevant to the study include:

- Process disruptions or system maintenance (e.g. flushes in collection system, unit processes off-line in treatment plant) that could cause samples to be non-representative;
- Catchments with significant transient population (tourism, day workers), where weekday samples could be very different that weekend samples.

Table 1: Information Checklist for WWTP Sampling

Data elements for sewershed assessment WWTP for sampling
• Date
• Plant Name, Address, Directions
• Contacts (name, phone, email)
• Population served
• Average flowrate (m^3/d)
• % Domestic inputs
• % Industrial / commercial / institutional inputs. Any significant individual ICI inputs?
• Maximum travel time in collection system (hours)
• Screening and Grit removal (yes/no, what type)
• Influent sampler location (upstream of any chemical addition or internal recirculation)
• Primary clarification (yes, no)
• Primary effluent sampler location
• Aeration details
• Solids Retention Time (SRT, days)
• Mixed Liquor Suspended Solids (MLSS) in aeration tank
• Secondary clarification (yes, no)
• Is WWTP operated to nitrify?
• Phosphorus removal (yes/no, chemical addition points)
• Tertiary treatment (filtration, other?)
• Disinfection (yes/no, what type)
• Final Effluent sampler location
• Plant Hydraulic Retention Time (HRT, hours)
• Receiving water body
• Primary sludge (PS) details
• Waste biological sludge (WBS) details (Is WBS co-thickened in primary clarifiers?)
• PS and WBS blended before treatment?
• Raw sludge sample location
• Solids treatment type
• Dewatering (centrate or supernate recirculates where?)
• Treated biosolids sample location
• Biosolids destination

Wastewater Sampling

Principles and Best Practices

Wastewater includes any used water. It may contain human waste, household wash water, commercial, institutional, and industrial inputs, inflow from storm water and other unintended cross-connections, and infiltration from groundwater seepage through cracks and joints.

Because of the variety of potential harmful components in wastewater, particularly infectious microorganisms from human waste, appropriate personal protective equipment (PPE) must always be worn when collecting wastewater samples and working with all associated equipment and supplies. Minimum PPE are disposable gloves and safety glasses; additional PPE could include face shield, facemask, disposable coveralls, rubber boots, or steel-toe boots depending on the sampling site and potential for splashing or spraying.

Wastewater discharges from households, institutions, commercial and industrial facilities are usually variable in both flow and composition. Wastewater flow in mixed-use sewersheds tends to follow a diurnal pattern, as illustrated in Figure 3.

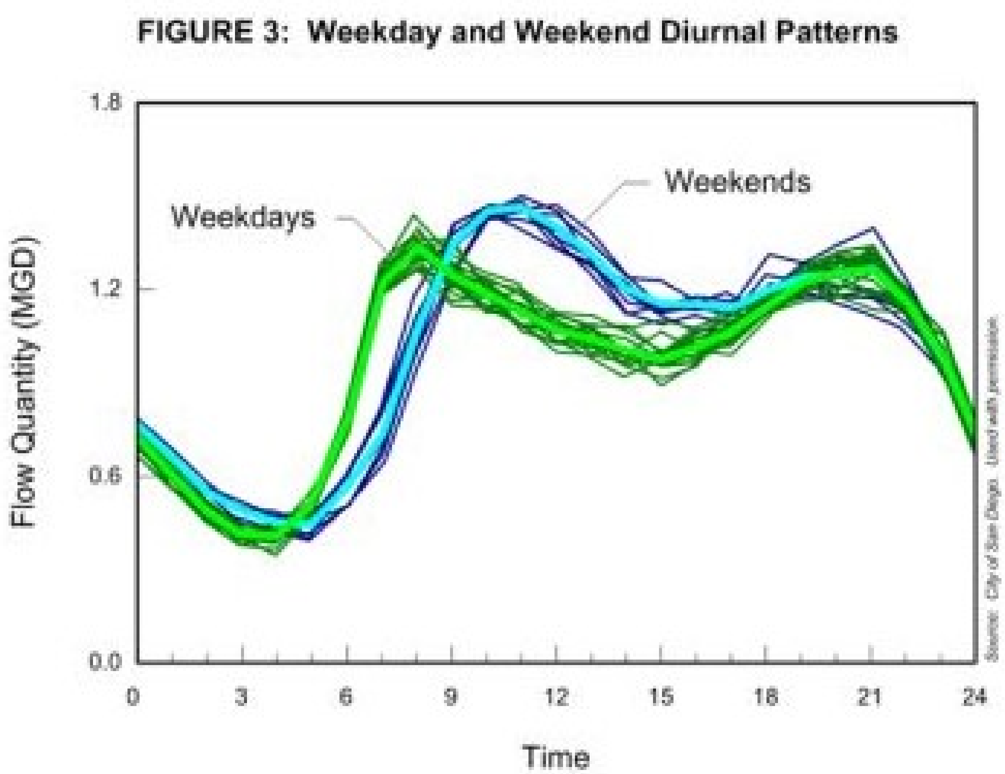


Figure 3: Example of diurnal wastewater flow pattern[34]

The study of either chemical or biological components in wastewater requires representative, consistent sample collection in order to draw appropriate conclusions. In the early 2000s when the issue of pharmaceuticals and personal care products (PPCPs) in wastewater was emerging, a wide variety of sampling details and techniques were included in journal publications. A comprehensive review of published sampling information[35] found that in many cases the sampling information was insufficient to allow replication of the study, and/or that inadequate design of the sampling method could lead to over-interpretation of data and misleading conclusions. Similarly, a review of studies of wastewater surveillance for SARS-CoV-2 RNA in wastewater[36] found that details regarding auto-sampler setup, as well as grab sampling time were poorly reported, which weakened the interpretation of results.

The ideal sampling technique for wastewater is a continuous side-stream that captures the entire variation in flow and composition[35,37]. However most wastewater studies and sampling points do not lend themselves to this option. This section provides a range of practical options for collecting wastewater samples in consideration of various real-world limitations.

Sampling Techniques and Equipment

Unless the goal of the study is to examine differences in wastewater characteristics during storm events, sampling for both chemical and biological parameters should be done during dry weather. Even in sewersheds with separate sanitary and storm sewers, there is always some degree of inflow and infiltration, and storm events change both the flow and composition of the wastewater: storm water dilutes the parameters of interest and also flushes a higher proportion of inorganic materials (sand, grit) into the flow. After a rain event, it is important to verify with the WWTP operators that flows have returned to dry weather levels; depending on the severity of the event and the characteristics of the sewershed this can take several days.

In the absence of continuous side-stream sampling, the cascade of preference for representative sampling of wastewater is[37,37]:

1. Flow-proportional composite with high sampling frequency (<15 min),
2. Equal-volume composite with high sampling frequency (<15 min),
3. Series of grab samples, manual compositing,
4. Individual grab samples.

How to Sample

There are three different sample collection methods used for epidemiological wastewater surveillance: composite sampling, grab sampling, and passive sampling. Composite sampling consist of pooling multiple grab samples collected at a set frequency over a set time period, which is typically 24 hours for wastewater surveillance. Composite samples can be collected either manually or using automated samplers with or without refrigeration capacity (that collect flow-weighted samples per X gallons of flow, or equal-volume timed samples as described above). Similarly, passive sampling occurs over a set time period but involves the use of a sorbent material submerged in a continuous flow. Passive sampling provides an overview of wastewater content over a designated time period. Grab sampling on the other hand constitutes rapid, manual collection that does not require automated equipment. Grab samples represent single moments in time and are largely influenced by daily fluctuations in the wastewater flow and composition. More details of each approach are provided below.

Composite Sampling

Composite samples are created by collecting aliquots of wastewater and blending them over a period of time. A variety of automatic samplers are available: HACH and ISCO are two popular manufacturers (Figure 4, Figure 5, respectively). Auto-samplers are available in portable, refrigerated, and all-weather models. Flow meters can be added for flow-proportional sampling, and multi-parameter in-line sensors (e.g. conductivity, dissolved oxygen) can be added to collect additional data. Portable auto-samplers are not refrigerated, but are lighter and easier to install in a wide variety of locations including manholes. All-weather auto-samplers are heavy and bulky, designed to be installed and remained in one location. Refrigerated auto-samplers can be moved between locations by a team of two physically capable people, and are the preferred equipment for most wastewater sampling because of the sample preservation provided by refrigeration.

Figure 4. HACH Sigma autosamplers. From left to right: AS950 Refrigerated Sampler; AS950 All-Weather Refrigerated Sampler; AS950 Portable Sampler[38]

Figure 5. ISCO autosamplers. Left and Right: ISCO 6712 Portable Sampler; Middle: ISCO 5800 Refrigerated Sampler[39]

Composite sampling with automatic samplers has limitations. The sampling location (channel, tank, wet well etc.) must have continuous and sufficient flow to keep the sample line submerged at all times during the sampling period. As shown in Figure 3, flows tend to decrease drastically during the night. The sample collection tubing needs to be protected from clogging by rags and other debris, preferably by installing the auto-sampler downstream of screening. Although auto-samplers have battery backup devices, these do not function reliably in colder weather, and do not provide power for the refrigerator. Access to 110V power is preferred.

Grab Sampling

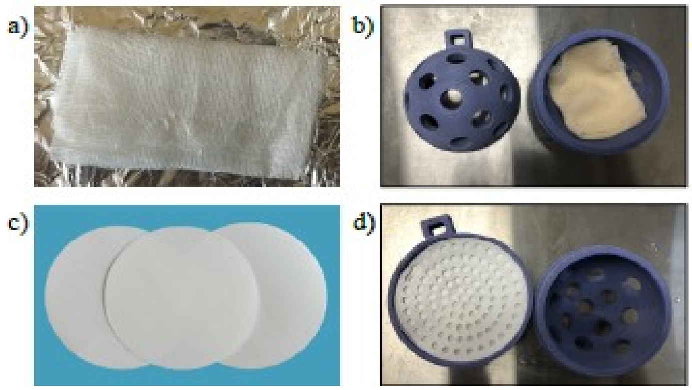
A grab sample is collected at one moment in time. Although grab sampling is generally considered as an inferior approach, it can be the only option in places where auto-samplers cannot be installed (e.g. due to intermittent flow or lack of electricity) or when the use of auto-sampler tubing will contaminate the samples. It is also the appropriate technique when the purpose of the study is to examine differences in wastewater composition over time. Grab samples can be collected with a bucket and rope, or with an extendable “dipstick” depending on access to the wastewater channel or tank.

Passive Sampling

Passive samples are collected over a set time period by submerging a sorbent material in a continuous flow of wastewater. Several passive sampling methods are readily available such as the COVID-19 Sewer Cage (COSCa), torpedoes, and modified Moore Swabs (tampon dunks), which utilize a variety of sorbent materials including electronegative membrane filters, cheesecloth or gauze, and cotton Q-tips. Table 2 provides details on the various passive samplers and methods currently being used in Canada for surveillance of SARS-CoV-2.

Table 2. Passive sampling techniques currently implemented in Canada

COVID-19 Sewer Cage (COSCa)



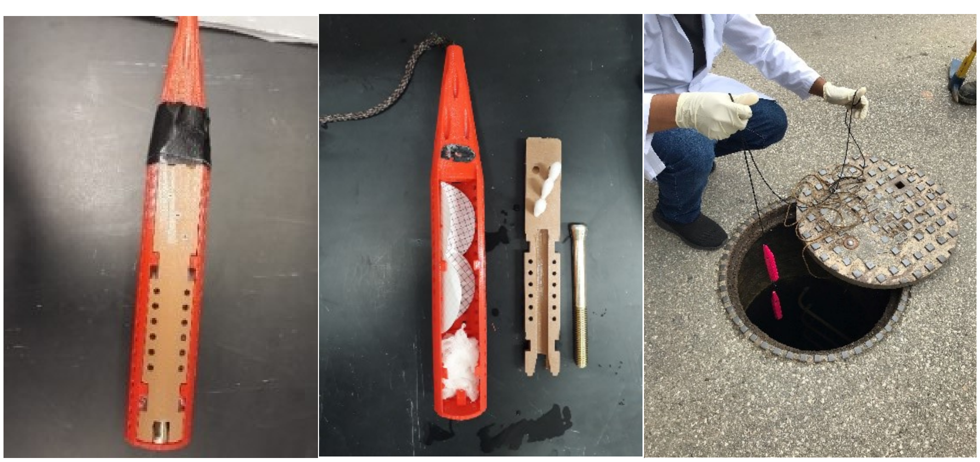
(Source: Wastewater Surveillance of SARS-CoV-2 using the COVID-19 Sewer Cage (COSCa) Standard Operating Procedure, Centre for Water Resources Studies, Dalhousie University)

The COSCa is a 3D-printed passive sampling cage designed for deployment in a wide range of sewershed sampling sites accessed through manholes, lift stations, and piping systems. The device is 10 cm in diameter and includes 26 holes, each 1.5 cm in diameter[40]. This figure shows cotton cheesecloth (a) inside a COSCa (b) and electronegative membrane filters (c) inside a COSCa insert (d).

Resources:

- Download 3D-print files: [COSCa device and insert]
- COSCa deployment SOP: [Deploying a COSCa in a manhole - YouTube]
- Hayes, E. K., Sweeney, C. L., Anderson, L. E., Li, B., Erjavec, G. B., Gouthro, M. T., Krkosek, W. H., Stoddart, A. K., and Gagnon, G. A., 2021. A novel passive sampling approach for SARS-CoV-2 in wastewater in a Canadian province with low prevalence of COVID-19. https://doi.org/10.1039/D1EW00207D

Torpedo



(Source: Dr. Peter Vanrolleghem and Dr. Dominic Frigon, CentrEau; Dr. Ed McBean, University of Guelph)

The torpedo passive samplers can house gauze, electronegative membrane filters, and cotton Q-tips. The small size of the sampler (15-20 cm long, ~3 cm diameter) allows for easy in-building installation and the narrow shape reduces the risk of clogging of piping systems. When deploying the sampler, it should lay flat in the flow with the pointed end directed upstream.

Resources:

- Download 3-D print files: [Passive Sampler - BoSL Wiki]
- Schang, C., Crosbie, N., Nolan, M., Poon, R., Wang, M., Jex, A., John, N., Baker, L., Scales, P., Schmidt, J., Thorley, B., Hill, K., Zamyadi, A., Tseng, K., Henry, R., Kolotelo, P., Langeveld, J., Schilperoort, R., Shi, B., and Mccarthy, D., 2020. Passive sampling of viruses for wastewater surveillance. *Environmental Science: Water Research & Technology,* http://dx.doi.org/10.13140/RG.2.2.24138.39367
- Habtewold, J., McCarthy, D., McBean, E., Law, I., Goodridge, L., Habash, M., and Murphy, H. M., 2022. Passive sampling, a practical method for wastewater-based surveillance of SARS-CoV-2. *Environmental Research*, https://doi.org/10.1016/j.envres.2021.112058
- E-poster on use of torpedo samplers in homeless shelters, Laval University: Learning Toolbox Viewer (ltb.io)

Modified Moore Swabs (Tampon Dunks)

(Source: Left: Sikorski, M.J. and Levine, M.M., 2020. 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): e00060-20. doi.org/10.1128/AEM.00060-20; Right: Dr. Mike McKay, University of Windsor)

The conventional Moore Swab is constructed using gauze folded onto itself in a pleated pattern to create a pad (A to D). The pad is then tied at the centre using a fishing line (E). A modified Moore Swab (tampon dunk) is similar to the conventional swab but rather than the use of a constructed gauze pad, the modified swab utilizes a feminine hygiene product.

Resources:

- Bivins, A., Lott, M., Shaffer, M., Wu, Z., North, D., Lipp, E.K., and Bibby, K., 2021. Building-level wastewater monitoring for COVID-19 using tampon swabs and RT-LAMP for rapid SARS-CoV-2 RNA detection. *Preprints*, 2021050381. doi: 10.20944/preprints 202105.0381.v1
- Corchis-Scott, R., Geng, Q., Seth, R., Ray, R., Beg, M., Biswas, N., Charron, L., Drouillard, K.D., D'Souza, R., Heath, D.D., Houser, C., Lawal, F., McGinlay, J., Menard, S.L., Porter, L.A., Rawlings, D., Tong, Y., Scholl, M.L., Siu, K.W.M., Weisener, C.G., Wilhelm, S.W., McKay, R.M.L. 2021. Averting an outbreak of Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) in a university residence hall through wastewater surveillance. *medRxiv* doi.org/10.1101/2021.06.23.21259176
- Liu, P., Ibaraki, M., VanTassell, J., Geith, K., Cavallo, M., Kann, R. and Moe, C., 2020. A novel COVID-19 early warning tool: Moore Swab method for wastewater surveillance at an institutional level. *medRxiv* doi.org/10.1101/2020.12.01.20238006
- Sikorski, M.J. and Levine, M.M., 2020. 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): e00060-20. doi.org/10.1128/AEM.00060-20

Passive sampling has several limitations. While passive sampling may be superior to alternative sampling methods for low detection events on a small scale, current approaches do not allow for comparable quantification for high detection events on a larger scale. Although recent studies have suggested that quantification may be possible with current passive sampling techniques[40,41], relating viral RNA concentrations eluted from the adsorbent material to the wastewater sample from which it was collected poses a challenge due to (1) the possibility that the sorbent material becomes saturated during the sampling period, and (2) the inability of accurately determining the amount of flow passing the sampling device. Because of these limitations, passive sampling is used primarily for determining presence/absence of infectious agents in wastewater on a local scale rather than quantification. Passive samplers can be deployed in locations with continuous and intermittent flow; however, heavy flow may impede the effectiveness of the sampler. Samplers are also susceptible to clogging from rags and debris if submerged in a location upstream of screening.

Sampling Sludge and Biosolids

Due to the retention times of 2 to 8 hours in primary and secondary clarifiers, primary sludge and waste biological sludge can be considered composited in-situ. Similarly, the retention times of 10 to 30 days in digesters and other solids treatment processes produce biosolids that are inherently composited. Additionally, sampling access points for these streams are usually closed pipes which cannot accommodate auto-samplers. Therefore sludge and biosolids samples are collected using grab techniques.

Sampling in the Collection System

The wastewater collection system resembles a watershed in that it includes small "feeder" pipes (tributaries), and large "main" or "trunk" pipes (rivers). Sampling in the collection system has more complications and limitations than within a WWTP. Maintenance holes can be located in streets or in parks. Depth to the water flow can be quite deep. Electricity sources may not be available. Access to the water can require confined space entry with its associated hazards and required training. These characteristics can make auto-sampler installation and security very challenging or impossible, necessitating the use of grab or passive sampling instead.

What to Sample

There are two main substrates for surveillance sampling:

1. For wastewater-based epidemiological surveillance, raw/untreated wastewater is the preferred sample. Untreated wastewater includes household waste (from toilets, showers, kitchen sinks), and waste from non-household sources (rainwater, industrial use). Evidence of good correlation has been demonstrated between changes in SARS-CoV-2 RNA concentrations in samples from wastewater influent and trends in reported cases of SARS-CoV-2 infection[42].
2. Primary sludge is constituted of suspended solids that settle out of wastewater during primary sedimentation. This may be the preferred matrix for indicators that are associated with the solid portion of wastewater, such as viral nucleic acid[43].

Consultation with WWTP Managers and Operators

WWTP managers and operators take great pride in their work and are interested in supporting wastewater research and monitoring. They possess detailed knowledge of the collection and treatment systems and can provide real-world perspective and recommendations to help accomplish the study goals. Study leaders and field personnel should always seek the advice of operators to identify the best sampling locations, and for details of auto-sampler installation to ensure it is installed in a location that (i) complies with the health and safety requirements of the WWTP, and (ii) provides the desired type of sample.

As publicly owned utilities, WWTPs operate on strict budgets. Their staff are fully occupied with their day-to-day tasks. It is essential to respect their time, i.e. do not assume that they can collect samples for your study in addition to their existing workload. If the WWTP manager agrees that their staff can collect samples, it is essential to engage directly with the operators to provide a detailed explanation of what types of samples are required and why. With this information and involvement, operators are more likely to make the additional effort to collect study samples appropriately.

How Often to Sample

The study leader will take the following factors into account when deciding on the sampling frequency:

- objective(s) of the surveillance, which may vary from early warning or monitoring the trends in the community, to screening for the indicator at a specific target site (long-term care facility, schools, hospitals, prisons, etc.)[20–22,44–46],
- availability of resources (field personnel, equipment, analytical capacity),
- characteristics of the wastewater flow, and
- geographical location of the wastewater treatment plant.

Sampling Volume

The volume of sample to collect depends on the sample type (wastewater *versus* sludge), or requirement to repeat measurements and/or measurement of biological variability, or if any additional tests are planned. If using auto-samplers, it is recommended to program a minimum volume of 100 mL per aliquot, then shake the bulk sample thoroughly and subsample the required volume. Auto-samplers are not precision instruments and the volume calibration becomes less accurate at lower aliquot volumes.

Sample Handling, Transportation, Storage, and Quality Control

By their nature, wastewater samples are very “active”, i.e. there is a high degree of biological activity that will cause the nature of the sample to change fairly rapidly. Wastewater samples also contain suspended solids, which are an integral part of the matrix. After collection, samples should be cooled to 4°C as quickly as possible, shipped cold using natural ice or ice packs, and using the most rapid available transportation. Upon arrival at the laboratory, they should be extracted as soon as possible. Standard Methods[47] recommends extraction within 7 days of collection when analyzing for trace chemical contaminants such as semi-volatile organics; however, holding times may vary depending on the target of interest. The samples must be shaken frequently and thoroughly during any sub-sampling in the field or laboratory to keep suspended solids in suspension and maximize sample homogeneity.

Composite, grab and passive sampling includes the use of consumables (tubing, bottles) and reusable containers and equipment. These containers and equipment must be tested to ensure that the sampling system is not introducing contaminants into the samples. Laboratory-grade water can be used to create Equipment Blanks by simulating a sampling event that includes all sampling materials: sample tubing, pump tubing, passive sampler sorbents and devices, collection containers and sub-sampling containers.

Wastewater Treatment Plan Metadata and Context

As discussed above, a wastewater study must be designed in the context of the collection and treatment system realities and details. Wastewater samples should always be characterized for conventional parameters to provide the context of wastewater strength and effectiveness of the treatment process. These parameters are listed in Table 3.

Table 3: Conventional wastewater parameters[47]

Parameter	Comments
Temperature - process	Indicator of microbial conditions for treatment
Temperature - sample	Confirmation of target storage temperature during sampling and sample transportation
pH	Indicator for general chemistry and microbiology
Alkalinity	Indicator of buffering capacity and nitrification
Total Suspended Solids (TSS)	Empirical gravimetric test, indicator of wastewater strength and treatment effectiveness, can be correlated with some chemical and microbiological constituents
Chemical Oxygen Demand (COD)	Measure of material amenable to oxidation under strong chemical conditions, indicator of wastewater strength and treatment effectiveness
Biochemical Oxygen Demand (BOD)	Measure of material amenable to oxidation under specific biological conditions, indicator of wastewater strength and treatment effectiveness
Total Organic Carbon (TOC)	Measure of total organic (reduced) carbon, indicator of wastewater strength and treatment effectiveness
Total Kjeldahl Nitrogen (TKN)	Measure of total organic (reduced) nitrogen
Ammonia nitrogen	Measure of nitrogen available for nitrification
Nitrate + nitrite	Measure of oxidized nitrogen, indicator of nitrification or denitrification
Measured average daily flow	Available from the WWTP, indicates the size of the system and confirms dry weather conditions or influence of storm events

Summary

Any study of wastewater constituents requires a thorough understanding of the collection and treatment system, in order to design a sample collection process that will answer the study questions. Sampling locations should be confirmed in consultation with WWTP operators, and described in details in all reports and publications. Likewise, sampling techniques (composite, grab, or passive) should be described in sufficient detail to be repeatable. Wastewater samples must be stored, transported, and handled appropriately to maintain their integrity.

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