Public Health Scotland

Evaluating the public health utility of wastewater-based surveillance of SARS-CoV-2 in Scotland: Technical Report

A Management information release for Scotland

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Contents

Executive Summary

In June 2020, a national Wastewater Monitoring Programme for COVID-19 was established in Scotland. The system was established to support the emergency COVID-19 pandemic response. At the time, the programme was found to have successfully monitored SARS-CoV-2 trends and informed modelling exercises to understand the spread and potential impact of COVID-19 in Scotland. There is now a need to reassess whether the system is effectively supporting the current public health needs of Scotland.

While the World Health Organization provide guidance on emerging best practices for wastewater surveillance, specific recommendations are not made owing to evolving methods and possible differences in performance or local needs. In Scotland, a change in the performance of the data has been observed since the introduction of the SARS-CoV-2 Omicron variant and there are known geographic variations in performance.

In view of the need for improved understanding, Public Health Scotland (PHS), in partnership with Biomathematics and Statistics Scotland (BioSS) and the University of Edinburgh (UoE), undertook an evaluation to formally assess the public health utility of wastewater-based surveillance of SARS-CoV-2 in Scotland.

The report, presented here, describes the approach, findings and conclusion, most notably that the Wastewater Monitoring Programme in Scotland has value in supporting three key epidemiological goals: (i) t*he description of broad temporal trends and geographic spread of COVID-19, (ii) the timely identification of new COVID-19 growth periods, and (iii) determination of predominant SARS-CoV-2 variants.* It is therefore recommended that COVID-19 wastewater monitoring activities continue, with periodic review to ensure they address the evolving public health need for COVID-19 and wider infectious respiratory disease surveillance. Caution is required, however, in the application of the system for informing immediate public health action, p*articularly when applied to smaller geographic areas.* A number

of recommendations are made for future improvements to ensure the reliability and validity of the system in Scotland.

1. Background

Wastewater-based epidemiology has been successfully employed to provide qualitative and quantitative measures of chemical and biological markers in sewage samples to inform on the health of individuals within catchment populations. Areas of application (also within the Scottish context) include the monitoring of pathogens, markers of antimicrobial resistance, usage of prescribed and illicit drugs, and exposures to industrial chemicals (1; 2; 3; 4).

In June 2020, a national Wastewater Monitoring Programme was established in Scotland to detect levels of SARS-CoV-2 RNA in the population using Polymerase Chain Reaction (PCR) testing methods (5; 6). This was Scotland's first wastewater-based pathogen surveillance system. Later, the European Commission recommended that Member States establish national wastewater surveillance of SARS-CoV-2 and its variants by October 2021 (7). In Scotland, the programme expanded further in February 2023 to include genomic-based surveillance of SARS-CoV-2 variants (5).

Although best practice guidance from the World Health Organization (updated in September 2023 (8)) outlines the situations and contexts in which wastewater-based surveillance could complement public health surveillance of SARS-CoV-2, it acknowledges the current lack of universal standards in methodology. In practice, the utility of the system is likely to vary, and the public health need, alongside clinical testing, has not been clearly defined in Scotland. Furthermore, there are no international or national guidelines on the genomic-based surveillance of pathogens in wastewater.

To maximise the public health utility of wastewater-based surveillance, careful consideration must be given to the generation of valid, reliable, and actionable

intelligence for informing public health decision making. Whilst wastewater-based monitoring of SARS-CoV-2 in Scotland has been successfully established (5), there is known geographical variation in performance (9) and an ongoing performance assessment is needed given the evolution of the SARS-CoV-2 virus. In view of this, Public Health Scotland (PHS), in partnership with Biomathematics and Statistics Scotland (BioSS) and the University of Edinburgh (UoE), undertook an evaluation of the public health utility of wastewater-based surveillance of SARS-CoV-2 in Scotland.

2. Aims and objectives

The aim was to assess the public health utility of Scotland's Wastewater Monitoring Programme for COVID-19 and its variants ('the surveillance system'), to recommend (i) whether to continue the testing of wastewater samples to estimate COVID-19 activity in the community and to monitor variants, and (ii) any future improvements to the system and requirements for its effective implementation. The system was evaluated in three parts against the following categories:

- Part A: **Purpose and operational description**;
- Part B: **System performance attributes**;
- Part C: **Performance of statistical indicators**.

3. Surveillance system evaluation approach

PHS has overseen the design and conduct of the evaluation, providing epidemiological and public health expertise. The approach followed standard approaches for conducting public health surveillance evaluations as developed by the ECDC and CDC (10; 11). Project partners from BioSS and UoE contributed technical knowledge and data analysis. See **Appendix A** for an overview of roles and responsibilities.

Contextual and technical information in preparation for the evaluation was collated from each of the operational arms of the surveillance programme – Scottish Government (SG), Scottish Water (SW), Scottish Environment Protection Agency (SEPA), BioSS, and NHS Lothian – through an email-based questionnaire (see **Appendix B** for details). A Scientific Advisory Group (SAG) was convened in June 2023 to oversee the conduct of the evaluation and final report. Membership of the SAG included academic and industry stakeholders: infectious disease epidemiologists, bioinformaticians, biologists, statisticians, modellers, and experts in the sewage system and operation of the Wastewater Monitoring Programme in Scotland, and with international representation from public health bodies providing expertise on similar wastewater surveillance evaluations (12) (see **Scientific Advisory Group** for details). The Scottish Government held observer status of the SAG and conduct of the evaluation.

4. Part A: Purpose and operational description

4.1. Importance

The wastewater surveillance system was formally established in June 2020 to provide regular outputs in response to the evolving pandemic situation. At the time, numbers of reported COVID-19 cases were growing globally, with significant concerns around the severity of disease and uncertainty in the likely population impact in Scotland. Public, scientific and political interest was very high. In June 2020 the EC established an EU alliance and community of practice for wastewater surveillance of COVID-19 (7) and on 17 March 2021, the EC adopted the recommendation that Member States establish national wastewater surveillance of SARS-CoV-2 and its variants by October 2021 (13). By March 2021 the wastewater system had contributed to informing the allocation of mobile testing units and modelling the epidemic trajectory in Scotland. The latter was especially important during the latter stages of the pandemic as access to widespread community testing was curtailed and information about changes in relative levels of infections in the population was limited.

4.2. Objectives and development

The surveillance system was initiated following a research project commissioned by CREW, the Scottish Government (SG) funded Centre of Expertise for Waters, in Spring 2020 as part of the emergency COVID-19 pandemic response (5). Contributors to the project included researchers from the Roslin Institute (University of Edinburgh) and the Scottish Environmental Protection Agency (SEPA), who developed and tested protocols for measuring SARS-CoV-2 RNA concentrations, and with wastewater sampling logistics provided by Scottish Water.

In December 2020, the SG identified an area of the Health Directorate that would take on responsibility for the policy aspect and operation of the programme and committed funding to support collection and testing of wastewater samples. BioSS were contracted in December 2020 to develop and implement statistical analysis of the PCR data and regularly report results to SG and public health stakeholders. The population covered by the surveillance system was expanded in 2021 and genomic-based monitoring of SARS-CoV-2 variants was developed by Gilbert and colleagues in February 2023 (5).

The main objective of the program was to monitor SARS-CoV-2 levels in the sewered Scottish population to support health boards, local authorities, and the SG understand localised risk. After developing an analysis and reporting strategy, BioSS have produced weekly reports on levels of SARS-CoV-2 in wastewater in Scotland. Data are also provided on Dashboards delivered by SEPA and PHS. The outputs from the programme have been used to inform modelling exercises, such as estimation of the R number and medium-term projections for hospital admissions and bed occupancy and deaths (14; 6).

4.3. Operational components

4.3.1 Sampling methodology

The primary component of the system is the wastewater sampling network delivered by Scottish Water. The main sampling sites consist of wastewater treatment works

(WWTWs) where auto-samplers were installed. A typical sampler collects a fixed subsample of around 10 ml every 15 minutes, producing a time composite sample of influent wastewater over a 24-hour period, although some variation in practice exists across the network. Generally, samples are refrigerated.

4.3.2 Surveillance sampling design

The programme initially piloted the sampling of 28 of the ~1,800 WWTWs in Scotland twice a week from May 2020, with the sites chosen to maximise geographical and population coverage. In April 2021 the sampling effort was increased to target over 90 WWTW sites, although with some variability week on week due to local circumstances. A median of 92 sites (ranging 48-98) were sampled between w/e 04 April 2021 and w/e 07 November 2021 (excluding an anomaly week when only 9 sites were sampled). The sampling effort was subsequently increased to target over 100 sites from November 2021, with some sites being sampled more than once each week. A median of 102 sites (ranging 24-113) were sampled between w/e 14 November 2021 and w/e 03 September 2023. See **Figure 1** and **Figure 2** for the weekly numbers of samples and WWTW sites captured.

During the period April 2021 to April 2022, a number of network samples were also collected by Scottish Water and analysed by SEPA as part of developments to address the objective of understanding localised risk. These grab samples were taken within the sewage network at near-source locations for specific settings of epidemiological interest at the time, for example near universities and hospitals. As these samples were not used for informing public health action or included in the routine weekly outputs, they are not included in the statistics reported here.

Figure 1. Total weekly numbers of wastewater samples collected in Scotland, w/e 19 July 2020 - w/e 03 September 2023

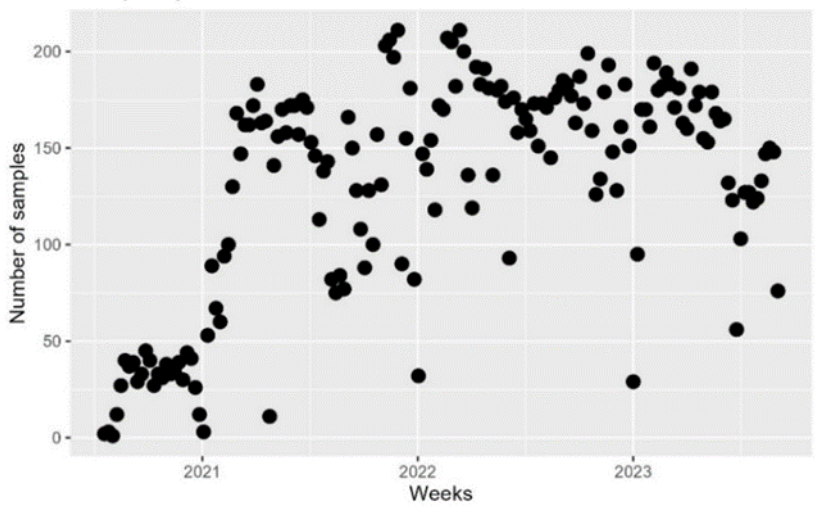
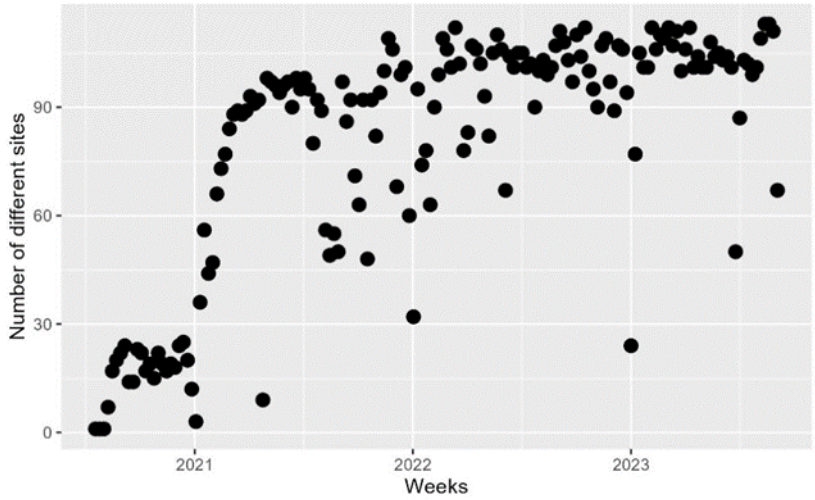


Figure 2. Total weekly numbers of wastewater treatment works sites sampled in Scotland, w/e 19 July 2020 - w/e 03 September 2023



The sampling design was re-developed at various points taking into consideration site populations, whether they are located within local authorities that would be otherwise sparsely covered, site-specific analysis failure rates, and practical limitations on sampling frequency: in August 2021 (the temporal and geographic distribution of additional sampling), in December 2021 (a sampling prioritisation scheme for periods of reduced sampling capacity), in March 2022 (a variation of the sampling prioritisation scheme under specified sampling coverages of 100, 200 or 300 samples), in April 2023 (a scheme of reduced sampling over the summer due to

increased routine demands on the SEPA laboratory), and in August 2023 (a sampling scheme for a return to 200 samples using the methodology as applied previously).

An approach was also developed by BioSS to select 90 samples per week for whole-genome sequencing by the NHS Lothian laboratory, prioritising sequencing of sites that maximise population coverage.

4.3.3 Laboratory processing

Each sample is separated for chemical analysis by Scottish Water (in particular, for the determination of ammonia levels via colorimetry), core quantitative real-time polymerase chain reaction (qPCR) analysis for SARS-CoV-2 RNA by SEPA, and for sequencing analysis by NHS Lothian via SEPA. SEPA conduct the qPCR analysis using “method 6” as described in Fitzgerald et al. 2021 (9). The method involves spiking each sample with porcine reproductive and respiratory syndrome virus (PRRSv) as a check for chemical inhibition. Samples are then clarified and concentrated by centrifugation. Viral RNA was initially extracted using the QiAmp RNA extraction kit before switching to MagMAX™ Wastewater Ultra Nucleic Acid Isolation Kit to allow the process to be automated, enabling a higher throughput of samples.

After elution, one-step qPCR reactions are conducted using Luna® Universal One-Step RT-qPCR Kit, focusing on the N1 gene. Concentrations of viral RNA are then computed and entered into a SEPA-run dashboard, together with other data like flow or ammonia concentrations. The overall process is repeated 2-4 times per week. A subset of samples are provided to NHS Lothian for genomic Illumina sequencing using the EasySeq™ qPCR SARS CoV-2 whole-genome sequencing kit (NimaGen).

4.3.4 Statistical analyses

The raw SARS-CoV-2 concentrations are measured in units of gene copies per litre. These measures are known to vary substantially, particularly at the scale of individual WWTW sites. Household drainage water is typically mixed with water from other urban sources, meaning samples will contain rainwater which dilutes the sample.

This variability is accounted for in analyses developed by BioSS, by controlling for the volumes of influent (known as ‘flow’) received by WWTWs (6).

Data on flow, or ammonia as a proxy alternative, is generated by Scottish Water on samples collected alongside samples collected for COVID-19 surveillance. Flow is controlled for directly where feasible, or otherwise using an approximation based on ammonia levels. The data are also adjusted by the population size covered by each WWTW catchment area and finally reported in units of million gene copies per person per day (Mgc/p/d).

4.3.5 Bioinformatics analysis

Sequencing data generated through SARS-CoV-2 Illumina amplicon sequencing of wastewater samples is processed to reconstruct relative lineage abundances over time and by WWTW sites and NHS Boards. The pipeline used, initially designed by BioSS, utilises *Freyja* (15), a software tool created specifically for analysing SARS-CoV-2 wastewater sequencing data. *Freyja* employs a ‘barcode’ library of lineage-defining mutations to represent each SARS-CoV-2 lineage in the global phylogeny. To encode each sample, *Freyja* stores the single-nucleotide variant (SNV) frequencies (the proportion of reads at a site that contain the SNV) for each of the lineage-defining mutations and models the relative abundance of each variant.

4.3.6 Quality control

At the laboratory stage, an internal control is added to each sample to detect inhibition. Further, a water control and statistical process control (SPC) are included to detect contamination and changes in recovery efficiency respectively. For the SPC process, quality control samples, containing a known quantity of SARS-CoV-2, are tested alongside surveillance samples to assess the ability of the PCR process to recover the virus quantity.

Changes in recovery efficiency may arise that affect an entire batch of simultaneously analysed samples. From mid-2022, divergences in SPC values were identified in relation to some sample results and the associated data points were

subsequently excluded. The use of SPC data to compute changes in analysis efficiency and adjust for data anomalies is an area of ongoing technical development.

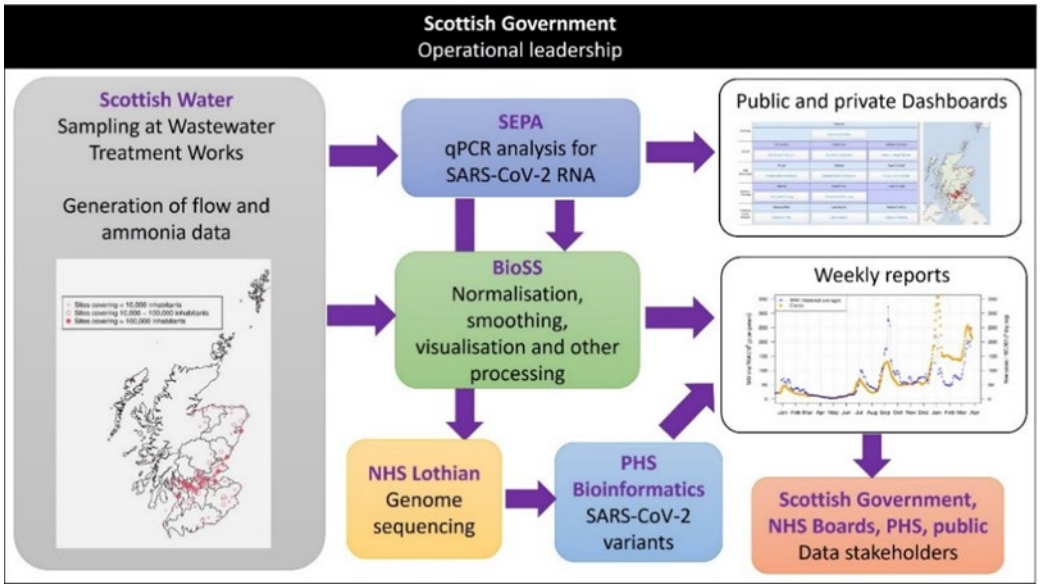
Non-routine processes are also conducted, such as repeat analysis and statistical modelling of samples indicating inhibition issues. At the PCR data analysis stage, automatic checks are conducted for improperly entered dates or site names, manual and automatic checks for the presence of outlier RNA or ammonia/flow levels, and detection of situations where coverage at a Local Authority level falls below 10%.

Currently, the laboratory service does not take part in an approved External Quality Assurance (EQA) scheme. Although there is no commercial EQA available for the testing of SARS-CoV-2 in wastewater, it is suggested that plans are developed for sample exchange with a similar service(s) to provide an alternative route to assurance.

4.3.7 Resource requirements

The delivery of the programme currently involves staffing across six organisations (**Figure 3**). Note the analysis and reporting of SARS-CoV-2 variants from genome sequencing data was transferred from BioSS to PHS in July 2023. A full review of the technical equipment required for the operation of the programme and costings are beyond the scope of this report. The amount of funding available to the programme and the current, year-on-year funding model are potential barriers to further development.

Figure 3. Wastewater Monitoring Programme components



5. Part B: System performance attributes

5.1. Simplicity

The operation of the system, established in response to the COVID-19 pandemic, is complex involving a partnership across six organisations. The system has increased in complexity over time with the evolving needs of stakeholder groups.

The transfer of data from the SEPA laboratory to BioSS is not fully automated. The downloading of data is usually straightforward, although there have been occasional issues with changed formats. The system is not embedded within the wider surveillance system infrastructure at PHS and so comparative data for informal validation is limited to publicly available sources (e.g., PHS dashboards).

The processes and methodology for PCR and genomic data wrangling, analysis and summary output production were developed specifically for the programme.

Developments continue, although this has been constrained by the prioritisation of routine report delivery and funding pressures. Information on volumes of influent received by WWTW's, known as flow (or ammonia levels if this information is unavailable), together with catchment area population sizes, are collected specifically for the programme and used to control for variability in SARS-CoV-2 RNA levels. The reliable interpretation of genomic sequence data derived from mixed samples such as wastewater is not well understood and requires specialist bioinformatic analysis.

The system is relatively simple with respect to quality assurance processes. A statistical process control procedure quantifies changes in recovery efficiency, which is used for informal benchmarking by BioSS analysts.

Although the generation of report outputs is now almost fully automated, some manual input is required to check influential data outliers and quality control data. System maintenance, including the analytical pipelines, requires significant attention with ad hoc changes often required at short notice. It is recognised by operational partners and data stakeholders that an end-to-end review of the current processes, set up in response to the emergency pandemic situation, is required to ensure the system is (i) streamlined where possible to remove bottlenecks and duplication of effort and (ii) continuing to address key public health needs in Scotland.

5.2. Flexibility

The surveillance system has flexibility in its design. Changes to the WWTW's selected for sampling and the sampling frequency can be made by SW, taking around three weeks to implement. Sampling in more remote locations, and modifying sampling to include network locations, can be challenging however due to resource constraints, physical accessibility, and health and safety considerations.

Currently, the laboratory capacity may limit the flexibility of the system for increasing the sampling and testing coverage. However, the programme does deliver population coverage of approximately 80% which is comparable to the 74% delivered by England’s Environmental Monitoring for Health Protection programme. It is recognised that achieving a higher population coverage may be difficult due to

technical issues, such as accessibility and private sewage networks that do not feed into the mainline system. (16)

The sampling, testing, analysis, and reporting processes have demonstrated flexibility in response to changes or challenges; for example, increases and decreases to sampling frequency and coverage, laboratory processes, new SARS-CoV-2 variants, comparative data sources, staff illness, new staff, and changes to budget allocation and budget uncertainty.

5.3. Acceptability

Wastewater-based surveillance enables a large proportion of the population to be captured in an unobtrusive, anonymous, and non-discriminatory manner, without the reliance on individuals interacting with and accessing the healthcare system. The system has produced reports reviewed by ministers and public health professionals for management purposes.

Public health acceptance of this approach was cautious during the height of the pandemic response given the extensive other measures of COVID-19 population prevalence that were available at the time, including the ONS infection survey (which was considered a gold standard measure) and widescale testing in the population (16; 17)). Other concerns included challenges with the reliability of outputs, with no formal validation, standards, or guidance to inform the effective application of this novel system for informing public health action in Scotland. Under a different scenario where there is an absence of alternative sources of community prevalence, the public health value of wastewater surveillance will be deemed greater, particularly as validation of the system and its outputs is prioritised in the near future.

5.4. PCR data quality

5.4.1 Completeness

The qPCR data generally have a high degree of internal completeness when received for analysis. Some issues can arise (e.g. changes to date formats and

duplicate data); however, these have been quickly resolved. The qPCR data relating to each composite wastewater sample may fail as a result of chemical inhibitors in the samples resulting in missing data. It is not possible to directly measure external completeness through a comparison of levels of SARS-CoV-2 RNA in wastewater and clinical testing-based systems, since the latter monitors the frequency of individual infections in the population.

5.4.2 Reliability

Reliability was assessed with respect to the ability of the system to collect, manage and provide results on levels of SARS-CoV-2 in wastewater without failure.

On average, 5.5% of samples had failed PCR results between January 2021 and February 2023, ranging on average from 2.6% to 8.1%. Generally, this failure of the qPCR relates to exceedance of the inhibition score and this rate of failure is typical for this type of material. Failure rates were overall below 4% in late Spring/Summer (May to August) and over 5% in the remaining months (September to April). This seasonal pattern was consistent across 2021 and 2022 and did not appear to be explained by reductions in sampling frequency or testing that occurred due to adverse weather conditions or festive holiday closures . The proportion of failed qPCR results also varied between WWTW sites; the top 20 sites with the highest failure rates had an average failure rate of 20% (ranging 12% - 50%) compared to an average failure rate of 3.3% across the remaining 102 sites. The reason for these apparent seasonal and site-specific effects with qPCR reliability requires further investigation.

Exceptionally high values of SARS-CoV-2 RNA levels can occasionally arise that are unverifiable by consecutive measurements or other clinical surveillance indicators, thereby affecting reliability of trends. This has been observed particularly following the introduction of Omicron in Scotland.

The laboratory service is not accredited at this stage and an inter-laboratory comparison highlighted issues with repeatability.

5.4.3 Validity

Validity represents the extent to which the system correctly determines the levels of infection in the population when compared to a gold standard data source, as typically measured by the sensitivity and specificity of the laboratory test and the statistical outputs from the system. The sensitivity and specificity of the PCR tests for detecting SARS-CoV-2 in wastewater samples is not quantified at this stage.

At the population scale, a number of technical difficulties with quantifying the sensitivity and specificity of the system for determining the presence or absence of COVID-19 cases or localised outbreaks have been identified: (i) the inability to compare SARS-CoV-2 presence or absence simultaneously in both wastewater and clinical samples derived from the same individuals, (ii) an inability to directly translate levels of SARS-CoV-2 RNA in wastewater into numbers of cases to enable direct comparison with other surveillance sources, (iii) differences in surveillance design in terms of sampling frequency and coverage, and (iv) the detection of SARS-CoV-2 in all time periods under investigation and geographical regions (i.e. no disease 'absence' to enable quantification of specificity).

The validity assessment will therefore focus on time series correlations and lags with other COVID-19 surveillance systems and ability to correctly detect periods of growth in COVID-19 activity. See **Part C: Performance of statistical indicators**.

5.5. Genomic data quality

5.5.1 Completeness

Missing data can arise for a number of reasons, including but not restricted to: samples without correct metadata (typically collection site and/or date), sequences that do not meet the quality control thresholds, for example owing to low viral concentration in the sample (note currently samples selected for sequencing are not PCR screened) and sequences that do not provide sufficient genomic information for the Freyja software to confidently assign a specific lineage (e.g., if the coverage in key lineage-defining positions is consistently low due to amplicon dropouts).

It is not possible to directly measure external completeness through a comparison of wastewater-derived genomic sequencing with the national clinical genomic surveillance system for SARS-CoV-2, since the latter monitors variants identified from individuals. The degree of any underreporting is reliant on the strategy for selection of wastewater samples for sequencing.

The current sampling strategy aims to collect 150 wastewater samples, of which 90 are sequenced. A subsampling algorithm prioritises samples from larger catchment areas, which may lead to a consistent underrepresentation of sampling sites located in less populated areas. Samples from larger catchment areas allow the sampling to more efficiently cover the population for a given amount of analysis effort. Further, samples from especially small catchments may be less consistent (or at least, more volatile) due to a smaller number of individuals contributing to viral shedding.

5.5.2 Reliability

Reliability was assessed with respect to the ability of the system to collect, manage and provide results from genomic sequencing of SARS-CoV-2 without failure. A number of areas of concern have been identified that could represent potential risks:

- Metadata is collected on paper, together with samples collected by Scottish Water, and sent to SEPA where data are transcribed into their LIMS system for sharing with the NHS Lothian laboratory;
- Potential effects of contamination;
- Potential problems in sequencing data transfer;
- Mid- to long-term availability and maintenance of software, tools, and database dependencies (e.g., lineage barcodes used by *Freyja*),);
- Changes in the ability to sequence samples (e.g., if Ct values increase due to lower viral loads, or primer efficacy drops due to a variant shift).

5.5.3 Validity

Validity represents the extent to which the SARS-CoV-2 variant frequency in wastewater is correct when compared to other data sources. The ONS infection survey formed the basis of a comparison at the population level, and the national clinical-based genomic surveillance system for analysis at subnational levels.

Analyses were carried out comparing population levels of SARS-CoV-2 variants detected in wastewater and the equivalent clinical data, based on data collected between 24 October 2022 and 30 June 2023. Relative abundance values were averaged across observed WWTW sites per sampling day and smoothed using 7-day moving averages. For comparison, relative frequencies for clinical data were calculated, also applying a 7-day moving average. The relative frequency (values from 0 to 1) of each lineage detected in wastewater and clinical samples across Scotland per collection day is shown in **Figure 4** and **Figure 5** respectively.

Figure 4 Wastewater sample-derived SARS-CoV-2 variant trends in Scotland, November 2022 - June 202

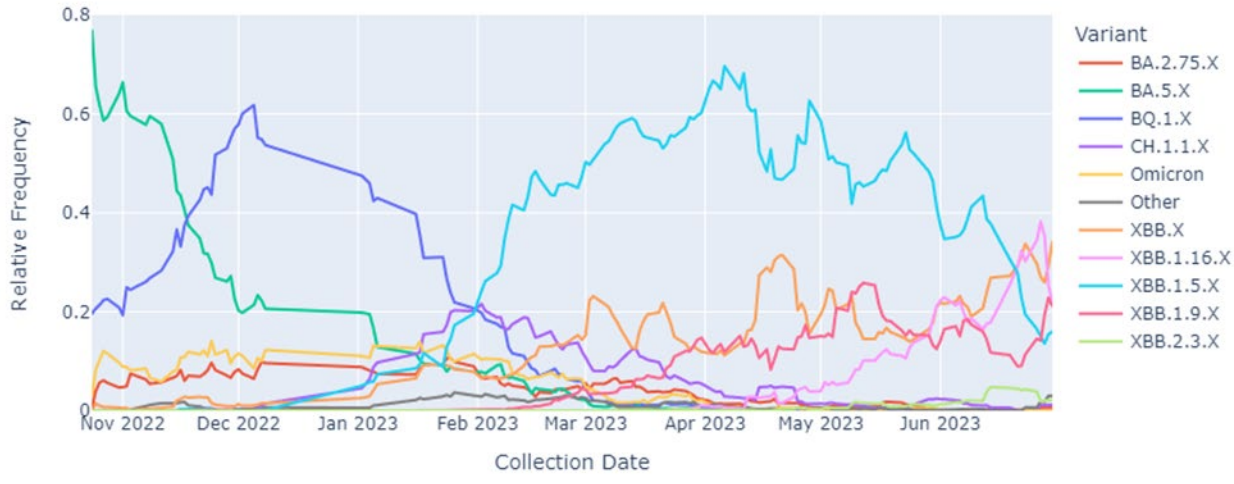
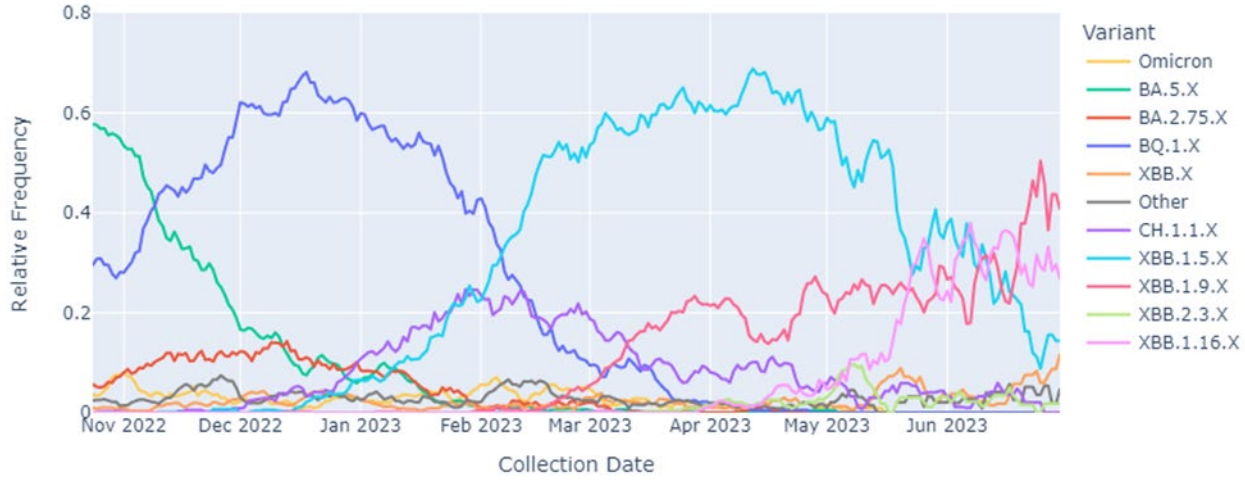


Figure 5. Clinical sample-derived SARS-CoV-2 variant trends in Scotland, November 2022 - June 2023



The relative proportions of variants over time in both data sources were similar in terms of the predominant variant in each period (i.e., successive "waves" of BA.5, BQ.1, and XBB.1.5). However, the specific variant abundances differed widely between the two data sources, with wastewater sometimes estimating substantially different proportions of specific variants compared to clinical samples (e.g., the estimated abundance was much lower in wastewater data than in clinical data for XBB.1.5 and the opposite for BQ.1). In addition, wastewater data are sometimes unable to differentiate between very similar variants, resulting in specific Omicron lineages can be defaulted to “Omicron” as an example (i.e., sequences identified as Omicron but not ascribed to a specific Omicron lineage) as shown from November 2022 to March 2023. Likewise, from February 2023 to June 2023, wastewater-derived data showed a larger proportion of XBB, which in clinical data is subdivided into different lineages within XBB. This may reflect a limitation of Freyja to assign sequence read data to individual lineages that differ in a small number of mutations, defaulting to a less specific lineage.

In conclusion, whilst the general trends in relative proportions of variants looked similar over time (e.g., majority variants), there are differences in the proportions of specific Omicron variants between wastewater and clinical data. Some emerging variants can be relatively easily detected from wastewater genomic data, but detection is challenging for others. Wastewater-derived sequencing data does not

provide an early detection of specific Omicron variants (as observed for specific variant signals within the study period such as CH.1.1, CH.1.1.1, XBB.1.5, XBB.1.5.7, and XBB.1.16, among others). Some variants will be difficult to identify from mixed samples due to the minimal distinguishing variation, potentially resulting in surveillance blind spots.

5.6. Representativeness

To date, the system has captured a maximum of 113 WWTWs in a given week, out of 189 WWTW sites in Scotland undergoing at least primary treatment serving 2,000 or more people. There are ~1,800 WWTWs in total across Scotland, including septic tanks treating household wastewater that are not covered by the COVID-19 surveillance programme; the smallest site in the programme serves 1,460 people. Remote, rural, and island communities are therefore less represented.

Examining data from w/c 27th May 2020 to w/c 30th August 2023, the maximum coverage achieved in a given week was 81% of the Scottish population served by the sewage network, representing 73% of the total Scottish population (**Figure 6**). When examining by NHS Board for a period of high sampling frequency (target of 90+ sites) the coverage ranged 15%-93% (**Table 1**). Further increases to site coverage are expected to provide minimal improvement to population coverage; for example, an increase from 80% population coverage to 90% would require an increase in WWTW sampling sites from ~100 to ~1800. As noted previously, similar coverage estimates are reported for England's Environmental Monitoring for Health Protection programme of 74% and it would be technically difficult to increase coverage further owing to technical issues such as accessibility and private sewage networks that do not feed into the mainline system. Details of the population coverage served by all SW wastewater assets are provided in Appendix C: Supporting data.

Given the aggregate nature of wastewater data, it is not feasible to assess the representation of the system according to individual-level attributes. For the genomic surveillance of SARS-CoV-2 variants, the percentage of sewered population covered by sequenced sites (averaged across sequencing runs) was 89.5%, ranging 23%-96% (**Table 1**).

Figure 6. Weekly summary of coverage, as a percentage of the Scottish population served by the national sewage system

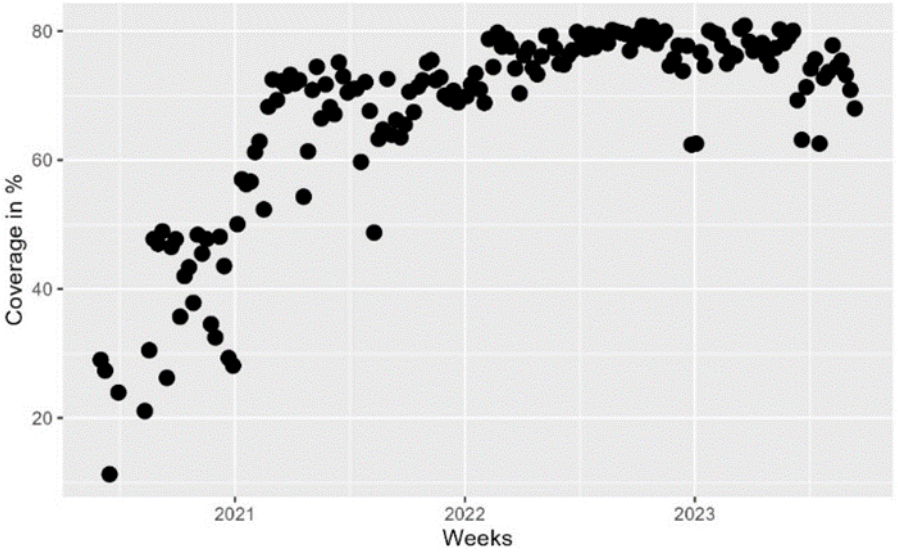


Table 1. Wastewater Treatment Works site coverage by NHS Board during a peak period of sampling

NHS Board1	Average no. sites sampled per week2	% coverage of sewered population in each Board by PCR data2	% coverage of Scottish population in each Board by PCR data2	% coverage of sewered population by genomic data3
Greater Glasgow & Clyde	9	94	93	96.4
Lothian	12.6	94	86	82.6
Lanarkshire	18.1	85	86	60.8
Grampian	10.2	76	66	72.6
Tayside	5.3	78	70	74.9
Fife	9.8	83	81	76.2
Ayrshire & Arran	7.1	87	87	82.1
Highland	8.1	54	39	41.9
Forth Valley	10.6	84	81	81.7
Dumfries & Galloway	5.8	50	36	39.3

NHS Board1	Average no. sites sampled per week2	% coverage of sewered population in each Board by PCR data2	% coverage of Scottish population in each Board by PCR data2	% coverage of sewered population by genomic data3
Borders	8.3	69	52	57.6
Western Isles	0.5	49	15	23.3
Shetland	0.7	60	25	42.3
Orkney	0.6	50	22	25.4

[1]Ranked in order of population size from high-to-low
[2]Sampled sites and coverage are for a period spanning October 2021 to August 2023
[3]Coverage relating to genomic data is for the period November 2022 to June 2023

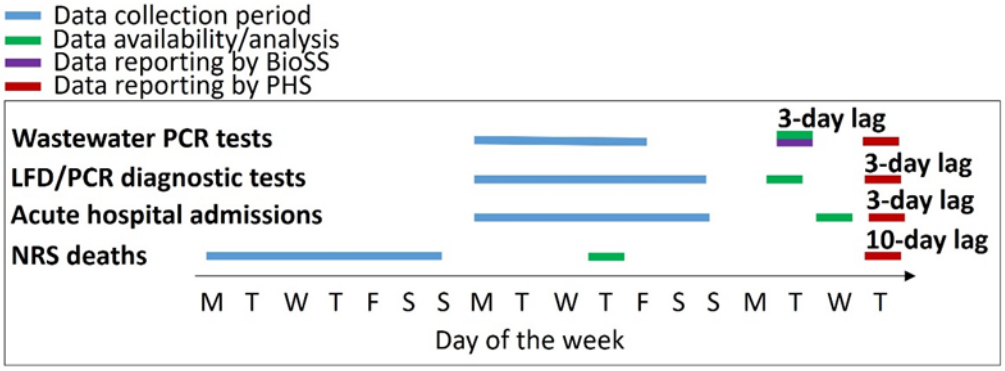
5.7. Timeliness

Wastewater samples are typically collected over a 5-day period (Monday to Friday), analysed at the SEPA lab by the following Monday, and statistically analysed and reported by BioSS on Tuesday. There is therefore a 3-day lag between the final sample collection dates and the data being reported by BioSS.

In comparison, COVID-19 case data (LFD or PCR diagnostic tests) are collected over a 7-day period (Monday to Sunday), extracted the following Tuesday and reported on Thursday. COVID-19 acute hospital inpatient admissions data are submitted to PHS for the previous week by Wednesday, when data are analysed for reporting on Thursday. There is a lag of 3 days between the testing and hospital admissions data collection and reporting by PHS. NRS deaths data are collated over a 7-day period (Monday to Sunday) and are available the subsequent Thursday.

In summary, wastewater PCR data are currently reported 2-days ahead of clinical COVID-19 surveillance data but are slightly more historic. The utility of wastewater signal as an early warning of changes in COVID-19 trends should not be hindered by the comparative timeliness of other surveillance indicators (**Figure 7**).

Figure 7. Comparing turnaround from data collection to data processing and reporting across COVID-19 surveillance systems



With respect to SARS-CoV-2 variants, a subset of wastewater samples collected over a one-week period undergo sequencing each week. Wastewater variant reports for SARS-CoV-2 are currently generated and circulated fortnightly on Thursday (previously weekly), incorporating bioinformatic analyses of samples collected and sequenced in the 2-4 weeks prior.

The typical lag between sample collection and variant reporting varies between 18 and 31 days. In comparison, SARS-CoV-2 variants reports from PCR-tested clinical samples are generated and circulated daily Monday to Friday, incorporating samples collected 2-weeks prior. The lag between sample collection and data availability can range 5-90+ days but with 94.6% returned within 30 days (based on most recent 6-month period). The typical lag between sample collection and reporting is on average 17 days for clinical samples collected during 2023.

In summary, SARS-CoV-2 variant data from clinical samples is reported more frequently than from wastewater, and the time between clinical sampling and reporting is typically shorter. Clinical sequencing data are incomplete but overall turnaround from sampling to data availability is shorter than for wastewater data.

5.8. Stability

The system has delivered weekly reports on levels of SARS-CoV-2 RNA trends from wastewater PCR testing for the SG and other stakeholders on each Tuesday from 12 January 2021. The frequency of reporting has evolved over time to meet the needs of

stakeholders at different stages of the pandemic response; 49 reports were produced in 2021, 50 reports were produced in 2022, and 34 reports in 2023 (out of a possible 38 as of w/c 09 October). Four reports were not delivered in the summer of 2023 due to a laboratory contamination issue.

The system currently delivers fortnightly (formerly weekly) reporting of SARS-CoV-2 variants aligning to PHS's requested reporting schedule, with 17 reports produced out of a possible 17 (10 by BioSS and 7 by PHS) since February 2023, as of w/c 09 October 2023.

A large decrease in SARS-CoV-2 levels was seen for samples collected over the period 21 December 2022 to 4 January 2023. This may have been a consequence of the freezing and subsequent thawing of samples collected over this period. An adjustment was therefore made to the analysis over this period, based on estimated divergences in the statistical process control. Sampling was also restricted over this period to the largest sites.

The delivery of sampling and PCR testing has been largely resilient throughout. The accessibility and physical context of certain WWTW sites can increase the chance that samples are not collected, may constrain sampling capacity, or affect sample quality through risk of chemical inhibition. There are rare cases where samples cannot be collected, for example owing to freezing conditions or broken samplers.

Capacity issues at the SEPA laboratory can cause analyse delays or reduced outputs, particularly during the periods of the year when SEPA has statutory duties to test 150 samples per week from bathing waters. Occasional unexpected issues, such as lack of water supply, laboratory reagents, and power cuts can prevent the laboratory from operating. The operation of the programme has been resilient to challenges faced through successive internal reorganisations, leading to staff moving on and subsequent loss of expertise.

5.9. Usefulness

The surveillance system has the potential to generate intelligence on population-level trends of COVID-19 activity in Scotland. However, levels of viral RNA in Scotland

cannot as yet be reliably translated alone into numbers of cases and population prevalence or stratified by disease severity, although these data have been used to inform estimates alongside other indicators of disease burden. Caution is therefore needed in the use of the system for estimating the burden of COVID-19 disease in Scotland. Variation in the presence of SARS-CoV-2 in the sewage network, owing to differences in shedding from the gastrointestinal and/or respiratory tract (e.g., disposal of tissues) between individuals, SARS-CoV-2 variants, and due to seasonal factors, is not yet well described.

Unverifiable fluctuations in (unsmoothed) SARS-CoV-2 levels in wastewater limit the usefulness of the system outputs from PCR testing for informing public health action in real-time. The system was used by an NHS Board to inform the allocation of mobile testing units during the pandemic response following a rise in wastewater signal (18). This aspect of the systems utility is unlikely of value outwith emergency public health response.

The system has value in the analysis of smoothed temporal trends in COVID-19 activity. These data have also been used to inform epidemiological models of SARS-CoV-2 transmission, following a transformation of RNA levels into measures of population prevalence (requiring assumptions of shedding rates at the individual level), in the absence of alternative sources of data for community infection. Such models were regularly reviewed by the UKHSA Epidemiology Modelling Review Group to inform consensus statements on the COVID-19 situation in the UK (19; 20).

The genomic sequencing of wastewater samples has the potential to confirm the presence of SARS-CoV-2 variants of concern in Scotland. There remain challenges however in the reliability of measures of presence and abundance of SARS-CoV-2 variants, and confirmation of absence, from wastewater sampling. Challenges also remain in establishing the minimum sampling strategy required to detect SARS-CoV-2 variants and monitor their spread geographically with a given level of confidence (see **Genomic data quality**). This is being addressed through review of the existing strategy.

5.10. Communication

The frequency of reporting meets the needs of public health professionals, SG, and the public. Normalised data (adjusted for flow and population) were reported by SG (fortnightly) and currently by PHS in public facing reports (weekly or monthly) and a Dashboard (weekly). Unadjusted data are currently available on public and private Dashboards hosted by SEPA. Weekly Management Information reports are also produced for public health and policy decision makers. Wastewater surveillance data presents a challenge for clear interpretation owing to the aggregated scale, uncertainty in the sources of variation, and fluctuating nature of the signal. It is critical that the specialist information is easily and accurately interpretable by public health professionals and policy makers who may potentially use the data for responding to incidents and informing public health action at a local or national level.

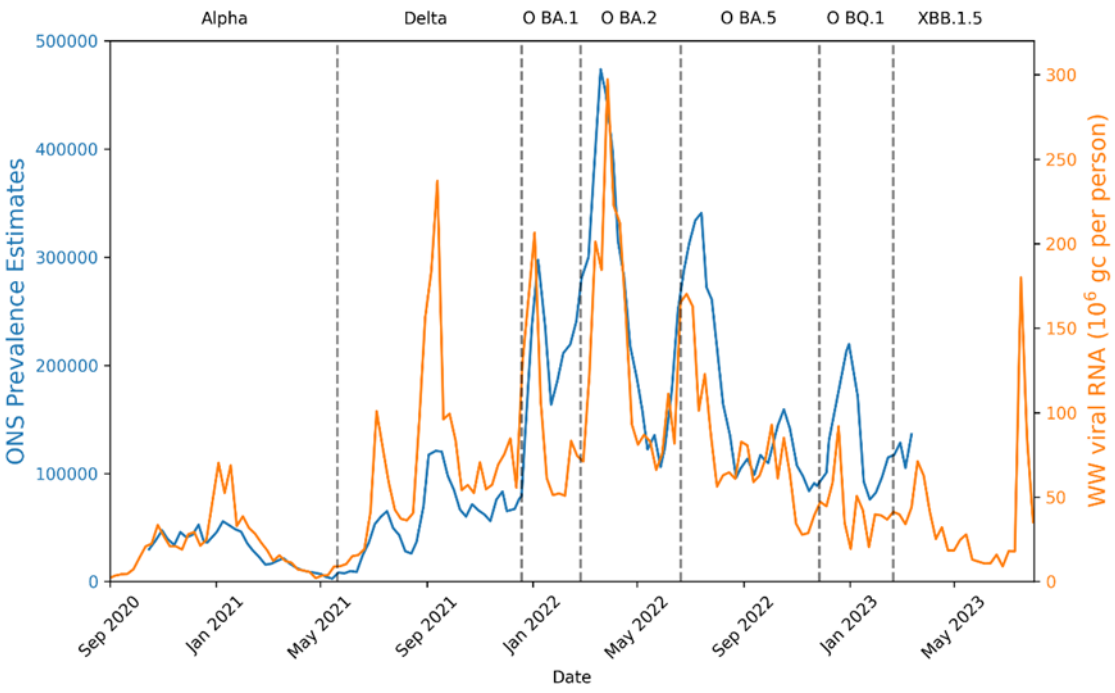
6. Part C: Performance of statistical indicators

6.1. Correlations with other surveillance indicators

An early study conducted in Scotland demonstrated a strong correlation between SARS-CoV-2 RNA in wastewater and COVID-19 case frequency and deaths for data up to January 2021, with significant variation found across wastewater sites (9). Here we investigated the performance of the system in more recent periods, allowing for comparisons across periods of coverage and SARS-CoV-2 variants.

The time series of weekly normalised SARS-CoV-2 RNA levels detected in wastewater, as reported routinely by the surveillance system, were compared against two other sources of community COVID-19 case trends (i) weekly ONS Infection Survey prevalence estimates and (ii) weekly clinical case numbers identified through LFD and/or PCR diagnostic testing. Normalised levels of SARS-CoV-2 RNA in wastewater fluctuate week on week to varying degrees (**Figure 8**), making their direct use for informing public health action in real-time challenging in the absence of any quantifiable confidence in apparent changes in trajectory.

Figure 8 Extended time series of COVID-19 activity in wastewater against ONS infection survey prevalence



Owing to these fluctuations in weekly levels of SARS-CoV-2 levels, statistical correlations were quantified for the period April 2021 to July 2022 following smoothing of the wastewater time series using a 7-day moving average (see **Figure 9** - **Figure 10**). See **Appendix C: Analytical methods** for details.

The overall national-level correlations with wastewater COVID-19 levels were 0.86 for ONS prevalence estimates and 0.70 for cases detected through diagnostic testing. During a period of medium WWTW, coverage when the Delta variant dominated (23rd May 2021 to 7th November 2021), relatively high correlations of 0.94 and 0.96 were found when comparing levels of COVID-19 in wastewater against ONS prevalence estimates and clinical cases respectively.

In contrast, relatively low correlations of 0.68 and 0.36 were found, when comparing levels of COVID-19 in wastewater against ONS prevalence estimates and clinical cases respectively, for a period of high WWTW coverage when Omicron variants dominated (from 19th December 2021 to 1st July 2022). Table 2 summarises the geographic variation in the correlation estimates against clinical testing data by NHS

Board. Correlations ranged from a low of 0.59 in NHS Highland to 0.80 in NHS Orkney and NHS Fife.

Figure 9. Temporal correlation between national COVID-19 activity from wastewater and ONS infection survey prevalence estimates

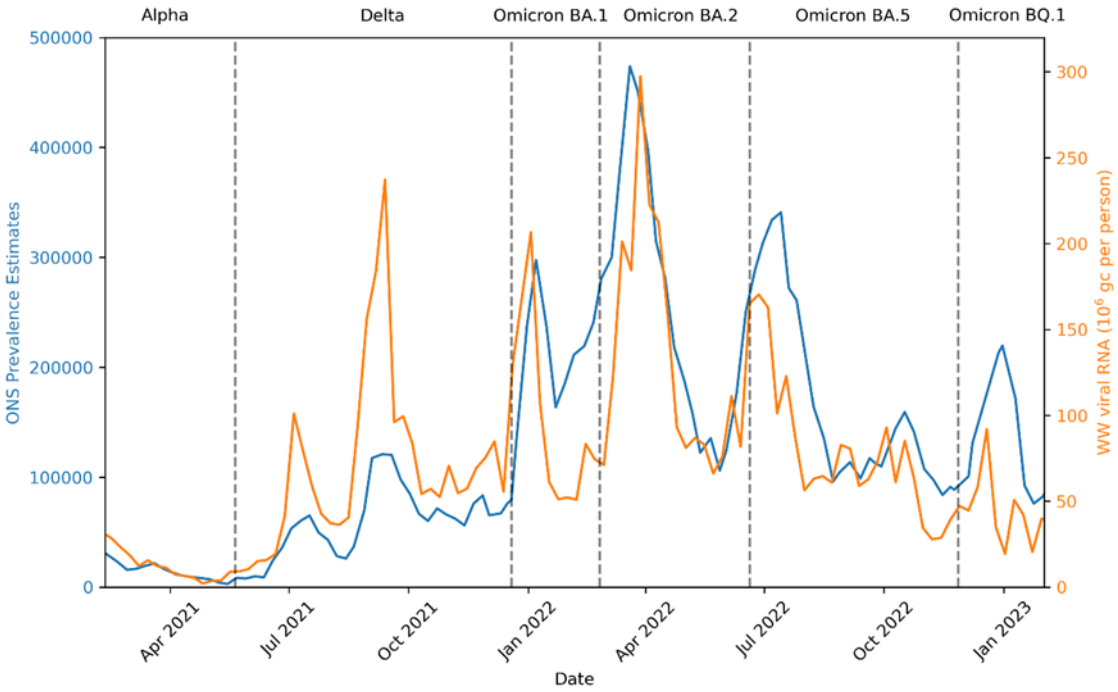


Figure 10. Temporal correlation between national COVID-19 activity from wastewater and cases identified through diagnostic testing

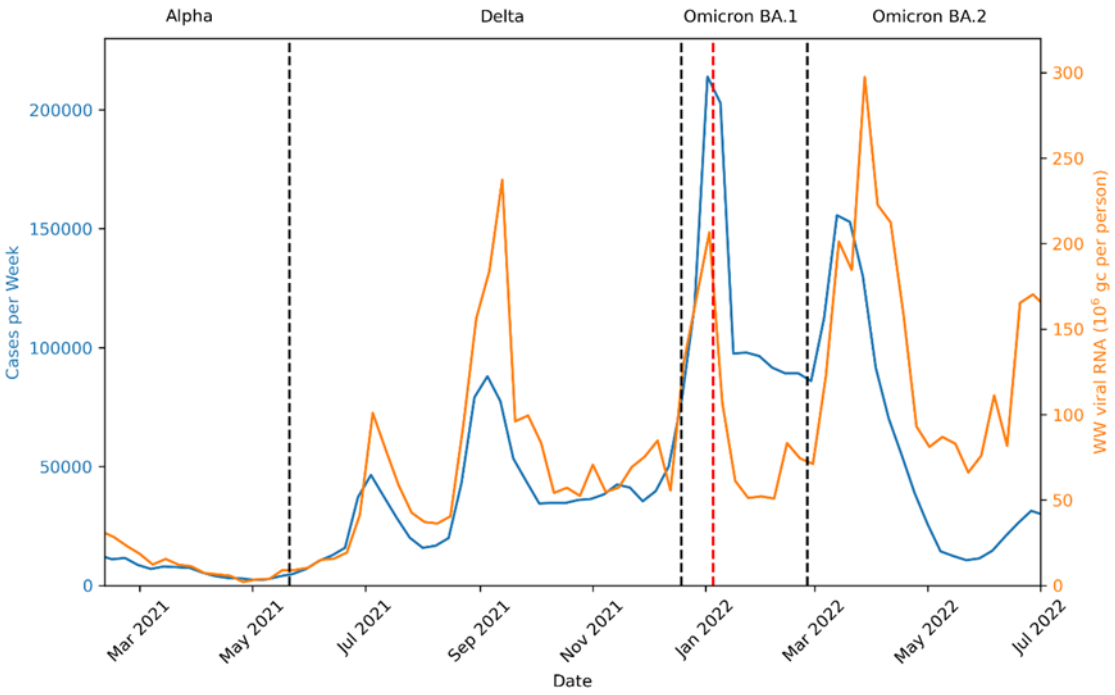


Table 2. Correlation between weekly COVID-19 activity detected in wastewater and testing surveillance nationally and by NHS Board

Location1	Med coverage + Delta (May 2021-Nov 2021)	High coverage + Omicron (Dec 2021-Jul 2022)	Overall period (Apr 2021-Jul 2022)
Scotland	0.95	0.28	0.74
Greater Glasgow & Clyde	0.96	0.34	0.67
Lothian	0.87	0.40	0.72
Lanarkshire	0.93	0.36	0.69
Grampian	0.94	0.53	0.74
Tayside	0.92	0.53	0.79
Fife	0.94	0.17	0.80
Ayrshire & Arran	0.90	0.03	0.61

Location1	Med coverage + Delta (May 2021-Nov 2021)	High coverage + Omicron (Dec 2021-Jul 2022)	Overall period (Apr 2021-Jul 2022)
Highland	0.91	0.29	0.66
Forth Valley	0.92	0.45	0.70
Dumfries & Galloway	0.89	0.17	0.76
Borders	0.67	-0.04	0.67
Western Isles	0.78	-0.23	0.68
Shetland	0.82	0.30	0.78
Orkney	0.95	0.28	0.85

[1] Ranked in order of population size from high-to-low.

6.2. Early warning of rising cases and severe disease

The performance of the system as an early indicator of a rise in COVID-19 cases, acute hospital admissions, and deaths was assessed for two periods. A comparison of wastewater trends against hospital admissions and deaths is shown in **Figure 11** - **Figure 12**. Nationally, the wastewater signal was found to lead ONS prevalence by 4 days (Delta period) and 2 days (Omicron period). Comparing against clinical cases however, the wastewater signal lagged by 3 days (Delta period) but not during Omicron. The wastewater signal was found to lead COVID-19 hospitalisations by 8 days (Delta period) and 4 days (Omicron period), and deaths by 27 days (Delta period) and 12 days (Omicron period) nationally. Some variation was seen by NHS Board. See **Table 3** - **Table 4** for results and **Appendix C** for methods.

Figure 11. National temporal correlation between COVID-19 activity from wastewater and COVID-19 acute hospital admissions

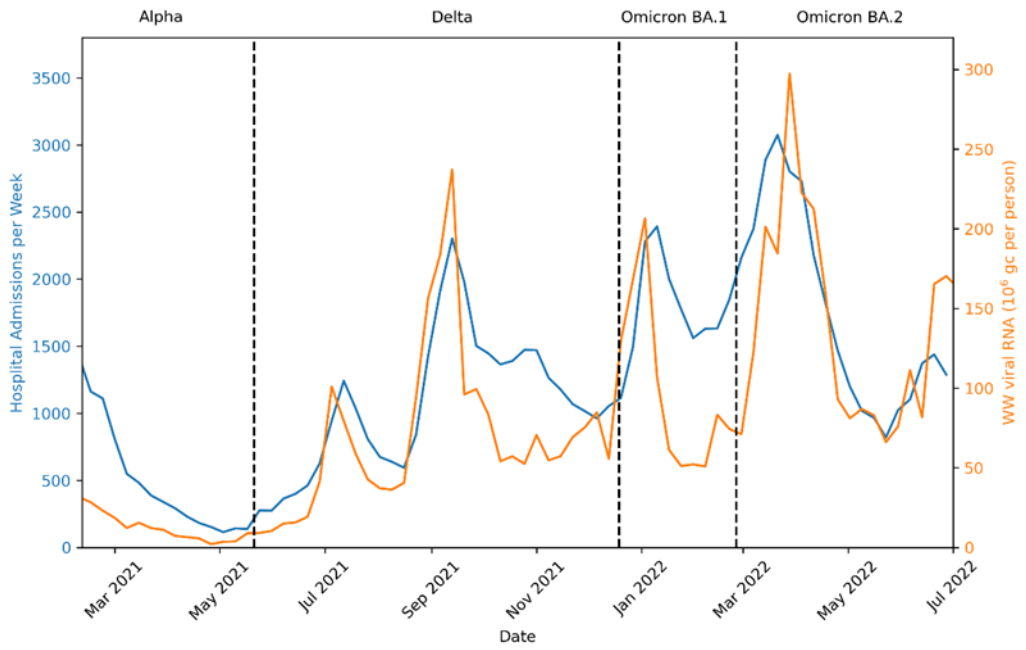


Figure 12. National temporal correlation between COVID-19 activity from wastewater and COVID-19 deaths

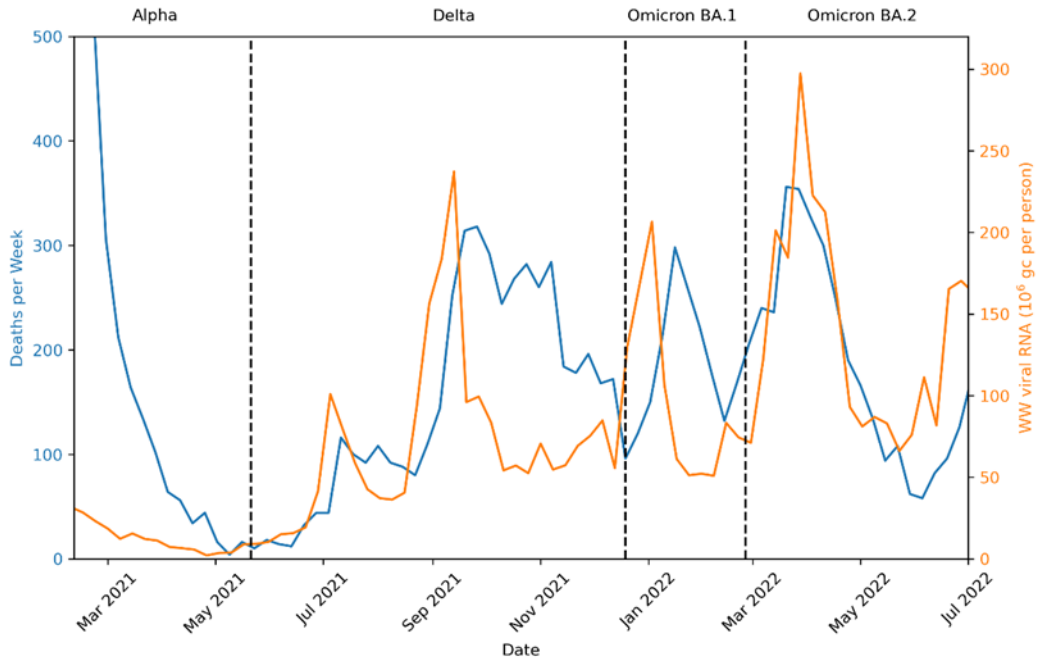


Table 3. Performance of wastewater surveillance as an early indicator of growth in COVID-19 cases and severe outcomes: period of medium sampling coverage and Delta variant dominance

Location[1]	Lag (in days) with COVID-19 cases[2]	Lag (in days) with COVID-19 hospitalisations[2]	Lag (in days) with COVID-19 deaths[2]
Scotland	3	-8	-27
Greater Glasgow & Clyde	3	-9	-30
Lothian	1	1	-13
Lanarkshire	6	-4	-11
Grampian	0	-7	2
Tayside	2	-7	-27
Fife	4	-3	-19
Ayrshire & Arran	0	-9	-30
Highland	7	-7	-6
Forth Valley	6	9	15
Dumfries & Galloway	3	-26	-25
Borders	0	-6	-18
Western Isles	-14	-3	-17
Shetland	8	-7	-26
Orkney	8	-8	-30

1. Ranked in order of population size from high-to-low
2. Cross-correlation analysis for describing the relationship between wastewater signal and comparative COVID-19 surveillance indicators: positive values indicate the wastewater signal lags; negative values indicate the wastewater signal leads.

Table 4. Performance of wastewater surveillance as an early indicator of growth in COVID-19 cases and severe outcomes: period of high sampling coverage and Omicron variant dominance

Location[1]	Lag (in days) with COVID-19 cases[2]	Lag (in days) with COVID-19 hospitalisations[2]	Lag (in days) with COVID-19 deaths[2]
Scotland	0	-4	-12
Greater Glasgow & Clyde	0	15	-17
Lothian	0	-1	-26
Lanarkshire	0	-12	-21
Grampian	0	2	-20
Tayside	0	-9	-20
Fife	-1	-1	-24
Ayrshire & Arran	0	6	-9
Highland	9	30	24
Forth Valley	0	21	22
Dumfries & Galloway	-4	-14	-24
Borders	0	-12	-24
Western Isles	-14	-1	-17
Shetland	-30	-4	-20
Orkney	9	-6	-22

1. Ranked in order of population size from high-to-low

2. Cross-correlation analysis for describing the relationship between wastewater signal and comparative COVID-19 surveillance indicators: positive values indicate the wastewater signal lags; negative values indicate the wastewater signal *leads*.

6.3. Retrospective detection of periods of growth in COVID-19 activity under maximised performance

The performance of the system for correctly detecting periods of growth in COVID-19 activity was assessed retrospectively applying a bespoke statistical modelling method for a period spanning April 2021 to July 2022. In these analyses, case trends identified through diagnostic testing were treated as the 'true' estimates. Altering the degree of improvement in model fit, through retaining or excluding data outliers, provided a range of possible estimates. Results are shown for the best possible system performance, whereby statistical models have been optimised in terms of the fit of the model to the data. See **Appendix C** for methodology details. At an NHS Board level, results are summarised for the WWTW sites that yielded minimal and maximal performance values (see **Table 5**).

At a national level, the maximum *sensitivity* and *specificity* of the system for detecting periods of growth in COVID-19 activity (where wastewater identifies a period of growth and decline that is matched, or not matched, in the clinical cases respectively) at a WWTW site level was 100% respectively. The minimal national *sensitivity* and *specificity* at a WWTW site level were 0% and 18% respectively.

Some variation in system performance for detecting growth in COVID-19 activity was found across sites and NHS Boards. *Sensitivity* estimates ranged at a WWTW site level from lows of 0% in NHS Highland to 100% in NHS Western Isles, with maximum values ranging 75% in NHS Orkney to 100% for 11 out of 14 NHS Boards. Similarly, *specificity* estimates at the WWTW site level ranged from lows of 18% in NHS Western Isles to 88% in NHS Shetland, with maximum values ranging 18% in Western Isles to 100% for 9 out of 14 NHS Boards. System performance appeared to broadly increase with increasing WWTW catchment population size (**Figure 13**).

Figure 13. Relationship between system validity in detecting COVID-19 growth and WWTW site catchment population size

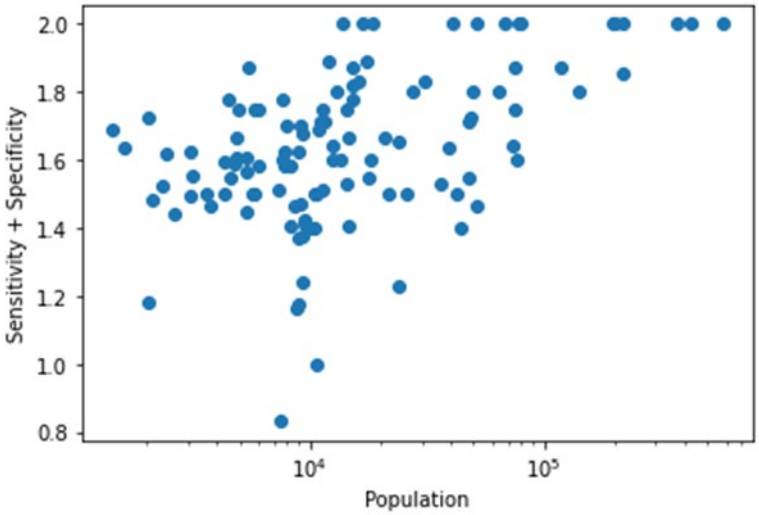


Table 5. Retrospective analysis of wastewater surveillance performance for correctly detecting growth in COVID-19 activity at a WWTW site level in Scotland, April 2021 - July 2021

Location[1]	Sensitivity range (%)[2]	Specificity range (%)[3]	Range PPV (%)[4]	Range NPV (%)[5]
Scotland	0-100	18-100	0-100	25-100
Greater Glasgow & Clyde	60-100	64-100	56-100	67-100
Lothian	38-100	56-100	45-100	44-100
Lanarkshire	45-100	22-100	13-100	45-100
Grampian	33-100	64-90	56-90	45-100
Tayside	57-100	58-100	50-100	63-100
Fife	60-100	50-100	38-100	67-100
Ayrshire & Arran	50-100	60-100	54-100	50-100
Highland	0-100	38-100	0-100	25-100
Forth Valley	45-100	50-100	50-100	33-100
Dumfries & Galloway	50-100	50-90	38-89	55-100

Location[1]	Sensitivity range (%)[2]	Specificity range (%)[3]	Range PPV (%)[4]	Range NPV (%)[5]
Borders	36-89	38-100	29-100	36-91
Western Isles	100-100	18-18	10-10	100-100
Shetland	89-89	88-88	89-89	88-88
Orkney	75-75	64-64	60-60	78-78

1. Ranked in order of population size from high to low
2. Sensitivity: proportion of predicted periods of growth in COVID-19 activity from wastewater that were corroborated by diagnostic testing data.
3. Specificity: proportion of predicted periods with no growth in COVID-19 activity from wastewater that were corroborated by diagnostic testing data.
4. Positive Predictive Value (PPV): proportion of 'true' periods of growth in COVID-19 activity from diagnostic tests detected by the wastewater system.
5. Negative Predictive Value (NPV): proportion of 'true' periods of no growth in COVID-19 activity from diagnostic tests detected by the wastewater system.

6.4. Real-time detection of periods of growth in COVID-19 activity under maximised performance

The performance of the system for correctly detecting growth in COVID-19 activity was also assessed in *real-time* for the period April 2021 to July 2022. Case trends identified through diagnostic testing were applied as the 'true' estimates.

In these analyses, the model was fitted to optimise wastewater signal performance, applying data up to the week before the current week of interest. The most recent weekly estimate of levels of COVID-19 in wastewater was then added as the real-time data point and retained if the model fit improved. In this way, the procedure removes data outliers. See **Table 6** for results and **Appendix C** for methods.

At a national level, the maximum *sensitivity* and *specificity* of the system for detecting periods of growth in COVID-19 activity in real-time (where wastewater identifies a period of growth and decline that is matched, or not matched, in the clinical cases respectively) at a WWTW site level was 100% respectively. The minimal national *sensitivity* and *specificity* at a WWTW site level were 14% and 57% respectively.

Some variation in system performance for detecting growth in COVID-19 activity was found across sites and NHS Boards. *Sensitivity* estimates ranged at a WWTW site level from lows of 14% in NHS Highland to 100% in NHS Shetland, with maximum values ranging 60% in NHS Orkney to 100% for 11 out of 14 NHS Boards. Similarly, *specificity* estimates at the WWTW site level ranged from lows of 56% in NHS Fife to 83% in NHS Western Isles and NHS Orkney, with maximum values ranging 73% in NHS Shetland to 100% for 10 out of 14 NHS Boards.

Table 6. Real-time analysis of wastewater surveillance performance for correctly detecting growth in COVID-19 activity at the WWTW site level, April 2021 - July 2021

Location[1]	Sensitivity range (%)[2]	Specificity range (%)[3]	Range PPV (%)[4]	Range NPV (%)[5]
Scotland	14-100	57-100	50-100	25-100
Greater Glasgow & Clyde	60-100	57-100	36-100	60-100
Lothian	43-100	60-100	43-100	29-100
Lanarkshire	60-100	60-100	43-100	63-100
Grampian	29-100	59-100	42-100	17-100
Tayside	43-100	69-100	60-100	43-100
Fife	60-100	56-88	44-83	67-100
Ayrshire & Arran	50-100	67-100	58-100	50-100
Highland	14-100	57-100	50-100	25-100
Forth Valley	50-100	67-100	57-100	29-100
Dumfries & Galloway	50-88	67-100	64-100	43-90
Borders	50-100	64-100	50-100	27-100
Western Isles	64-64	83-83	88-88	56-56
Shetland	100-100	73-73	67-67	100-100
Orkney	60-60	83-83	75-75	71-71

1. Ranked in order of population size from high-to-low

2. Sensitivity: proportion of predicted periods of growth in COVID-19 activity from wastewater that were corroborated by diagnostic testing data.
3. Specificity: proportion of predicted periods with no growth in COVID-19 activity from wastewater that were corroborated by diagnostic testing data.
4. Positive Predictive Value (PPV): proportion of 'true' periods of growth in COVID-19 activity from diagnostic tests detected by the wastewater system.
5. Negative Predictive Value (NPV): proportion of 'true' periods of no growth in COVID-19 activity from diagnostic tests detected by the wastewater system.

7. Study limitations

A full assessment of all variations in system performance and the causes is beyond the scope of this evaluation. It should also be noted that the evaluation has focused on assessing system validity for both PCR and genomic data sources historically. It is possible that system performance under current or future epidemiological conditions, for example following the introduction of new SARS-CoV-2 variants, may differ from that examined here. The statistical results present a broad and relatively simple view of overall performance and are subject to additional systematic uncertainty owing to the complexity of the temporal trends in both the wastewater data and the comparative COVID-19 data sources. A cost-benefit analysis, and the wider potential utility of wastewater surveillance beyond the context of COVID-19, are outwith the scope of this evaluation. Furthermore, SARS-CoV-2 is the only marker currently routinely tested for by Scotland's Wastewater Monitoring Programme for which suitable data are available for validating system performance.

8. Discussion

The wastewater-based surveillance of SARS-CoV-2, operated by Scotland's Wastewater Monitoring Programme, was initiated in response to the COVID-19 pandemic. The programme was established rapidly under lockdown conditions, with the design and delivery aimed to meet the evolving needs of the pandemic. The system met its intended objectives of monitoring SARS-CoV-2 levels across Scotland and understanding localised risks.

The importance and usefulness of the system has changed over time, alongside developments in other COVID-19 surveillance systems and the evolving epidemic situation. The public health utility of the system is greater in the absence of other community surveillance systems for COVID-19 infection trends, although it is important to note that these alternative sources are needed for ongoing validation of system performance, particularly with evolution of new SARS-CoV-2 variants. Ongoing public health input will further improve the usefulness of the system going forwards.

The current operational structure of the system, as developed in response to the COVID-19 pandemic, is complex and does not support direct integration of the system with other COVID-19 surveillance systems or public health governance infrastructures. Rationalisation of the multiple data dissemination routes may be warranted, together with guidance on the limitations and appropriate interpretation of the data, to support its use by public health professionals and decision makers.

The system has flexibility in the sampling design and may be adaptable with evolving epidemiological needs. Challenges remain, however, in establishing an optimal sampling design, with sufficient statistical power to deliver the objectives of the programme whilst maintaining cost-effectiveness, particularly with respect to monitoring SARS-CoV-2 variants. For example, what statistical distribution best captures levels of SARS-CoV-2 or variant presence in the population? How does this vary under different prevalence conditions? What is the minimal target level of SARS-CoV-2 for detection? What level of precision is tolerable? A cost-benefit analysis is beyond the scope of this evaluation however the findings may be used to inform such an analysis in future.

Acceptability of the system is good overall, although public health professionals remain cautious owing to limited quality assurances, with a lack of integration of the system with clinical pathogen testing governance infrastructure. Regular fluctuations in the reported wastewater data also complicate the use of the data for informing near real-time public health decision making. The representativeness, timeliness, and stability of the system is reasonable. The assessment of data quality with respect to validity and reliability is challenging to determine without quantification of PCR test performance and a lack of a suitable gold standard measure. The laboratory service

does not currently take part in an approved EQA scheme, although alternative routes to quality assurance are feasible.

In assessing validity at the national level, the smoothed wastewater signal was found to be strongly correlated with other indicators of community infection levels during the Delta variant wave. However, a shift in the wastewater signal curve was apparent following the introduction of Omicron in Scotland. Similarly, levels of SARS-CoV-2 in wastewater preceded rises in COVID-19 hospitalisations and deaths, but not consistently following Omicron. The reason for variation in system performance owing to the circulating SARS-CoV-2 variant is not known but could potentially include differences in the incubation period, tissue tropism, relative levels and duration of shedding, co-circulation of other respiratory infectious diseases, and the temporality of shedding between SARS-CoV-2 variants and also body systems (upper versus lower respiratory tract and gastrointestinal tract). The system does not routinely convert levels of SARS-CoV-2 RNA into estimates of population prevalence, a process reliant on assumptions of SARS-CoV-2 shedding/presence in the gut (or presence in the sewage system through other means), which may vary across individuals as well as by SARS-CoV-2 variants.

When optimised with statistical modelling procedures, the system shows variable performance in retrospectively identifying periods of growth in COVID-19 activity (determined against ONS infection survey nationally and clinical case data across NHS Boards). As evidence in Section 6.3, this variation appears dependent on the size of WWTW site catchments, with excellent performance found for larger populations but poor performance for smaller populations. This pattern was consistent across NHS Boards and across retrospective and real-time analyses. The reason for this apparent population-size dependent variation in performance is unknown, although it is anticipated there may be many contributing factors, such as differences in local level infection prevalence, the proportional contribution of commuters, the volume of wastewater influent flow, and local sewage infrastructure. Previous analyses have also found differences in the statistical slope of the relationship between COVID-19 case frequency and wastewater signal across wastewater sites (9). A challenge remains in determining thresholds of significant growth or decline that may trigger decision-making processes, and the associated

acceptable levels of false positive or negative alarms to inform appropriate usage at local and national levels.

The assessment of variation in performance at NHS Board and site levels was conducted by comparing to the clinical testing data, in part because the “gold standard” comparison to ONS surveillance data is not possible at this resolution. It should be noted that clinical testing data are inherently biased towards individuals seeking and accessing diagnostic tests while wastewater surveillance is not affected by this type of testing bias. In the context of COVID-19, the purpose of clinical testing has evolved over time, with a focus on testing symptomatic individuals by the end of September 2022 as Scotland transitioned out of the emergency pandemic response. Whilst there is anticipated variation in case ascertainment over time (based on a previous analysis (21)), these biases are not expected to impact significantly on the timing or shape of the COVID-19 activity trends evaluated here.

The utility of the system for detecting specific SARS-CoV-2 variants is dependent on the novelty of the associated genetic mutations and is largely useful in determining variant presence rather than geographic spread under the current sampling regime, depending on the novelty of the genetic profile of the variant. Comparison with clinical data shows that wastewater surveillance has limited utility for accurately identifying specific sub-variants and quantifying their relative abundance. Although the sequencing technology is very similar (amplicon-based Illumina whole-genome sequencing using the same primer sets), clinical samples are obtained through a nose swab from a single patient, whereas wastewater samples comprise biological data from multiple individuals. Although, there are differences in the representativeness of samples, with clinical data currently predominantly targeting hospitalised cases. Therefore, direct sample-to-sample comparisons are not possible, although an assessment may be feasible through temporal and/or geographical aggregation at a population scale, albeit with some anticipated confounding owing to the different sampling designs.

Greater confidence can be given to variant classifications from clinical samples, since they contain an almost clonal SARS-CoV-2 population from which a full genome is reconstructed generating a mutational profile across the full genome. In contrast, wastewater-derived genome sequences consist of smaller fragments of the

SARS-CoV-2 genome comprised of multiple viral populations that cannot be concatenated to reconstruct a full genome. Specific software (in this case, the program *Freyja*) is needed to model which variants are present at a relative abundance based on the frequencies of mutations known to be present in each variant described so far. Information on the lower limit of detection of the technique is required, and a minimum abundance cut-off to determine variant presence. However, limits of detection are SARS-CoV-2 variant specific and depend on the uniqueness of the mutational profile with respect to co-circulating variants. The lack of detection of a given variant in one sample cannot rule out its presence, preventing a reliable estimation of specificity. Whether such differences are due to limitations of the wastewater system, or biases in the clinical data, is yet to be determined. A possible approach to evaluating the performance and detection thresholds of *Freyja* would be to conduct an External Quality Assessment, in which mock mixed samples including known abundances of different variants, are sequenced to test if the current methods detect those abundances correctly.

Finally, it should be noted that the performance of wastewater-based surveillance for monitoring the presence and spread of pathogens is not expected to be the same in all countries and settings owing to several factors, including differences in sewage system infrastructure, sample collection and testing methods, and the underlying epidemiology of the disease, including the distribution of circulating SARS-CoV-2 variants. The findings presented here are therefore specific to Scotland and may not apply to other contexts. Given the ongoing evolution of the SARS-CoV-2 virus, and apparent variation in system performance depending on the SARS-CoV-2 variants in circulation, ongoing evaluation will be critical to ensure continued reliability and accurate interpretation of wastewater surveillance system outputs. Cost implications may however limit the nature and availability of comparative data sources and feasibility of undertaking such evaluation exercises.

9. Conclusion

A Wastewater Monitoring Programme was successfully established in Scotland to monitor COVID-19 activity at national and localised levels during the COVID-19

pandemic response. The quality of intelligence generated by the system for monitoring trends in COVID-19 activity, and presence of SARS-CoV-2 variants, is dependent on the specific variants in circulation, and with variation in performance across locations. The value of the system is in trend analysis, and potentially in informing predictive and scenario modelling exercises. Owing to the fluctuating nature of the data, caution is needed in interpreting the outputs without statistical smoothing techniques, and in using the outputs to inform immediate public health action. Caution is also needed in comparing the timing and magnitude of activity across different periods of SARS-CoV-2 variant dominance, and in ascertaining the presence of novel SARS-CoV-2 variants with indistinguishable genetic profiles.

10. Recommendations

Following the completion of the evaluation and reviewing the objectives, the project team concluded that the Wastewater Monitoring Programme in Scotland has value in supporting the following epidemiological goals:

1. *The description and understanding of broad temporal trends and geographic spread of COVID-19 in Scotland, with caution currently in the interpretation when applied to smaller populations and when comparing across different periods of dominating SARS-CoV-2 variants;*

2. *Timely identification of new periods of growth in COVID-19 activity, with caution currently in the interpretation when applied to smaller populations and with consideration that the* data may not yet be sufficient for informing immediate public health action*;*

3. *Determination of predominant SARS-CoV-2 variants in Scotland, and detection of novel variants, with caution that they require sufficiently distinguishable mutational profiles currently.*

4. As a result, it is recommended that the above activities continue, albeit with periodic review to ensure they address Scotland's evolving public health

needs for COVID-19 surveillance and the wider infectious respiratory disease surveillance landscape.

A number of recommendations are also made for future improvements to the system to ensure reliable and valid use in Scotland, including:

- *Operational simplicity: streamlining of the operational, analytical, and reporting of data is anticipated to improve efficiencies across the system, such as the integration of data quality assessments with other microbiological services, and analysis and reporting with existing COVID-19 surveillance in Scotland;*

- *Quality assurances: laboratory processes should be accredited to ensure alignment with existing governance of clinical testing-based pathogen surveillance systems, to improve acceptance and confidence by public health professionals and decision makers;*

- *Sampling strategy: the selection of WWTW sites should focus on consistent inclusion of larger catchment areas in particular to enhance the reliability of surveillance outputs, for both PCR testing and genomic surveillance of variants, and should aim to deliver the public health objectives of the surveillance programme in a cost-effective manner;*

- *Validity of use for monitoring trends: levels of SARS-CoV-2 from PCR testing should be smoothed when applied to support retrospective epidemiological investigation of the temporal and geographic patterns of infection, and analytical modelling techniques are required for interpretation of growth in COVID-19 activity to support decision making in real-time;*

- Validity of use in modelling exercises to estimate R or predict future COVID-19 cases, hospitalisations and deaths under different scenarios: while wastewater data has already provided inputs to support modelling of these outcomes, further work is needed to validate the performance of wastewater data compared to other data sources;

- *Detection of novel SARS-CoV-2 variants: further work is needed to refine the bioinformatic pipelines and improve the discriminatory power of wastewater-based genomic surveillance;*

- *Ongoing validation: it is important that this relatively new system undergoes frequent re-assessment of performance, especially following the introduction of new SARS-CoV-2 variants into Scotland;*

- *Usefulness: the utility of the system should be considered alongside existing COVID-19 surveillance systems and should avoid duplicated effort. Further consideration may be needed to the prioritisation of this surveillance resource across other biological or chemical markers of public health importance. A cost-benefit analysis is warranted to ensure value for money.*

11. Protection of human subjects

This evaluation was undertaken as part of the COVID-19 programme of Public Health Scotland, in line with the necessary associated regulations and guidelines. The retention and processing of information on individuals was conducted by Public Health Scotland as part of COVID-19 surveillance in Scotland in the context of emergency data processing (**https://www.informationgovernance.scot.nhs.uk/covid-19-privacy-statement/**), including the Civil Contingencies Act 2004, the NHS (Scotland) Act 1978 and the Public Health (Scotland) Act 2008, and under Articles 6(1)l, 9(2)(h), 9(2)(i), 9(2)(j) of the General Data Protection Regulation. Surveillance data was shared with NHS Scotland according to the Intra NHS Scotland Data Sharing Accord (**https://www.informationgovernance.scot.nhs.uk/wp-content/uploads/2020/06/2020-06-17-Intra-NHS-Scotland-Sharing-Accord-v2.0.pdf**). Ethics approval and informed consent was not required for this work which was based on pre-existing surveillance data for the Scottish population. The access and processing of clinical and wastewater surveillance data was conducted under Data Protection Impact Assessment information governance approval.

12. Data ownership

Scotland's Wastewater Monitoring Programme is operated by SG in partnership with SW and SEPA. Data are available through SEPA and PHS Dashboards.

13. Source of funding and conflicts of interest

This evaluation study was funded as part of the delivery of Public Health Scotland's Infectious Respiratory Diseases Plan (**Scotland's Infectious Respiratory Diseases Plan 2023–2024 (publichealthscotland.scot**). PHS have led the conduct and objectives of the evaluation and provided expert epidemiological and public health input. At the time of project initiation, and for the key period under evaluation for the PCR testing component, PHS were stakeholders of wastewater surveillance reports for SARS-CoV-2. The role of PHS in Scotland's Wastewater Monitoring Programme has subsequently evolved with the transitioning of the programme and strategic planning being conducted jointly between SG and PHS. Biomathematics and Statistics Scotland (BioSS) were funded by PHS to lead the drafting of the operational description of the system. Opinions were also sought from BioSS relating to operational attributes of the system. The University of Edinburgh were funded by PHS to lead the analytical components of the evaluation for the PCR testing data.

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Appendix A. Team and stakeholder contributions

- Public Health Scotland
- Biomathematics & Statistics Scotland
- University of Edinburgh
- Operational leads
- Scientific Advisory Group

Evaluation components	First draft lead	Key input	Review and feedback
Background, aims & objectives			
A1. Importance			
A2. Objectives and development			
A3. Operational components			
B1. Simplicity			
B2. Flexibility			
B3. Acceptability			
B4. Data quality (PCR tests)			
B5. Data quality (genomics)			
B6. Representativeness			
B7. Timeliness			
B8. Stability			
B9. Usefulness			
B10. Communication			
C1. Correlations			
C2. Lags/early warning			
C3. Growth/decline - retro			
C4. Growth/decline - real-time			
Study Limitations			

Evaluation components	First draft lead	Key input		Review and feedback
Discussion				
Conclusion				
Recommendations				

Appendix B: Stakeholder questionnaire

Evaluating the public health utility of wastewater-based surveillance for monitoring SARS-CoV-2 in Scotland: Stakeholder Questionnaire

August 2023

Background information

1. Which organisation do you represent?
2. What is your role in the organisation?
3. What is your organisation's role in the WW Monitoring Programme for SARS-CoV-2 in Scotland?

Surveillance system purpose *(for SG)*

Please comment on the importance of the health-related event under surveillance, considering the context at the time of the system development including the political climate. See **Appendix A** for further guidance.

Surveillance system performance against key attributes

To assist with an understanding of the surveillance system performance, please could you comment on each of attributes outlined below, as relevant, to the best of your knowledge.

Please consider each of the following operational aspects of the surveillance programme when completing your answers:

- The conception, rationale, objectives and design of the programme (including sampling and reporting mechanism);
- The components of the system, including the end-to-end process from sampling to PCR testing and genomic sequencing and reporting;
- The resources used to operate the system and any barriers or enabling factors to service delivery.

1. Simplicity *(for all)*

Please comment on the simplicity of the structure and operation of the Wastewater Monitoring Programme for SARS-CoV-2 in Scotland. See **Appendix B** for example content.

2. Flexibility *(for all)*

Please comment on the flexibility of the Wastewater Monitoring Programme for SARS-CoV-2 in Scotland. See **Appendix C** for example content.

3. Acceptability *(for all)*

Please comment on the acceptability of the Wastewater Monitoring Programme for SARS-CoV-2 in Scotland. See **Appendix D** for example content.

4. Data quality – PCR *(for SEPA, BioSS)*

Please comment on the quality of the PCR data generated by the Wastewater Monitoring Programme for SARS-CoV-2 in Scotland, in terms of data completeness and validity, including PCR test sensitivity and specificity. See **Appendix E** for example content. Quantitative assessment will be performed by the project team as feasible.

5. Data quality – genomic *(for NHS Lothian, BioSS)*

How do you consider the quality of the genome sequence data generated by the Wastewater Monitoring Programme for SARS-CoV-2 in Scotland, in terms of data completeness and validity. See **Appendix E** for example content. Quantitative assessment will be performed by the project team as feasible.

6. Usefulness *(for SG)*

Please comment on the acceptability of the Wastewater Monitoring Programme for SARS-CoV-2 in Scotland? See **Appendix F** for example content.

7. Communication *(for SG)*

Please comment on the ease of communication, in terms of frequency, format of reporting, and use of different media for distribution of information about the Wastewater Monitoring Programme for SARS-CoV-2 in Scotland and the public health outputs generated.

Appendix A: Importance

The public health importance of a health-related event and the need to have that event under surveillance can be described in several ways. Health-related events that affect many persons or that require large expenditures of resources are of public health importance. However, health-related events that affect few persons might also be important, especially if the events cluster in time and place (e.g., a limited outbreak of a severe disease). In other instances, public concerns might focus attention on a particular health-related event, creating or heightening the importance of an evaluation. Diseases that are now rare because of successful control measures might be perceived as unimportant, but their level of importance should be assessed as a possible sentinel health-related event or for their potential to re-emerge. Finally, the public health importance of a health-related event is influenced by its level of preventability.

Parameters for measuring the importance of a health-related event:

- indices of frequency (e.g., the total number of cases and/or deaths; incidence rates, prevalence, and/or mortality rates); and summary measures of population health status (e.g., quality-adjusted life years [QALYS]);
- indices of severity (e.g., bed-disability days, case-fatality ratio, and hospitalization rates and/or disability rates);
- disparities or inequities associated with the health-related event;
- costs associated with the health-related event;
- preventability;
- potential clinical course in the absence of an intervention (e.g., vaccinations); and
- public interest.

Preventability can be defined at several levels, including primary prevention (preventing the occurrence of disease or other health-related event), secondary prevention (early detection and intervention with the aim of reversing, halting, or at least retarding the progress of a condition), and tertiary prevention (minimizing the effects of disease and disability among persons already ill). For infectious diseases, preventability can also be described as reducing the secondary attack rate or the number of cases transmitted to contacts of the primary case. From the perspective of surveillance, preventability reflects the potential for effective public health intervention at any of these levels.

[Adapted from CDC]

Appendix B: Simplicity

The simplicity of a public health surveillance system refers to both its structure and ease of operation. Surveillance systems should be as simple as possible while still meeting their objectives. Consider the following:

- amount and type of data necessary to meet the surveillance objectives e.g. to establish that the health-related event has occurred;
- amount and type of other required data eg. alternative surveillance data sources for validation purposes;
- number of organizations involved in collecting, analysing and disseminating data;
- level of integration with other systems;
- method of collecting the data, including number and types of reporting sources, and time spent on collecting data;
- amount of follow-up that is necessary to update data;
- method of managing the data, including time spent on transferring, entering, editing, storing, and backing up data;
- methods for analysing and disseminating the data, including time spent on preparing the data for dissemination;
- staff training requirements; and
- time spent on maintaining the system.

[Adapted from CDC]

Appendix C: Flexibility

A flexible public health surveillance system can adapt to changing information needs or operating conditions with little additional time, personnel, or allocated funds. Flexible systems can accommodate, for example, new health-related events, changes in case definitions or technology, and variations in funding or reporting sources.
In addition, systems that use standard data formats (e.g., in electronic data interchange) can be easily integrated with other systems and thus might be considered flexible.
Please consider examples where the public health surveillance system has been adapted to another disease (or other health-related event), a revised case definition, additional data sources, new information technology, or changes in funding to help assess this attribute.

[Adapted from CDC]

Appendix D: Acceptability

Acceptability reflects the willingness of persons and organizations to participate in the surveillance system. Acceptability refers to the willingness of persons in the sponsoring agency that operates the system and persons outside the sponsoring agency (e.g., persons who are asked to report data) to use the system. To assess acceptability, the points of interaction between the system and its participants must be considered.

Some factors influencing the acceptability of a particular system are:

- the public health importance of the health-related event;
- acknowledgment by the system of the person's contribution;
- dissemination of aggregate data back to reporting sources and interested parties;
- responsiveness of the system to suggestions or comments;
- burden on time relative to available time;
- ease and cost of data reporting;
- the ability of the system to protect privacy and confidentiality;
- public health legislative requirements for data collection and case reporting; and
- participation from the community in which the system operates.

[Adapted from CDC]

Appendix E: Data quality

Data quality reflects the completeness and validity of the data recorded in the public health surveillance system. Examining the percentage of "unknown" or "blank" responses to items on surveillance forms is a straightforward and easy measure of data quality. Data of high quality will have low percentages of such responses. However, a full assessment of the completeness and validity of the system's data might require a special study.
Data values recorded in the surveillance system can be compared to "true" values through for example, a review of other sources of public health intelligence. In addition, the calculation of sensitivity and predictive value positive for the system's data fields might be useful in assessing data quality. Please note that quantitative analysis of system performance will be carried out by the project team as deemed technically feasible.
Quality of data is influenced by the performance of the screening and diagnostic tests (i.e., the case definition) for the health-related event, the clarity of hardcopy or electronic surveillance forms, the quality of training and supervision of persons who complete these surveillance forms, and the care exercised in data management. A review of these facets of a public health surveillance system provides an indirect measure of data quality.

[Adapted from CDC]

Appendix F: Usefulness

A public health surveillance system is useful if it contributes to the prevention and control of adverse health-related events, including an improved understanding of the public health implications of such events. A public health surveillance system can also be useful if it helps to determine that an adverse health-related event previously thought to be unimportant is actually important. In addition, data from a surveillance system can be useful in contributing to performance measures, including health indicators that are used in needs assessments and accountability systems.
An assessment of the usefulness of a public health surveillance system should begin with a review of the objectives of the system and should consider the system's effect on policy decisions and disease-control programs. Depending on the objectives of a particular surveillance system, the system might be considered useful if it satisfactorily addresses at least one of the following questions.

Does the system:

- detect diseases, injuries, or adverse or protective exposures of public importance in a timely way to permit accurate diagnosis or identification, prevention or treatment, and handling of contacts when appropriate?
- provide estimates of the magnitude of morbidity and mortality related to the health-related event under surveillance, including the identification of factors associated with the event?
- detect trends that signal changes in the occurrence of disease, injury, or adverse or protective exposure, including detection of epidemics (or outbreaks)?
- permit assessment of the effect of prevention and control programs?
- lead to improved clinical, behavioural, social, policy, or environmental practices? or
- stimulate research intended to lead to prevention or control?

Usefulness might be affected by all the attributes of a public health surveillance system. For example, increased sensitivity might afford a greater opportunity for identifying outbreaks and understanding the natural course of an adverse health-related event in the population under surveillance. Improved timeliness allows control and prevention activities to be initiated earlier. Increased positive predictive value enables public health officials to more accurately focus resources for control and prevention measures. A representative surveillance system will better characterize the epidemiologic characteristics of a health-related event in a defined population.

[Adapted from CDC]

Appendix C: Supporting data

Table B.1. Number of Scottish Water wastewater assets and population coverage by NHS Board in Scotland

NHS Board	Number of assets covering >1000 people	Estimated total % coverage from assets covering >1000 people	Number of assets covering >500 people	Number of assets covering >500 people
Greater Glasgow and Clyde	12	99	13	99
Lothian	24	92	30	93
Lanarkshire	33	100	41	100
Grampian	40	86	52	88
Tayside	29	89	44	92
Fife	27	98	34	99
Ayrshire and Arran	23	99	28	100
Highland	44	72	73	79
Forth Valley	25	96	34	98
Dumfries and Galloway	19	72	29	77
Borders	17	75	25	80
Western Isles	1	30	11	58
Shetland	2	41	8	59
Orkney	2	44	4	50

Appendix D: Analytical methodology

Statistical correlations between signals including lags

As described in section 6.1, levels of SARS-CoV-2 RNA in wastewater (WW) were normalised by flow and catchment population before proceeding with the statistical analyses. The normalised WW data are shown in **Figure 8** - **Figure 12**.

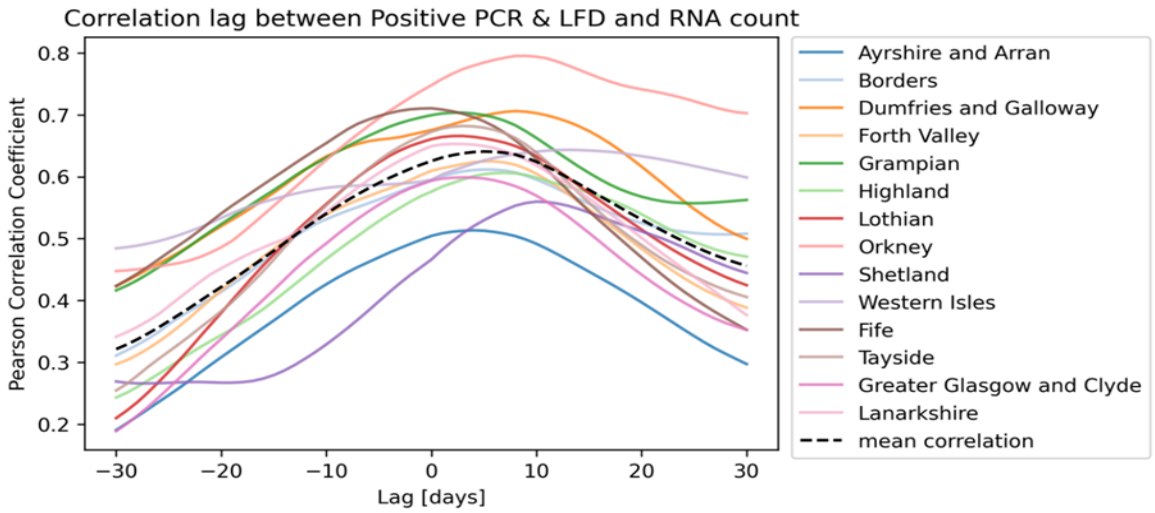
Due to the fluctuating levels of SARS-CoV-2 in WW across sampling dates, the data were smoothed using a 7-day rolling average for the cross-correlations in order to match the temporal resolution of the available comparison data, and to reduce undue loss of correlation due to high frequency noise in the signal. For dates with missed sampling, the missing data was interpolated by applying the SARS-CoV-2 RNA level reading available for the closest date. This smoothing was applied for each site.

When aggregating the WW signal to NHS Board level, the weighted mean was taken of all the normalised RNA values, with the weight for each WW sampling site catchment area to be equal to the census population size identified as residing within the catchment area and NHS Board.

Other COVID-19 surveillance data (cases, hospitalisations, deaths) were aggregated to WW sampling site and NHS Board levels through postcode linkage. All other data sources were provided in terms of occurrences aggregated to one week (Monday-Sunday), with the exception of the ONS infection survey for which the seven-day period varied.

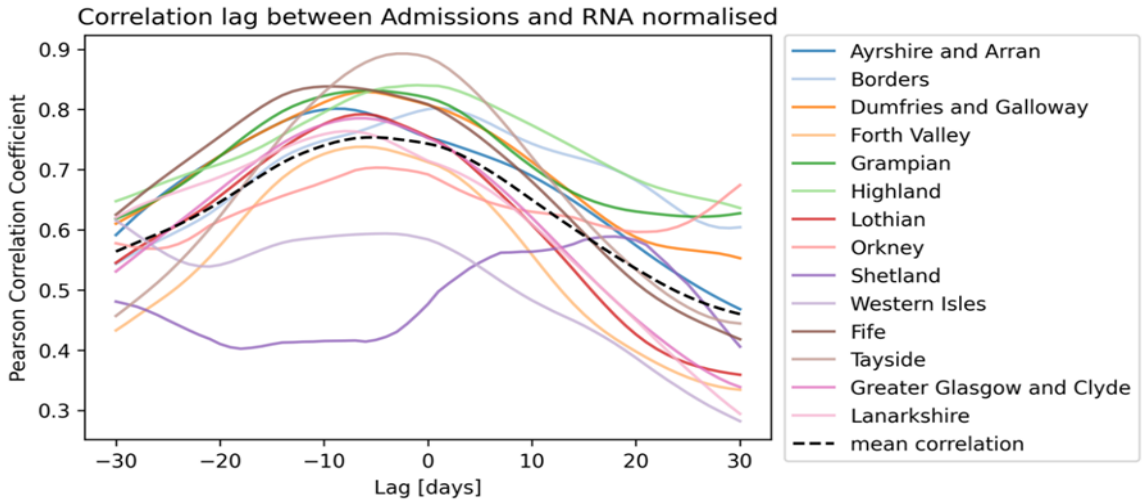
The time series cross-correlation analyses were undertaken by estimating the Pearson Correlation of the smoothed WW signal and comparison signal, within each included timeframe. In addition, the WW signal was shifted by one day increments a lag chosen which maximised the Pearson correlation between the two signals. Comparison of cases to the normalised WW signal showed a strong and consistent relationship across all NHS Boards (**Figure D.1** and **Figure D.2**) while the correlation was both weaker, and the lag more variable, when comparing to hospital admissions across different NHS Boards.

Figure D.14. Correlations between community testing for SARS-CoV-2 (PCR+LFD) and WW signal



Considering the period between May-2021 and March-2023 across Scottish NHS Boards, showing a strong preference for the lag with the highest likelihood. The mean lag at the most likely point shows some variation, lying within the range of +/- 10 days.

Figure D.15 Correlations between COVID-19 hospital admissions and WW signal.



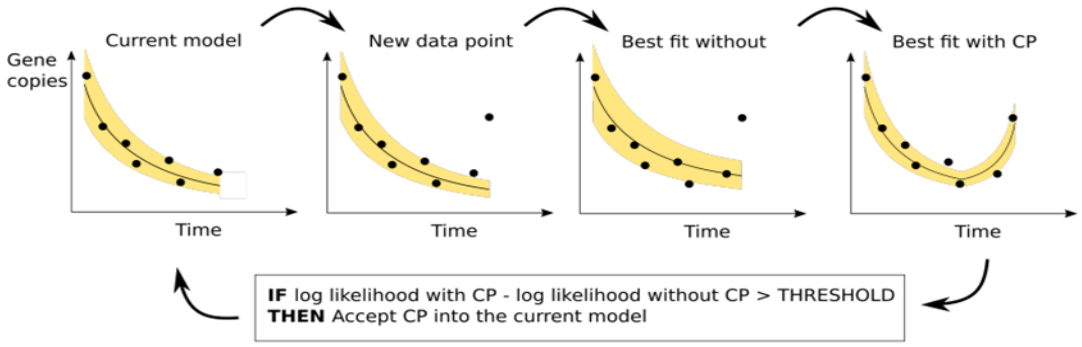
Considering the period between May-2021 and March-2023 across Scottish NHS Boards, showing a weak preference for the lag with the highest likelihood. The mean lag at the most likely point shows considerable variation, though the mean lag is approx. - 10 days (i.e. WW signal typically leads by 10 days approximately).

Statistical modelling to determine sensitivity and specificity of system to detect periods of growth

An approach was applied aimed at determining periods of growth of COVID-19 activity from the WW signal (22). This analysis includes a fit of the variability in SARS-CoV-2 shedding rates into wastewater, providing a better estimate of the uncertainty in WW detection when prevalence is low, compared to more standard statistical methods. This correction is due to the evidence that shedding of SARS-CoV-2 is highly heterogeneous (23) and with evidence of substantial differences between SARS-CoV-2 variants (24).

The analytical approach simultaneously fit the observed signal to a combination of two factors – a series of positioned 'spline points' where the growth rate changes (and may be positive or negative), and the value of the 'index of dispersion' of shedding of SARS-CoV-2 into wastewater per person which are used to generate a likelihood function of how likely the model and parameters are given the observed data. Maximising the likelihood identifies the parameters that best fit the data. The index of dispersion is a well-known parameter equal to the variance-to-mean ratio of the signal. An index of one implies a normal distribution, with large numbers being over-dispersed, and numbers below one, under-dispersed. Evaluating the WW and comparative signals from other COVID-19 surveillance data, the model is fitted by comparing the observed rise and fall of the signal over time, against the fitted function (a combination of spline points and growth/decay rate – see **Figure D.3**).

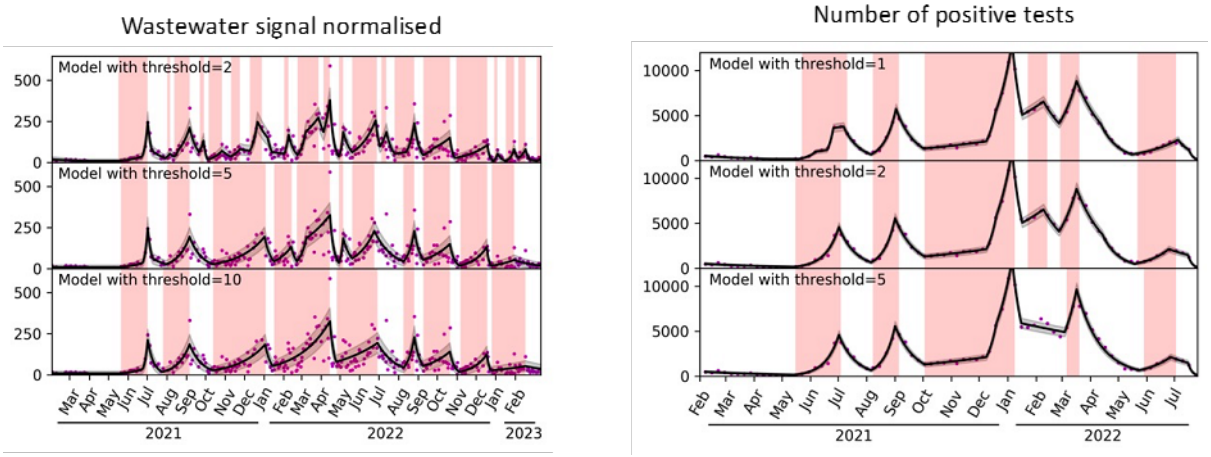
Figure D.16 Schematic of the fitting procedure.



A model consisting of a set of spline points, with a fitted exponential growth/decay curve between each spline. As new points are added (e.g. in real time), the model fit is adjusted to maximise the likelihood under the statistical model, potentially including the addition of a new 'historical' spline point to account for the new data.

As the data for each additional time point is added, the fitted function is adjusted to minimise the likelihood, including possibly 'backtracking' (i.e. introducing a spline point at an earlier date than the most recent one) and adding a new spline point at an earlier time. New spline points are identified by comparing the likelihood of the model with and without an additional spline point (and considering possibly all time points between the previous spline point and the current time). If the difference exceeds a specified threshold, then the spline point is added. The threshold is arbitrary; choosing a higher threshold creates a smoother fitted function with fewer spline points; the method is used to fit both the WW signal, and the comparison signals (see **Figure D.4**).

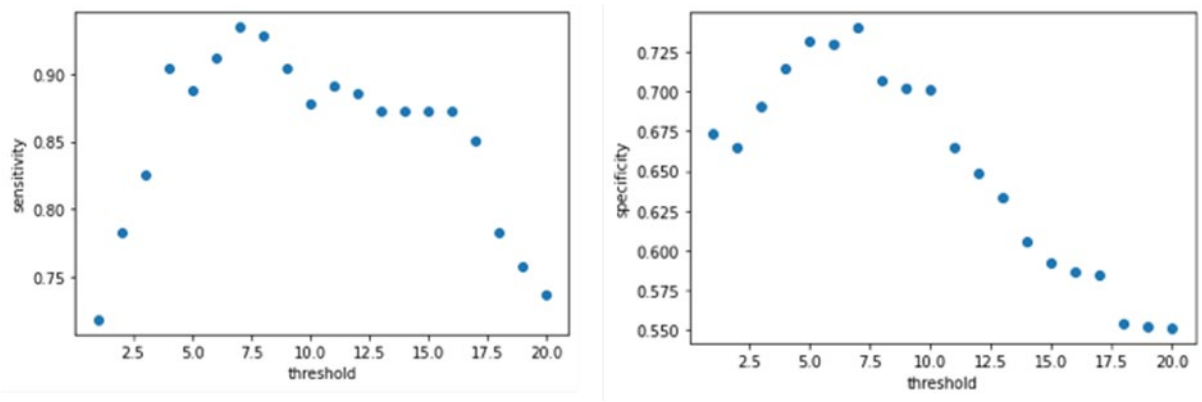
Figure D.17. Comparison of the fitted model to the data for all of Scotland from 2021 to 2023.



On the left, fit of the WW data; on the right, fit of the combined number of positive PCR and LFD tests. In pink shows periods of growth, in white periods of decay. As the model threshold increases, the number of fitted spline points are reduced.

The sensitivity and specificity of the model were determined by comparing the number of times new spline points were added in the WW signal to a 'gold standard'. For these analyses, prevalence estimates generated by the ONS Infection Survey were used as the gold standard when considering all of Scotland, however these data were not available at finer NHS Board or WW sampling site levels. At these finer scales, comparisons were made to the community PCR and LFD testing. When the model fitted to the WW data does not identify a period of growth (i.e. no new spline point) but the model fitted to the gold standard data identifies a period of growth and then a decline again (or vice versa), then the model is said to have generated a false negative. When the model fitted to the WW data identifies a growth and then a decay period (or vice versa) without the gold standard data identifying any new spline points at all, this is a false positive. The number of false negatives and positives are then used to quantify sensitivity (= true positive/(true positive + false negative) and specificity (= true negative/(true negative + false positive)) (see **Figure D.5**).

Figure D.18 Change in sensitivity and specificity for WW signal when compared to community tests for the final fitted model.



Peak sensitivity and specificity occur at very similar threshold values (i.e. ~ 7.5)