Learnings from wastewater surveillance techniques during the pandemic – detecting SARS-CoV-2 in sewage –

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ABSTRACT

The ColoSSoS Project – Collaboration on Sewage Surveillance of SARS-CoV-2 – was created by Water Research Australia (WaterRA) in 2020 in collaboration with water utility, health department and research partners in response to the COVID-19 pandemic. Since its inception, this ground-breaking research project has been tracking and monitoring the presence of the virus that causes COVID-19 and its persistence in the Australian sewerage network. The original intent to unequivocally share sampling and analysis methods, new findings and communication between health departments, research laboratories, universities, private consultancies and global partners, all brought together by WaterRA, in near real time while the pandemic was underway, has been a true manifest of the underpinning agility by all, to achieve outcomes for the communities.

The challenge was fully embraced: The ColoSSoS project was able to develop validated scientific methods used for sampling (and analysis) whereby the latter could, subsequently, be translated into established operational requirements for successful sewage sampling for SARS-CoV-2. This would have not been possible without the collaboration across 50 organisations who openly shared examples from across Australia and abroad demonstrating the efficacy of wastewater surveillance. The ongoing effort of validation gained the respect of health authorities and resulted in their confidence that monitoring SARS-CoV-2 could service as an important, additional tool providing another line of evidence that could be used in the early detection of COVID-19 outbreaks within the community. It allowed health departments across Australia to integrate reliable detections of the SARS-CoV-2 virus RNA in sewage with clinical health data for COVID-19. The impact of ColoSSoS was immediate and is testament to the 'better-together' approach and unique position in the water sector that WaterRA holds as a hub where industry needs are met by research 'know-how'.

This presentation illustrates how the sampling methods could be developed and implemented within four weeks of COVID-19 being declared by The World Health Organization (WHO) as a global pandemic. Throughout the past couple of years, the step-by-step methods have been further refined (1) for different sampling strategies (grab sampling, composite samplers, auto samplers, passive samplers) and (2) for different areas within a water utility (Wastewater Treatment Plant, Network Site, Other (eg, septic tank arrangements). Flow charts can now be used by operators to guide them through a process that allows for sample collection with appropriate quality assurance steps.

1.0 INTRODUCTION

The ColoSSoS Project – Collaboration on Sewage Surveillance of SARS-CoV-2 – was instigated by Water Research Australia (WaterRA) at the onset of the pandemic in 2020. It was a unique project, and a first of its kind in Australia, that embraced the opportunity (and challenge) to openly share scientific knowledge across 50 collaborators world wide (ranging from utilities, health departments, research institutions), to fast track the development of the most appropriate sampling (and analysis) methods in sewage surveillance to detect and monitor the virus. The work produced trustworthy results, which gained the confidence of the health departments and ultimately fostered the development a pragmatic sewage sampling protocol for operators.

Water Research Australia's innovative and collaborative investigation developed novel methods for the detection of SARS-CoV-2 in wastewater, by detecting the virus RNA. Utilising WaterRA's strong public health research collaborations, it built on infectious disease surveillance and mitigation efforts, such as the Global Polio Eradication Initiative.

2.0 DISCUSSION

The ColoSSoS project was able to develop validated scientific methods used for sampling (and analysis) whereby the latter could, subsequently, be translated into established operational requirements for successful sewage sampling for SARS-CoV-2.

2.1 Sampling methods

Four commonly used sampling methods were described for their use of sewage sampling:

1. **Grab samples:** collected at one location and at one point in time (i.e. one full sample container). Two or more grab samples can be taken from one location at different points in time and homogenised (i.e. mixed) to a composite sample. This composite sample is representative of one location over the period of time that the original grab samples were taken.
2. **Composite samples:** generated by a minimum of 2 grab samples taken at least 5 minutes apart during the morning peak inflow period. Generally the more samples that are taken to form a composite, the more representative it is.
3. **Auto-sampler samples**: taken over a higher time frequency (e.g., 12 hours) to generate a composite sample and stored within the auto-sampler unit.
4. **Passive samples**: Described in the ISO-5667 as selective accumulation of a sample over time. Deployed for 48 hours then collected and sent to lab for analysis.

Figure 1: *Composite sampler*

Figure 2: ***"Torpedo" passive sampler developed by Dave McCarthy (Monash University)***

With the complexity of networks and wastewater intake catchments as well as the time commitment to collect samples at a potentially high frequency, the use of passive samplers has gained popularity. Passive samplers are left at the same location, collecting the virus load over a period of time (e.g., one month). The deployment of passive samplers has been described in the following publication: from "Standard Operating Procedure for Passive Sampling in Wastewater" – D. McCarthy, M.Nolan, R.Poon, S.Wilson, C.Schang, J.Black, N.Crosbie.

The laboratories have to have the capacity to process the resulting membranes collected from the samplers, and some training material of how this can be best achieved has been useful. For

example, some organisations have been able to use 3-D printing for building their own passive samplers, and for processing and analysing the resulting samples.

2.2 Wastewater sampling methodology decision process

As every utility and every site may have unique circumstances that dictate what sampling methodology should be used, WaterRA developed a decision-tree to help operators in the field. Decision trees for sewage sampling for SARS-CoV-2 were developed for three broad categories. Separate decision trees are available for sampling from:

1. Wastewater treatment plant
2. Network site
3. Other (e.g., septic tank)

The following decision-tree format forms part of WaterRA's Report on ColoSSoS (in draft).

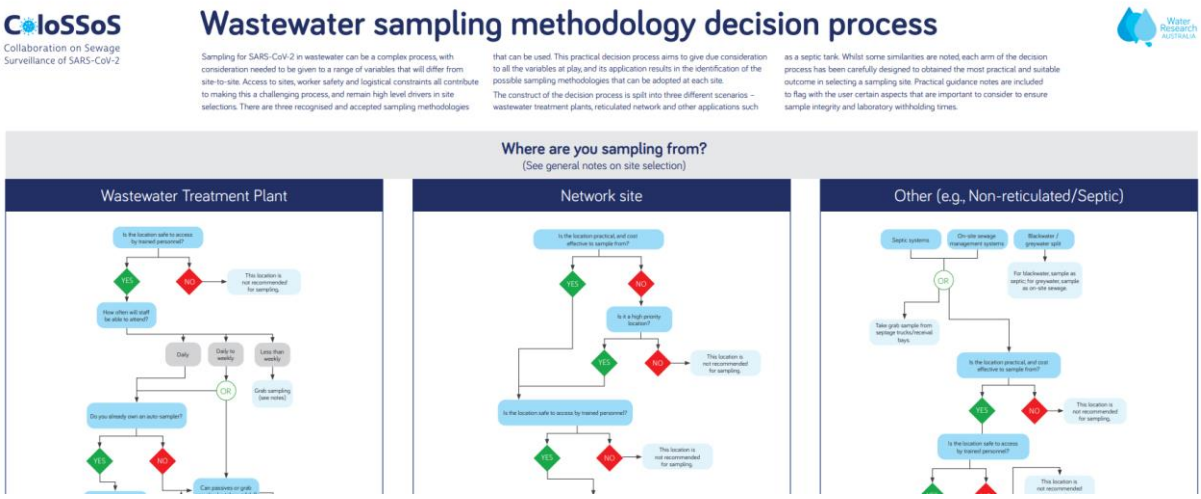

Figure 3: ***Excerpt of the decision tree trilogy for waste water sampling methods***

3.0 CONCLUSION

The pandemic created the need to not only monitor clinic results to stay ahead of the community spread of COVID, but also consider using sewage surveillance techniques to provide early warning signs. The ColoSSoS project has been able to leverage around 20+ years of health and water-based research undertaken by WaterRA and its predecessor – the CRC for Water Quality and Treatment in a very critical time of the pandemic. It enabled the development of pragmatic surveillance processes and tools in a rapid manner (within 4 weeks of the declaration of the pandemic), deriving benefit from the international collaboration that ensued. Water utility operators can use these fit-for-purpose tools according to the requirements within their respective facilities.

4.0 ACKNOWLEDGEMENTS

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5.0 REFERENCES

D. McCarthy, M.Nolan, R.Poon, S.Wilson, C.Schang, J.Black, N.Crosbie. “Standard Operating Procedure for Passive Sampling in Wastewater”

Water Research Australia. Fact sheet. SARS-CoV-2 - Water and Sanitation. WaterRA_FS_Coronavirus (1).pdf

Water Research Australia. Fact Sheet – Improving current enteric viral surveillance through wastewater monitoring WaterRA_FS_4519.pdf

Water Research Australia. ColoSSoS Member reports.