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In Focus

# Wastewater monitoring for SARS-CoV-2

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**Abstract.** Wastewater monitoring (WM) of SARS-CoV-2 from sewers was applied throughout the world early in the COVID-19 pandemic. Sharing of protocols and experiences in WM of SARS-CoV-2 by national and international researchers and practitioners has been vital to ensuring the sensitivity and specificity of the methods. WM has been a valuable adjunct to human clinical testing, and when positive results occur in sewage, community testing has been increased. WM findings allow public health officials to track and respond to the impacts of loosening lockdown restrictions, demonstrating when return to normal social activities might occur without a resurgence of rapid community transmission, and they are particularly useful in areas with low human case numbers and/or low clinical testing rates. New research is required to address several practical knowledge gaps, for example, sampling protocols, prediction of case prevalence from viral numbers by modelling, and determination of detection limits. Communication to the Australian public of WM of SARS-CoV-2 has been via interactive, visual dashboards. Once SARS-CoV-2 vaccinations are introduced, WM could help track the underlying circulation of the virus in the population, the spread of known variants and its future evolution.

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## Introduction

Wastewater monitoring (WM) or sewage surveillance involves the interrogation of domestic wastewater for chemical and biological markers of human activities[1]. It is applicable to a range of organisms, genes and chemicals excreted or shed by humans. In WM, wastewater analyte concentrations are converted to daily per capita quantities excreted to derive insights into the health of a wastewater catchment's population[2].

WM has long been used in the form of wastewater-based epidemiology (WBE)[3] to inform infectious disease surveillance[3], for example, in the Global Polio Eradication Initiative, but the most established application is for investigating illicit[4] and licit drug use[5]. For WM to be most useful as an epidemiological tool in a disease outbreak, rapid, sensitive and quantitative data on the infectious agent are required, and findings must be synchronised with public health data about the disease status in the community.

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## Coronavirus disease 2019: COVID-19 and WM

Even before severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the coronavirus disease (COVID-19) it causes was declared a pandemic, the scientific community had already initiated a multi-pronged response. It was sprinkled with surprises, such as the rapid global uptake and implementation of WM to help inform COVID-19 control strategies, for example, the European Union Umbrella Study[6] and the COVID-19 WBE Collaborative[7]. WM was raised as a SARS-CoV-2 observation option to the Australian Government early in the first global epidemic wave (early 2020) through an Australian Academy of Science, Rapid Research Information Forum[8]. As researchers pivoted to weave WM into the national COVID-19 response[9], this timely communication explained its potential and limitations[8].

With any new application of WM, the key is to develop and validate reliable analytical approaches. Applying WM for SARS-CoV-2 presented a number of substantial challenges. Wastewater as an analytical matrix is chemically and biologically complex. A 'typical' urbanised community may be releasing tens of thousands of different chemical pollutants from their everyday activities in homes, hospitals, commercial and industrial settings. Some of these decrease the WM sensitivity, for example, by accelerating the decay of SARS-CoV-2 shed from infected people, or by inhibiting the polymerase chain reaction (PCR), a method that typically enables very low concentrations of the virus to be detected.

As soon as the genetic code of SARS-CoV-2 was published, researchers commenced developing diagnostic and screening tools. Reverse transcriptase–quantitative PCR (RT-qPCR) assays were rapidly developed for clinical screening. Given the complexity of the matrix it was imperative to confirm that the methods adapted for wastewater analysis were accurately detecting SARS-CoV-2 and no other coronaviruses. Researchers confirmed that the RT-qPCR detection method worked in wastewater by sequencing the generated products[10]. Efforts then turned to innovations in sewer sampling techniques and improving the recovery of the virus from wastewater to enhance the detection sensitivity. Assays were compared and information shared.

During protocol development, international collaboration played an important role as sample collection, storage and processing tips and findings were rapidly shared[7]. Town-hall video meetings[6] united researchers from across the globe as they explained their own local progress and challenges. Meetings organised by European research leaders saw groups from as many as 20 different countries share results and lessons learned in the same conference call. An open-access platform, the NORMAN SCORE database, is an example of data sharing[11]. Importantly, we note that although SARS-CoV-2 RNA can be detected in wastewater, there has been no evidence of COVID-19 transmission via this route[12].

## WM of SARS-CoV-2 in Australia

In Australia, many water utilities and universities joined forces with state health departments under the umbrella initiative of Water Research Australia's **Col**laboration **o**n **S**ewage **Su**rveillance **o**f **S**ARS-CoV-2 (ColoSSoS) project[13]. ColoSSoS established working groups where interested parties could come together to discuss challenges and share ideas and developments. As the second Australian COVID-19 wave took off in July/August 2020, the Victorian Government Department of Health and Human Services (DHHS, recently renamed the Department of Health) invested significant resources to step up the state's WM capacity. A strong partnership between the University of Queensland and the Commonwealth Scientific and Industrial Research Organisation demonstrated the application of SARS-CoV-2 monitoring in wastewater as a potential COVID-19 screening tool[14]. Nationwide, activities grew and teams focused on different aspects such as the potential for early warning of outbreaks in remote communities (including mine site worker camps), for transport risk analysis, and monitoring and preventing cross border transmission. Researchers involved in this process noted that the openness in sharing protocols and ideas was essential for the rapid national response and operationalisation. Rapid publication turnaround by dedicated academic reviewers and journal editorial teams, and the increasing availability of pre-print publications was also instrumental.

A key implementation hurdle was how to turn WM data into actionable insights for public health agencies; this remains the subject of important discussions in Australia and abroad. In Victoria, a key step came in the development of a workflow diagram and decision support matrix to help guide health authorities, initially determining how to classify a positive detection and what it meant (DHHS, personal communication). Additional genetic targets were added to the assays to make them more specific and informative. The next question was: how should a positive detection be managed in terms of public health response? As the second Victorian wave took off, the implementation of WM took on a new urgency. The workflow model supported an active response[15]. For instance, where wastewater tested positive for SARS-CoV-2 in regional centres with no known clinical cases, the Victorian DHHS issued a public health warning, encouraging people to be vigilant of social distancing, to attend clinical testing facilities if they had any COVID-19 symptoms, and the testing capacity was also increased locally by deploying mobile testing facilities.

Throughout 2020, methods were continually improved. Rapid development of passive sewer sampling devices[16] allowed teams to deploy samplers at the sub-catchment scale where difficult sewer access (e.g. on-road manholes) prevented the collection of 24-h composite samples. The aim was to further enhance the likelihood of virus detection. Ongoing linking and modelling of wastewater and clinical caseload data in Australia and overseas verified that WM was highly sensitive, able to detect a single infected person shedding the virus in catchments of tens of thousands inhabitants[17]. With methods and sensitivity established, an increasingly important task became how to communicate to the public what WM entailed, how it was done, and what the data meant. Internal and external communication needs are different, but each can be conveyed effectively through dashboards and visually informative graphical displays. Example dashboards are from the governments of Victoria[15] (Figure 1), Queensland[18] and New South Wales[19].

## The future

As data sets develop, so does the potential for retrospective modelling of wastewater and clinical data to interrogate the strengths, weaknesses, biases, and future potential of WM. Many examples from across the globe have shown that this is a complex but sensitive approach that, when carefully established and optimised, closely tracks clinical case numbers[20,21]. This imparts the potential to monitor spatial change in distribution and prevalence of the virus over time.

Wastewater-derived data do not replace clinical surveillance but are very useful as an added line of independent evidence. There is clear benefit in applying WM in a precautionary manner and as an additional means of alerting the public to local risk levels and the need for vigilance amid possible signs of (re)emerging outbreaks. WM findings allow public health officials to track and respond to the impacts of loosening lockdown restrictions, facilitating normal social activities to happen as much as possible without a resurgence of rapid community transmission, and they are particularly useful in areas with low case numbers and low clinical testing rates. WM can also be used to verify decreasing community transmission as a result of interventions and to provide confidence in relaxing lockdown staging. WM can contribute to financial reductions; the cost of strict COVID-19-related economy-wide lockdowns in the United States were estimated at US$11.5 billion per day[22]. In the future, WM offers great potential to be used to good effect in tracing and confirming the end of outbreaks and eradication of disease, and it will play an important role in characterising the extent of vaccine penetration and efficacy[23]. Finally, ethical challenges around community application of WM findings need to be seriously considered in any future WM program.

The rapid progress and deployment of WM in the COVID-19 pandemic has clearly demonstrated the future potential for this field. New research is needed to address practical knowledge gaps and determine whether models can effectively be used to back-calculate virion copies to predict case prevalence in specific wastewater

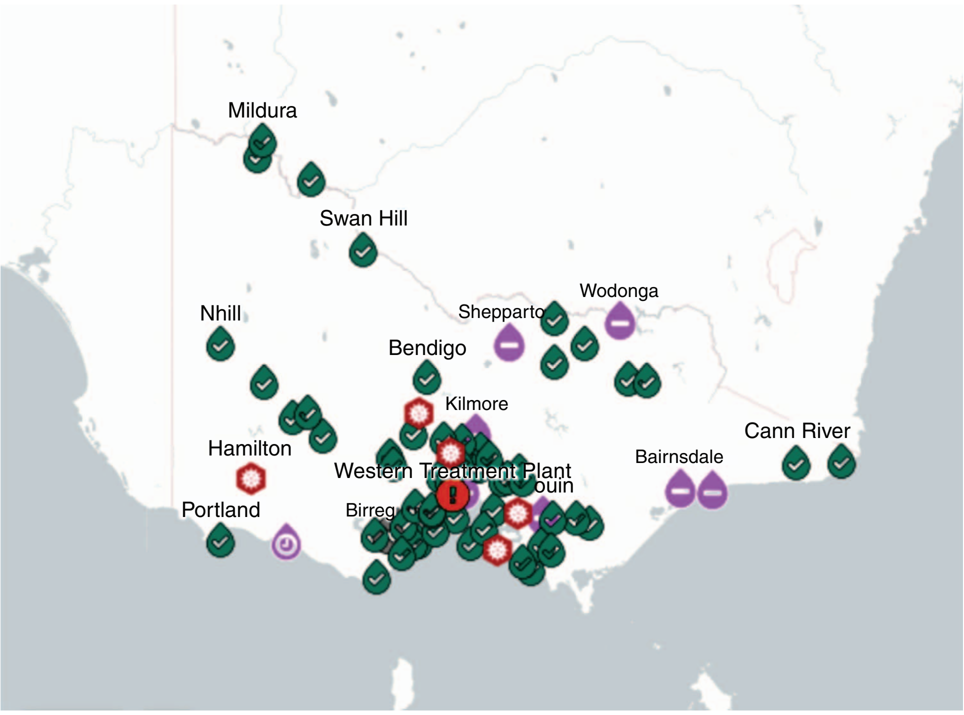


Figure 1. Map of detection of SARS-CoV-2 in Victorian wastewater samples from 30 January 2021 (Victorian Department of Health and Human Services). Tick in green teardrop = no detection; exclamation mark in red circle = anticipated detection; virus symbol in red hexagon = unexpected detection (source: https://www.dhhs.vic.gov.au/wastewater-monitoring-covid-19).

catchments/sub-catchments[2]; predict expected viral loads under different outbreak scenarios and in different catchments; and determine detection limits. Advances should also be in increasing sampling resolution to capture smaller population sizes, sampling methods to capture high-priority targets (e.g. quarantine hotels, nursing homes, schools), methods to survey septic tanks and holding tanks from in-coming international flights[24], and developing capacity to identify high-risk viral variants (such as UK B.1.1.7[25]) through novel diagnostic methods or whole genome sequencing and data analysis[26]. Other likely developments may include the integration of COVID-19 pandemic data with analysis of other health biomarkers, including pharmaceutical residues such as antivirals and antibiotics (used for secondary infections), or with stress markers and drug use patterns[27] that have likely varied in response to lockdown and other interventions. Once SARS-CoV-2 vaccinations are introduced, WM could help track the underlying circulation of the virus in the population, the spread of known variants and its future evolution. Some researchers holding collections of archived wastewater are also investigating important questions surrounding the early emergence of the virus. For example, a publication from Italy suggests[28], based on positive wastewater sample analysis, that the virus was already circulating in northern Italy in December 2019, prior to the previously supposed onset of the outbreak linked to the Wuhan live animal market in China.

The COVID-19 pandemic is set to become a significant milestone in the developing field of WM for infectious disease surveillance, but another critically looming pandemic, antimicrobial resistance (AMR), is already emerging as the next WM frontier[1,29]. AMR is a multifaceted One Health challenge[30] and WM could play a critical role in the global response[31] to it.

## Conflicts of interest

The authors declare no conflicts of interest.

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## Joint Biography

The co-authors on this paper share deep interests and wide-ranging, overlapping competences in the wastewater-based epidemiology (WBE) field. COVID-19 has brought them together, and along with other researchers and technologists, they are exploring funding options to cement their collaboration and take Australia into the next era of WBE.

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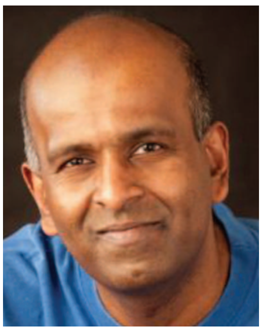

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