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Developing fit-for-purpose quality assurance measures for sewage surveillance during a pandemic

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Abstract: The potential of using sewage to monitor for SARS-CoV-2 infections at a facility or within a community was demonstrated early during the pandemic. However, health authorities require confidence in the quality of such monitoring data when making public health decisions, especially in a low disease prevalence setting. This paper describes the approach used to establish an assay for SARS-CoV-2 detection in sewage and the associated controls used for quality assurance.

Keywords: quality assurance; COVID; molecular testing

During the original outbreak of severe acute respiratory syndrome novel coronavirus (SARS-CoV), some patients developed diarrhoea and shed SARS-CoV in faeces, in some cases for many weeks after respiratory symptoms had resolved (Cheng *et al.* 2004). SARS-CoV RNA was detected in sewage from hospitals treating SARS patients, although culturable viruses could not be detected (Wang *et al.* 2005). In the context of the SARS-CoV-2 pandemic, the SARS-CoV findings were invaluable, providing the basis to risk assess the safety of domestic sewage and identifying a potential avenue for monitoring COVID in the community. The feasibility of this approach was strengthened early in the pandemic by the finding that up to 40% of COVID patients excrete SARS-CoV-2 in faeces (Wolfel *et al.* 2020) and the detection of SARS-CoV-2 in sewage, with the amounts of virus RNA in sewage increasing proportionally with COVID case numbers (Medema *et al.* 2020). This work demonstrated the proof of concept for using sewage to detect potential COVID cases within a facility, such as an airport, or within a community.

To use such information to inform public health responses, authorities need to have confidence in the results, and the data need to be in a format that can be readily interpreted and acted upon. Normally, diagnostic tests are rigorously validated, often documented within a standard, taking a large investment in time and resources to achieve. In a pandemic, such an approach was not feasible, due to supply chain disruptions affecting equipment and reagents, staff movement restrictions, and time pressure to implement support tools to better manage vulnerable populations. These challenges required a flexible approach to the development or adoption of methods to detect SARS-CoV-2 in sewage, including adaptation to changes in the availability of key reagents, and the use of appropriate quality assurance metrics to ensure reliability of results. Quality assurance is particularly important for sewage samples, because these can vary from inputs such as industrial effluent.

Ideally, controls measure performance of the entire process, from sample preparation to enumeration. In practice, finding the “perfect” process control is not a simple task. In the case of SARS-CoV-2, this would ideally be another coronavirus or enveloped RNA virus with a well-validated quantitative assay to measure process performance. During the pandemic, it was not possible to source these, due either to Australian import restrictions or supply chain limitations. We opportunistically selected the non-enveloped bacteriophage MS-2 as a process control because of availability and usage as the internal control assay in commercial SARS-CoV-2 diagnostic assays. Disadvantages of this control include presence in sewage at variable levels and

structural differences compared with SARS-CoV-2. However, it is much smaller than SARS-CoV-2 and does not associate with particles or surfaces and so is likely to be a conservative control.

Metrics were developed to track different aspects of assay performance, such as RNA extraction and lab contamination using spiked lab blanks (Figure 1), overall process performance using trend analysis of the MS-2 Ct values for samples (Figure 2) and the long-term reproducibility of the Ct values obtained for the qPCR TWIST standards (Figure 3). These types of controls, in addition to the usual positive or no template controls used in qPCR, allowed the identification of outliers which provided triggers for repeating sample analyses and for further investigation to determine and address the cause. This approach provided robust data that strongly correlated with clinical results (Figure 4), demonstrating that it is possible to produce meaningful and robust results using fit-for-purpose controls under non-ideal (ie. pandemic) conditions.

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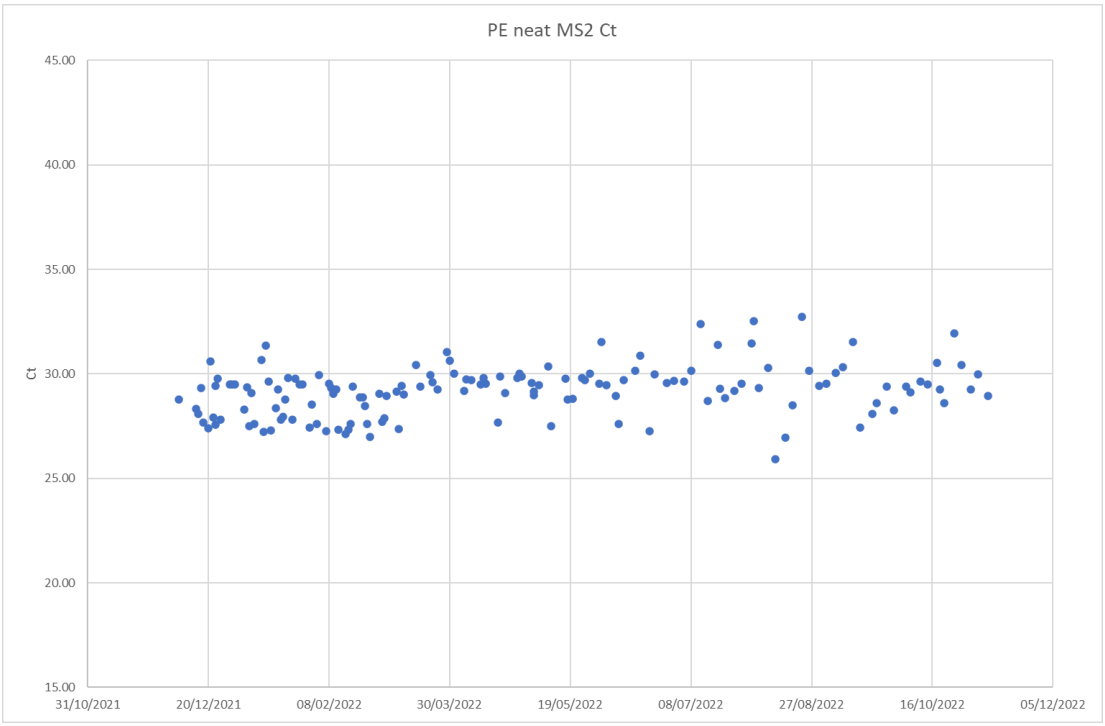

Figure 1. Trending the extraction of MS-2 phage spiked into "lab blanks".

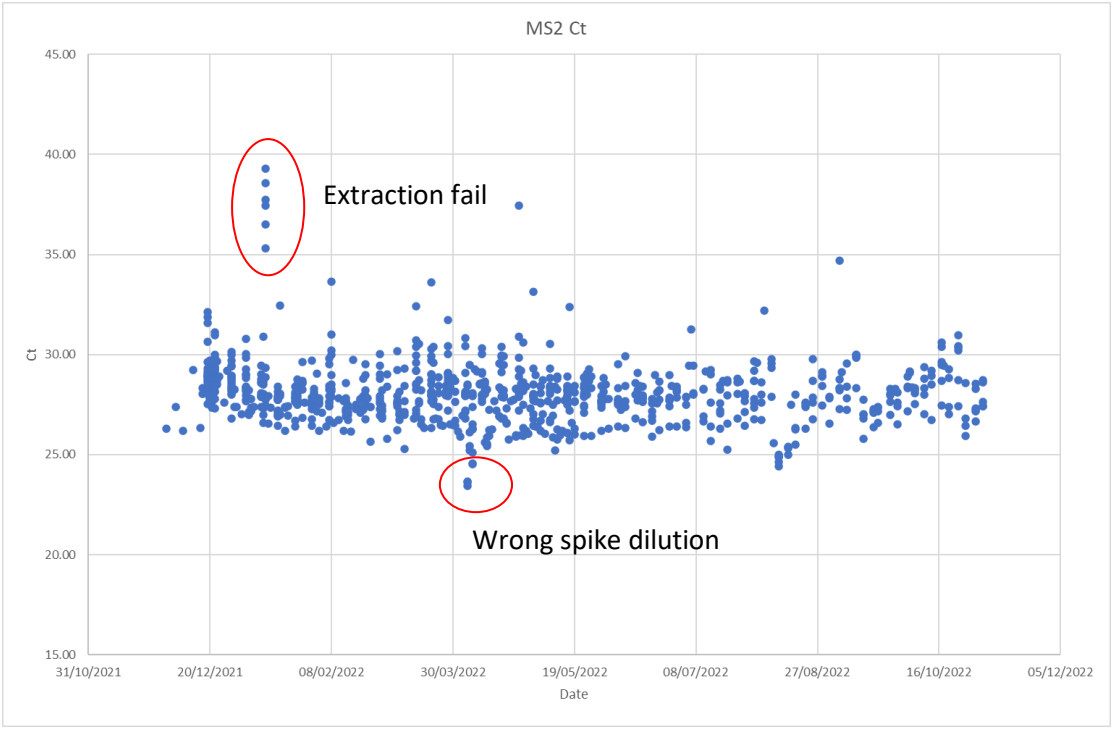

Figure 2. Trend analysis of sample concentration and extraction of combined indigenous F-RNA and spiked MS-2. Examples of exception events are highlighted.

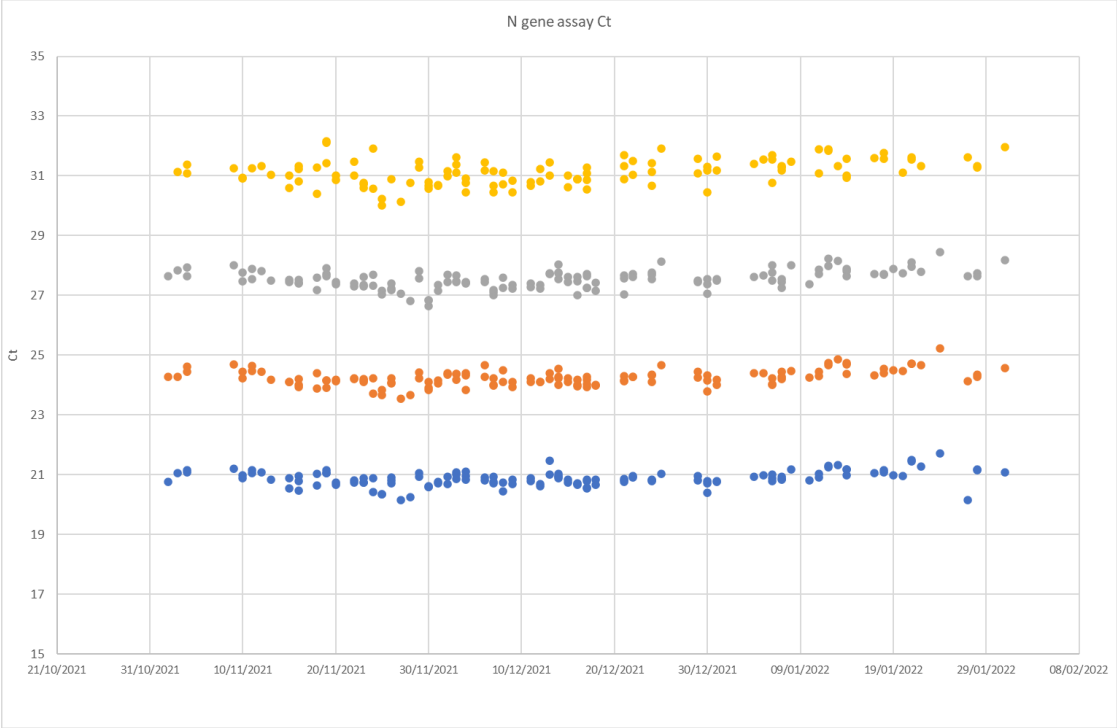

Figure 3. Trend analysis of N gene assay qPCR TWIST standards.

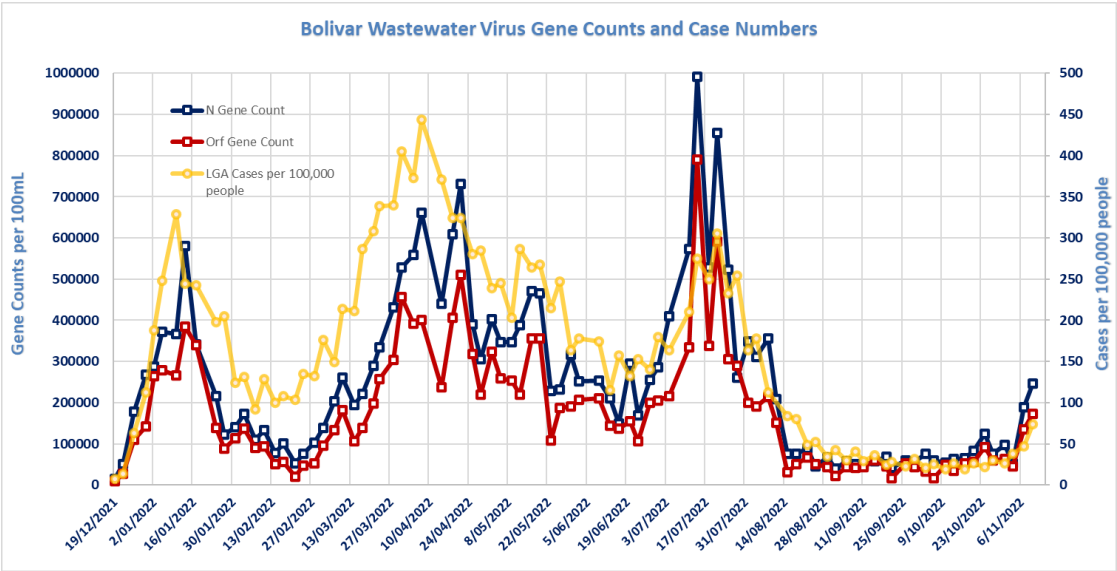

Figure 4. Comparison of COVID cases / 100,000 people with the N and Orf gene counts from an Adelaide wastewater treatment plant.