Wastewater COVID-19 Monitoring in the UK: Summary for SAGE – 19/11/20

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Abstract

This paper provides a technical overview of wastewater-based epidemiology and its application for COVID-19, a summary of the UK Wastewater Programmes and presentation of some results and data use-cases from the English programme.

Key Questions

1. Wastewater can be used as a binary indicator when infection levels are low or as an indicator of prevalence in areas with larger outbreaks. What would be the recommended primary use for wastewater:
   a. in areas of high prevalence to optimise the delivery of mass testing or to guide the tightening or easing of NPIs?
   b. in areas where cases are low to determine risk is still low in these areas?

2. There is a need for innovation in sampling and viral RNA detection to allow automation and improve the quality of data from near-source tracking (i.e., sewer pipe outside a school, etc). How can sewerage network data be used strategically to prioritise wastewater-based epidemiology (WBE) in networks that generate 'good' surveillance data, and can existing data be used to generate a better understanding of why a sewerage network generates 'bad' surveillance data?

1 Introduction to Wastewater Based Epidemiology and SARS-CoV-2

Most viruses that are harmful to human health affect the respiratory and gastrointestinal tract. It is therefore common for people infected by viruses to shed them in faeces at which point they enter the wastewater system. A range of studies have previously used this wastewater to provide an integrated signal to monitor the amount of disease circulating in the human population. This has included the routine surveillance for poliovirus in London as well as other enteric viruses such as Norovirus, Hepatitis A and E, Adenovirus, Rotavirus etc., at other locations in the UK (though there is no present system of monitoring for these pathogens). In the latter case, the monitoring of water either entering or leaving the sewage treatment works has been used to (i) estimate the amount of disease circulating in the population, (ii) evaluate the efficiency of wastewater treatment at removing pathogenic viruses and bacteria, and (iii) quantify the subsequent discharge of harmful viruses and bacteria into fresh- and marine waters and the potential risk they pose to human health. Many of the approaches used to measure non-enveloped viruses were easily repurposed to monitor the presence of the enveloped virus, SARS-CoV-2. This led to some early collaborative studies between Bangor, Newcastle and Edinburgh universities to monitor the levels of SARS-CoV-2 in sewage from a range of UK cities. These formed the basis of the national surveillance programmes in England, Scotland and Wales. This research was also mirrored in other European countries (e.g., Netherlands, Italy and Spain).

It is now clear that SARS-CoV-2 RNA is shed in faeces from many infected individuals[1]. The potential exists for sampling human waste at several different stages, including:

- Immediately at the sewer exit from an individual building or location, or within a small facility such as a septic tank (near-source tracking)
- Within a major sewer pipeline
- At intermediate sewer infrastructure such as pumping stations or holding tanks
- At the influent to a sewage treatment works (STW)
- In the wider environment

[1] Wu, Y. *et al*. *Lancet Gastroenterol. Hepatol.*, **5**(5): 434-435 (2020). "Prolonged presence of SARS-CoV-2 viral RNA in faecal samples."

Each option comes with advantages and disadvantages, including practical issues such as; access, safety of personnel, sample variability and representation, likelihood of blockages.

The approaches set out below broadly describe the process used in current wastewater monitoring for SARS-CoV-2 in the UK, and elsewhere. Similar approaches could be used for other waters such as rivers, lakes and marine; however, detectable numbers in those waters are expected to be many times lower and, to date, there is no evidence of viable SARS-CoV-2 in surface waters[2], although virus RNA has been detected in freshwaters subject to wastewater overflow events[3].

2 Technical details

2.1 Sample collection

There are two modes of sample collection from water or wastewater. A grab sample is a snapshot of virus load at a single point in time. It is usually taken at peak flow with the aim to increase the likelihood of capturing the most representative measure of shed virus (i.e. flow volume representing the point at which the majority of households have used the bathroom). Or an autosampler can be used to collect regular samples over a set period (e.g. 24 hours) in preselected time windows optimised for the location, or in proportion to flow. Samples can then either be kept separate to provide a time series showing how concentrations change during the day or combined to provide a composite sample. The latter may be pragmatic where concentrations are highly variable or dependent on diurnal human behaviour, as is the case with wastewater.

2.2 Sample transport, storage, handling and preparation

Samples need to be kept cold (4 - 6 ^{o}C) until they can be analysed to prevent degradation of RNA. Where autosamplers are used, they enable the sample to be cooled. Shipping needs to maintain refrigeration. Samples of wastewater must be handled in secure Category 2 laboratories with suitable safety cabinets.

2.3 Sample concentration

RNA from SARS-CoV-2 is highly dilute in aquatic matrices. In the UK-based wastewater monitoring programmes, wastewater samples undergo initial clarification by centrifugation to remove larger particles and then viral RNA is concentrated either by a filtration or a precipitation step[4].

2.4 Laboratory assays

All laboratories have adopted the use of quantitative Polymerase Chain Reaction with a reverse transcriptase step (RT-qPCR) to target specific genes in the virus (e.g. N1 gene in England & Wales, N1 and Sarbeco E genes in Scotland). So long as the samples are kept cold (4 °C), tests in several laboratories have shown that the viral RNA does not quickly degrade in the sample[5]. RT-qPCR measures the number of RNA copies in the sample, and data is reported as gene copies per litre of wastewater sample collected.

2.5 Normalisation

It is helpful to know how many people are contributing to the wastewater so that detected amounts of RNA can be compared or 'normalised' to faecal load. Several options exist that may allow estimation of relative human faecal load including biomarkers, such as human mitochondrial DNA or RNA and

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[2] Jones, D. *et al*. *Sci. Total Environ.*, **749**:141364 (2020). "Shedding of SARS-CoV-2 in faeces and urine and its potential role in person-to-person transmission and the environment-based spread of COVID-19."

[3] Rimoldi, S.G. *et al. Sci. Total Environ.*, **744**:140911 (2020). "Presence and infectivity of SARS-CoV-2 virus in wastewaters and rivers."

[4] Ahmed,W. *et al. Appl. Environ. Microbiol.,* **81**(6):2042-2049 (2015). "Comparison of concentration methods for quantitative detection of sewage-associated viral markers in environmental waters."

[5] Ahmed, W. *et al. Env. Res.*, **191**:110092 (2020). "Decay of SARS-CoV-2 and surrogate murine hepatitis virus RNA in untreated wastewater to inform application in wastewater-based epidemiology."

crAssphage[6], organic or inorganic compounds, such as ammoniacal nitrogen, and contaminants, such as pharmaceuticals. Simple flow volume can also be used. None of these normalising approaches are ideal, but they can be combined to provide a more stable signal[7]. At present, Scottish data is flow normalised, while that in England and Wales is reported without any normalisation.

2.6 Interpretation and limitations

Wastewater detection of SARS-CoV-2 is particularly useful where significant numbers of asymptomatic infections occur. Once COVID-19 is established in the population, the wastewater might be used to provide a reliable confirmation of infection rates falling and provide access to populations where interaction with formal healthcare is limited[8].

We are limited in an understanding of the fate of the virus and viral RNA in sewers. It is known that colder temperatures favour preservation and survival, and that dissolved O_2 decreases survival, but precise degradation rates are lacking and variable according to the pumping system of each STW.

Present measurements and comparison with T&T suggest that detection is typically at least as good as 1 infected person in 1000, though this is dependent on the local plumbing, and on the significant variability in faecal shedding rates of SARS-CoV-2 from individuals.

Currently, an approximate time window of 24 hours between sample collection and lab reporting is usual across all laboratories in the programmes.

Sequencing wastewater samples can be used to identify diversity of SARS-CoV-2 strains between and within cities. We are working with COG-UK to scope a project that will compare the wastewater sequences across the country, irrespective of testing numbers. The Welsh programme has had some success with early sequencing work from wastewater samples.

3 Overview of Wastewater Surveillance Programmes across the UK

Wastewater analysis for SARS-CoV-2 RNA has been underway in the UK since early 2020, with the earliest detections in serendipitously samples taken originally for polio surveillance[9]. From early summer 2020 separate surveillance efforts in England, Scotland and Wales have shown that SARS-CoV-2 RNA can be reliably quantified in samples from treatment works and from within sewer networks. Detection of faecal shedding of virus in the UK and worldwide has established wastewater-based epidemiology as a viable surveillance approach. Infectious SARS-CoV-2 from wastewater is difficult to recover, despite several attempts, and the assessment of the risk of viral transmission in wastewater are presently considered very low[10].

In March 2020, the ONS and Defra supported a grant application from a collaborative study between Bangor, Newcastle and Edinburgh universities to monitor the levels of SARS-CoV-2 in sewage from a range of UK cities. In April, a working group was formed by the ONS and JBC to work towards a national monitoring system with membership across sectors. With strong support from academics and water companies, the English wastewater monitoring programme was launched as a collaboration between the Defra Group and the JBC with continued support from the ONS. The programme began sampling at 44 wastewater treatment works across England in July 2020. Since then, several pilot studies have been undertaken to determine the effectiveness of wastewater monitoring to identity outbreaks in

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[6] Park G., *et al. Emerg. Infect. Dis.*, **26**(8):1731-1739 (2020). "CrAssphage as a Novel Tool to Detect Human Fecal Contamination on Environmental Surfaces and Hands."

[7] O'Brien, J. *Env. Sci. Technol.*, **48**(1):517-525 (2014). "A Model to Estimate the Population Contributing to the Wastewater Using Samples Collected on Census Day."

[8] Ahmed, W., *et al. Sci. Total Environ.*, **728**:138764 (2020). "First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community."

[9] Martin, J. Viruses, **12**(10):1144 (2020). **"**Tracking SARS-CoV-2 in Sewage: Evidence of Changes in Virus Variant Predominance during COVID-19 Pandemic."

[10] Kitajima, M. *et al.*, *Sci. Total Environ.*, **739**:139076 (2020). "SARS-CoV-2 in wastewater: State of the knowledge and research needs."

key infrastructure, and the effectiveness of wastewater monitoring as an indicator of prevalence within wastewater catchments. The JBC and Defra Group co-lead on wastewater monitoring in England and are currently working with the EA to increase lab capacity to enable the expansion of the English surveillance programme to cover 80% of the population via monitoring at treatment works. In addition, monitoring within catchments by sampling at pumping stations was initiated during the Liverpool mass testing and will be expanded to cover eight core cities plus Greater London. These data provide key insights for mass testing and for local response and readiness. Sample locations are selected using a COVID-19 risk index which include key insights on occupation and health risk in collaboration with geospatial at the ONS; these are used at a local level within cities and when prioritising sampling from wastewater treatment works. The programme continues to support monitoring in key infrastructure for example in schools and hospital accommodation.

Also, in March, samples were taken by Scottish Water from six wastewater treatment works in Scotland to explore the potential of using wastewater treatment plant influent to detect SARS-CoV-2 shedding within the community. Positive initial results from work at the Roslin Institute (funded by the Centre of Expertise for Waters) were used by SEPA to inform the development of a Scottish national wastewater surveillance network of 28 sites covering just under 50% of the population. Whilst sampling by Scottish Water under this programme started in May, most available results date from the beginning of August onwards, although archived samples from May, June and July are being used to complete the Scottish dataset.

Across all programmes, viral RNA data in the wastewater is being used along with complementary data, such as catchment area, sewer hydrology and sample inorganics such as ammonia and orthophosphate, to explore the relationship between SARS-CoV-2 RNA levels in STW influent and clinical cases in the population.

The Welsh wastewater monitoring programme began in March 2020 and initially focused on major urban centres across the North and South of the country (e.g. Wrexham, Bangor, Cardiff), alongside sites in England where tourist traffic to Wales is high (e.g. Liverpool, Manchester). Initially, this consisted of a weekly monitoring campaign in which the levels of SARS-CoV-2 in wastewater were monitored alongside a range of other water quality parameters. This data was mapped against the incidence of COVID-19 cases as well as COVID-19 related deaths. In addition, work was targeted at specific locations where there was a very high local incidence of COVID-19 cases (e.g. associated with food processing plants of Anglesey and Wrexham). In addition, work was also undertaken to optimise the methods used to extract and quantify the virus from wastewater. Taken together, this work confirmed that wastewater provided a practical, likely unbiased and cost-effective way to monitor the incidence of COVID-19 within the community. These pilot studies formed the basis of the Wales national surveillance programme which was expanded to encompass 20 sites across the country, covering 70% of the population. This work is being undertaken jointly between Bangor University, Cardiff University, Public Health Wales and Welsh Water. Sampling is currently being undertaken three times a week. This data was used to provide evidence of tourism-related entry of SARS-CoV-2 into Wales and was part of the portfolio of evidence supporting the imposition of the recent national lockdown. In addition, work is being undertaken to monitor the levels of SARS-CoV-2 in wastewater leaving the Bangor University halls of residence. This monitoring campaign was able to detect COVID-19 cases before symptoms have become evident in the student population (and cases formally confirmed). This also provided evidence that the approach can be used at a very local scale to inform local management decisions on COVID-19 control.

4 Programme results and data use-cases

The data generated by the programme report raw N1 gene copy number per sample reaction volume and the corresponding concentration per litre (gc/L). Associated bacteriophage biomarker genes (CrAssphage, ɸ6) have also been recorded by the laboratories.

Presently, the English National Surveillance Programme samples from 44 STW (covering approximately 23% of the population of England), with data available since 22/07/2020, sampling at a frequency of four measurements per week. Uniformly, the presence of SARS-CoV-2 has been detected across all sites. A key indicator of the sensitivity of wastewater monitoring is the alignment with disease prevalence in the community shedding into the catchment served by the STW where the sample is taken. We have incorporated data from the National Test and Trace programme to understand the relationships between the aggregated wastewater data and positive cases in the population. Given the large uncertainty due to multi-factorial and unresolved challenges in the wastewater samples, it is promising to observe that across 44 sites in the pilot study there is good alignment with wastewater and T&T time-series dynamics (Figure S1, Appendix 7.3). That is, where positive case rates are high, viral concentration in the wastewater is high, evaluated as being above the Limit of Quantification (LOQ), and where case numbers are low, viral load is below the Limit of Detection (LOD). Time series from different sites are not directly comparable due to idiosyncrasies of the sewerage systems.

To date, there has been no evidence of lead-lag across all sites, which we attribute to several factors including: testing prevalence in a catchment; asymptomatic cases; STW pumping systems. There may be indication of wastewater leading testing in some areas, but this question remains open for further analysis (See Appendix 7.2).

A valuable use-case when the wastewater and T&T signals do not covary is to understand whether this is indicative of incomplete test coverage in the community or attenuation of the viral signal in the wastewater due to confounding factors such as hydrological or biochemical perturbations in the network.

- **Liverpool** – Data from Sandon Docks STW indicates that SARS-CoV-2 concentrations in wastewater track the dynamics of positive case rates in both the magnitude and direction of the disease. Liverpool represents a site with a sharp increase in positive cases from mid-September to late October 2020 (Figure S2, Appendix 7.3)
- **Plymouth** – A distinct peak in virus load at the STW with no corresponding increase in the existing low positive case number was observed. The source was identified to be an isolated outbreak where positive case data was not included in the reported T&T numbers (Figure S3, Appendix 7.3)
- **Small City** – Sampling of wastewater upstream of the STW provides potential for greater measurement acuity. Low viral concentrations were identified for five consecutive days, after two weeks of ND (not detected) results at a site receiving wastewater from a multi-occupancy residential building. A positive case was subsequently reported, suggesting that low shedding rates from individuals are identifiable. An order of magnitude increase in wastewater viral concentrations was observed and further tests revealed two positive individuals (Figure S4, Appendix 7.3)
- **Scotland** - At Orkney STW (population equivalent 7750), virus was detected in the wastewater where less than 10 positive cases had been recorded in test numbers and Public Health Scotland were notified

The establishment of wastewater as a medium for the surveillance of the pandemic provides a case for the democratisation of the data for use by other stakeholders. Wastewater sampling is to be used to support the Mass Testing programme across eight core cities in England (including Greater London). Wastewater testing will be deployed 1-2 weeks ahead of diagnostic tests to understand trends in incidence and will help inform where to deploy diagnostic testing. The scope of sampling will be to collect and process samples from up to 300 sites daily, covering key portions of the cities at a geography allowing local authority to pinpoint interventions. In Exeter, we are working with local health and academic stakeholders to understand how wastewater surveillance can be used in areas with a high proportion of asymptomatic individuals, e.g. universities. We have a funded project with members of SPI-M that will explore opportunities for integrating the wastewater data with epidemiological modelling, accounting for uncertainty in the measurements.

Our datasets are available through several sources. JBC, DEFRA[11], and SEPA[12] host dashboards that visualise and map the wastewater data against other strategic datasets. The raw, assured and quality checked wastewater data is available to accredited researchers via the ONS Secure Research Service[13], and an overview of the data will soon be made available on the Coronavirus dashboard[14]. These dashboards provide near real-time tools for monitoring the current state and evolution of the wastewater prevalence across the country via user-friendly maps, plots and interactive features. They are used to help flag issues, guide executive decisions and eventually to inform the general public. By combining several sources of data for the whole country, including wastewater measurements, positivity rates, and statistical and geospatial information from ONS, the JBC dashboard provides a comprehensive and unifying platform to provide quick insights and orient subsequent decisions and analysis.

5 Operationalising the WW Programme and future plans

In England the National Surveillance Programme, upstream and near-to-source pilots in Exeter, Pendle and Southampton, and School's testing pilots have been operational for several months (See Appendix 7.1). The pilots continue to generate significant scientific insights and address gaps in the understanding of wastewater epidemiology. We are expanding the English National Programme, sampling treatment works to cover 80% of the population, and supporting mass testing in eight core cities and Greater London, scaling work we started in Liverpool, with further expansion proposed.

In Scotland, the network has been kept under weekly review. As COVID-19 Protection Levels in Scotland are designated at a Local Authority Level, the network is currently under review to see how it can best provide discrimination at a Local Authority level.

In Wales, wastewater monitoring is now also being used to support mass testing at national infection hotspots to inform local decision making on where and when to test (e.g. Merthyr Tydfil). The approach is now being upscaled and new automated technical work streams are being implemented. The work is also being expanded to include other viruses of public health concern (e.g. Influenza A/B, norovirus, hepatitis A/E).

5.1 Additional use-cases

Beyond identifying community-scale hotspots, wastewater monitoring can be applied at building-scale for the management of outbreaks in discrete populations. Learning from our work in the small city, upstream pilot and in schools, we are investigating the potential to use building-scale monitoring to prioritise the distribution of mass-testing, for example in prisons and critical points in the food supply chain.

As vaccines are distributed, it will be important to maintain a pulse-check in areas of high risk and potential low uptake. Wastewater monitoring presents a unique opportunity to track the effects of vaccination across a range of population sizes in a non-invasive way.

5.2 Technology development

The development of an automated and on-site technology for sampling, processing and analysis of SARS-CoV-2 in wastewater is underway. A workshop with key academic and industry partners led to

[11] https://help.cbas.cloud/local_lockdowns_tool/

[12] https://informatics.sepa.org.uk/RNAmonitoring/

[13] Research accreditation for access to SRS: https://researchaccreditationservice.ons.gov.uk/ons/ONS_registration.ofml

[14] https://coronavirus.data.gov.uk/

the identification of three key areas for development focused on (i) the automation of analysis in the field to enable detection and/or quantification of SARS-CoV-2 RNA near to the point of sampling, (ii) the development of ‘real time’ automated detection and/or quantification of SARS-CoV-2 RNA at the point of sampling, (iii) the development of technology that could be integrated into the network for wider health monitoring, for example at pumping stations.

6 Summary

The detection of SARS-CoV-2 viral RNA in wastewater has rapidly become a significant tool to address the ongoing pandemic. The scientific and epidemiological insights that can be derived from the wastewater data are dependent on the acuity and extent of the sampling, laboratory analyses and the level of environmental determinism and stochasticity driving variability in the measured signal. The UK Wastewater Surveillance Programmes across England, Scotland and Wales have been actively monitoring wastewater since early summer 2020, building up an evidence base that has provided knowledge and support to the wider efforts for managing the pandemic. Wastewater surveillance is a reliable, timely and cost-effective method to serve the needs of public health. Wastewater based epidemiology in the UK has a long and notable history, dating back to the identification, by John Snow, of wastewater as the source of cholera outbreaks in the mid-19th Century. The current work presented here anticipates the use of wastewater to track disease at scale as a proactive measure to ensure public health resilience beyond this pandemic.

7 Appendices

7.1 A. Current and Prospective Projects

Project aims and summary	Organisation	Status
Exeter and Pendle • Can the source of viral RNA be pin pointed to a precise upstream location? • Can wastewater data meaningfully impact local testing, tracing and containment?	JBC, DEFRA, EA	In pilot phase
Southampton • Is wastewater a leading indicator? • Time differential between viral detection in wastewater and diagnostic testing results? • How does accuracy vary?	JBC, DEFRA, EA	In pilot phase
TERM • Determine if wastewater monitoring can act as an early indicator of outbreaks in schools • Quantify how wastewater monitoring in schools relates to serological testing in the same populations?	JBC, Middlesex University	In pilot phase
NPI Effectiveness • Determine what the '*art of the possible*' is in terms of data products, outside of standard public health data, that can be derived to support analysis of people's movements and behaviours in order to determine the compliance and effectiveness of NPIs.	Innovation & Partnerships	Evaluating commercial bids
WW Mass Testing – Next Gen Technology • The current process from sampling to results is very time consuming and has several manual and logistical elements. • We are looking for innovative solutions to provide complete end to end system improvements, or improvements to a significant part of the system.	Innovation & Partnerships	Refining exam question and route to market
Predicting healthcare needs • Determine whether and to what extent healthcare needs (e.g. testing or hospital beds) can be predicted by integrating wastewater data with test and trace data. • What is the prediction horizon?	JBC, DEFRA, Imperial College London	Started 16/11/20
Newcastle University MEng Projects x 2 • Determine if wastewater monitoring can be used to identify under-representative community testing • Can wastewater act as a proxy for disease prevalence as infection declines and community testing scales back?	Newcastle University, JBC, DEFRA	Started 23/11/20

• Can factors that drive wastewater measurement uncertainty be determined from hydrological and biochemical data? • Is convergence/divergence of wastewater measurements with Test & Trace data explainable within or between sites?		
Control engineering and wastewater surveillance • The sensitivity of any control method in the Covid-19 case to the delays round the loop. This is especially so when there are uncertainties, and delays due to human analysis, decision making, and behaviours in the loop. The benefits of reducing these delays. • The observation problem and the use of data fusion/data reconciliation combined with modelling to reduce uncertainties and reduce observation delays • The use of expert elicitation with control methodology to systemise and optimise COVID-19 management • The use of WBE to provide both early warnings and to measure prevalence of infection in a population. The need for rapid and representative sampling/assay with fast delivery and analytics of results. • The problems of scaling up the use of effective WBE to be a powerful tool in the management of the pandemic	Spirocontrol, CEH, JBC	Preliminary discussions
SPI-M • Can uncertainty in wastewater data be determined and attenuated to provide good estimation of virus prevalence and R number? • How can wastewater data be incorporated into epidemiological models in a strategic manner?	LSHTM, Exeter Uni., JBC, DEFRA	Preliminary discussions

In addition, the UK now has several UKRI-funded projects that address elements of wastewater-based epidemiology, including:

- N-WESP; a large consortium of nine universities and a UKCEH aimed at developing and refining the sampling, analytical and modelling methodologies needed to execute a national wastewater-based epidemiology (UKCEH lead)
- Development of a low-cost paper-based device for rapid and on-site analysis of SARS-CoV-2 in sewage (Cranfield University lead)
- Monthly samples from London Beckton STW have been tested at NIBSC for the presence of SARS-CoV-2 RNA since December 2019 using RT-qPCR and nested RT-PCR assays targeting different genes (NIBSC lead)
- Building an Early Warning System for community-wide infectious disease spread; SARS-CoV-2 tracking in Africa via environment fingerprinting (Bath University lead)
- Assistance on WBE provided to national surveillance efforts in Spain, Paraguay, India, Egypt and Central and South America

7.2 A note on the predictive ability of WBE

The analysis of wastewater-surveillance data is challenging, especially for a national programme encompassing multiple sites. The effect of sewerage systems on the recovered RT-qPCR signal can vary substantially across sites, due to differences including dilution, hydraulic effects, and water chemistry. Furthermore, the lag between Test and Trace and wastewater data may differ due to the topology of the sewerage network and the resulting different residence times. Determining to what extent WBE has predictive ability in the context of SARS-CoV-2 will require combining the expertise of epidemiologists and insights from wastewater specialists with robust statistical methodology. However, even a simple correlative analysis (See Fig. S1) indicates a significant correlation between wastewater and Test and Trace data, suggesting that more sophisticated approaches, such as distributed lag models[15], could provide a useful complementary signal to prioritise the allocation of scarce resources.

7.3 Supplementary figures

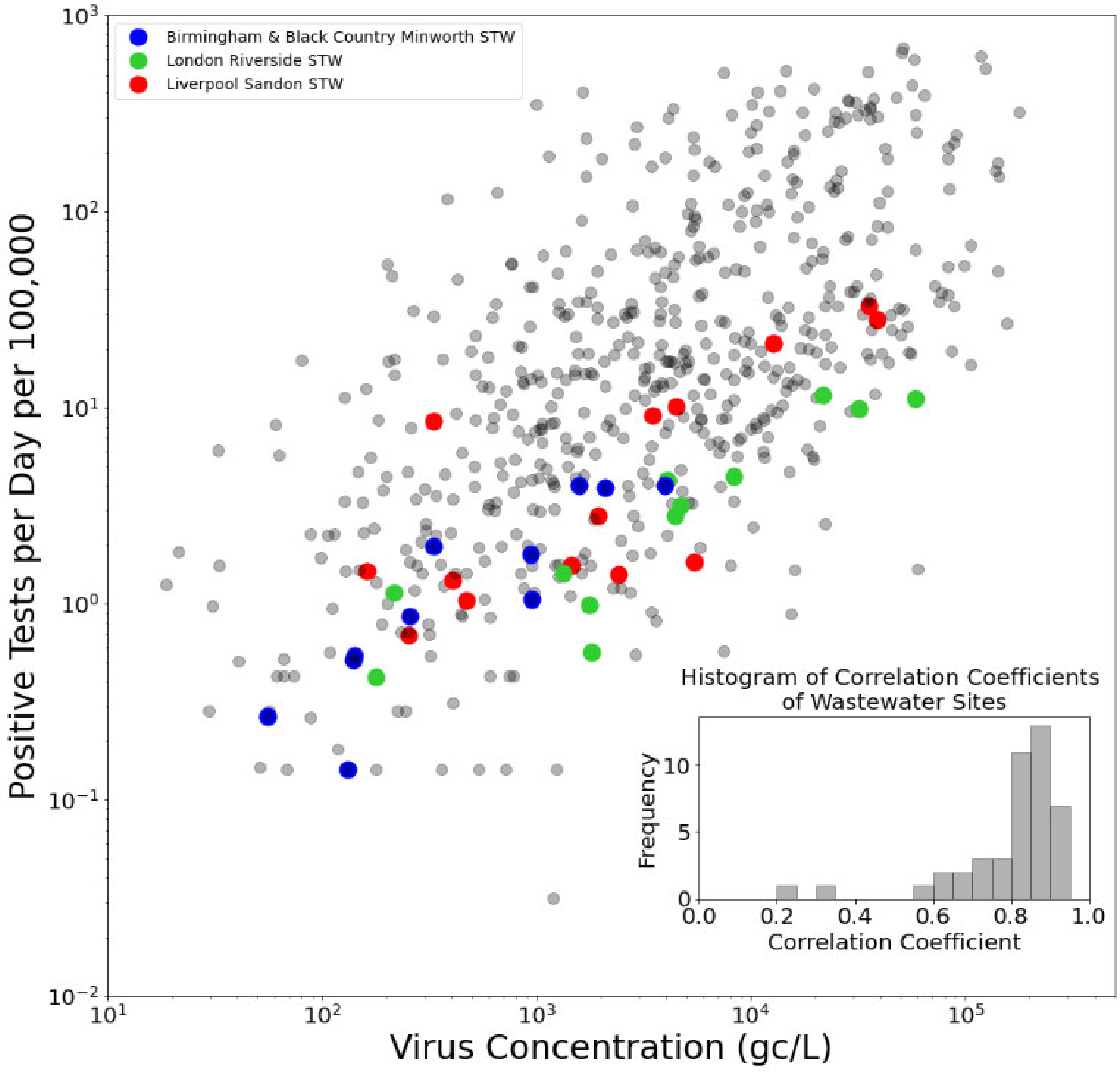

Figure S1 SARS-CoV-2 RNA concentration (gc/L) versus number of positive tests per 100,000 population. Data reports 1-week averages from the start of the English National Surveillance Programme in England. Three sites representing STWs with high correlation to positive cases in the community are presented in colour. A histogram of Pearson r correlation coefficients of

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[15] Peccia, J. *Nat. Biotechnol.*, **38**:1164-1167 (2020). “Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics.”

the individual wastewater sites is presented in the inset to the figure. The average value of the correlation coefficients is 0.80 ± 0.14. When the data from all STWs are combined the correlation coefficient is 0.42, with a one-sided p-value of 4×10^{-28}. The correlation coefficient for the combined data is much lower than the correlation coefficient for individual sites because there are factors of variation between sites such as hydrology, testing strategy, and catchment area size that are currently unaccounted for. Conversely taking the average of individual correlation coefficients provides an upper bound for the correlation. It can be said that there is a correlation with a high statistical certainty and that the correlation should lie between 0.42 and 0.8.

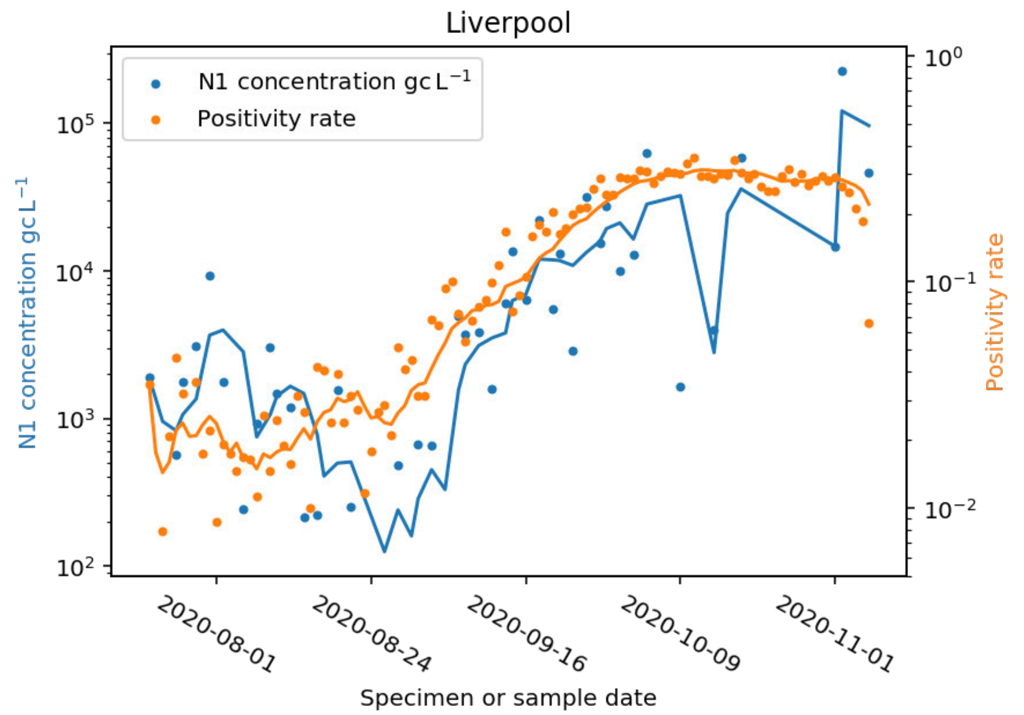

Figure S2 Time-series of measured SARS-CoV-2 N1 gene copies per litre at Liverpool Sand Docks STW (blue circles) with a 7-day rolling average. The positivity rate (number of positive tests / total tests per day, orange circles) are shown with a 7-day rolling average. Liverpool is an area with high prevalence from mid-September until late October. Wastewater data maps well with the change in positivity rate over the surveillance period.

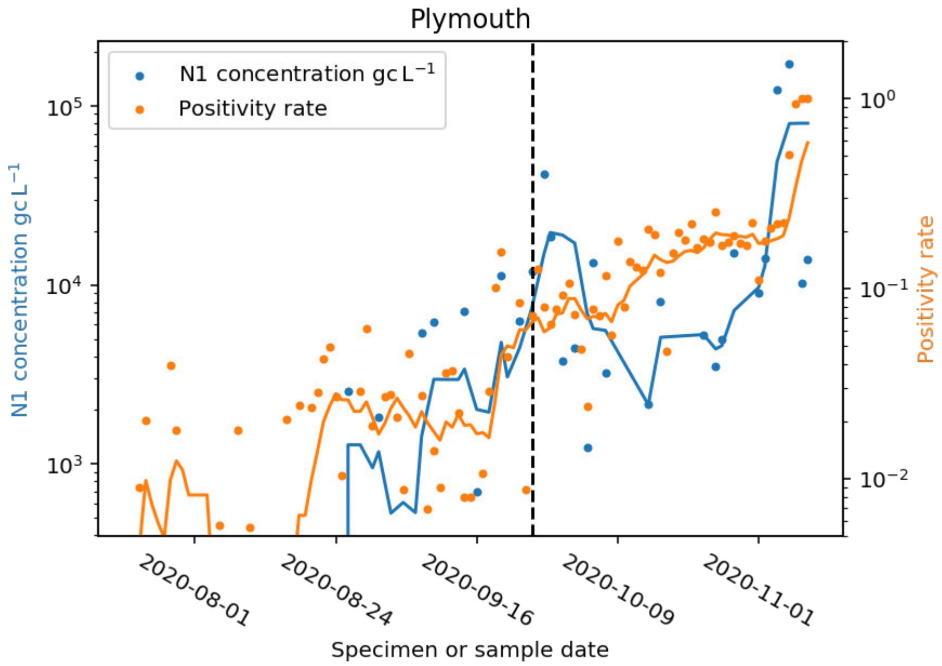

Figure S3 Time-series of measured SARS-CoV-2 N1 gene copies per litre at Plymouth Central STW and test positivity. The dashed line indicates the day wastewater was transported from a vessel docked at Devenport dockyard that subsequently reported an outbreak of SARS-CoV-2. These positive cases were not recorded in Pillar 2 Test & Trace. The subsequent spike in wastewater virus numbers can be linked directly to this outbreak as city-wide infection rates remained low and stable over this period. Recent positivity rates should be interpreted cautiously: the reporting of positives may be prioritised, leading to inflated positivity rates.

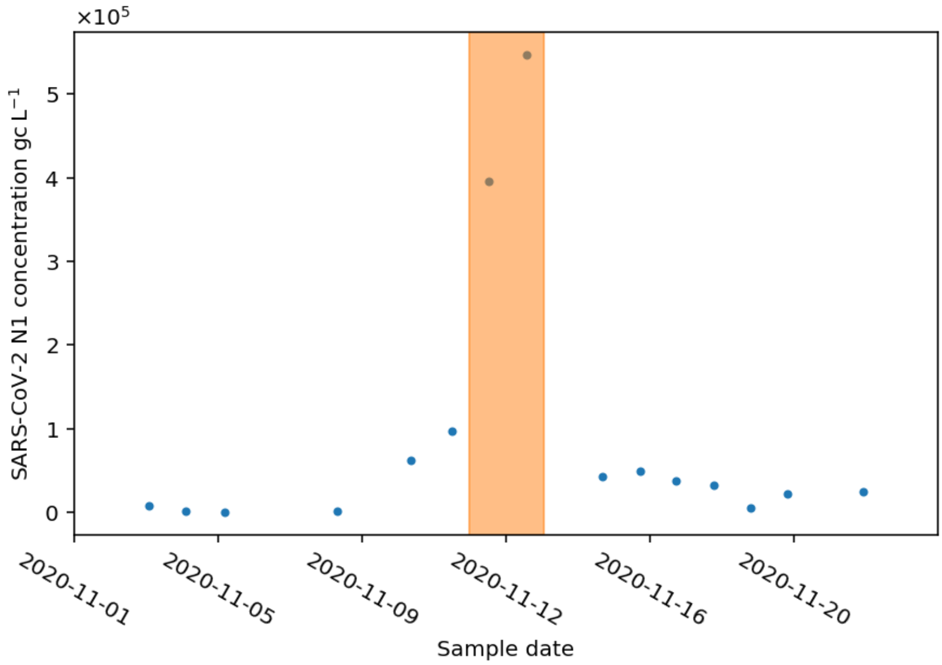

Figure S4 SARS-CoV-2 N1 gene copies per litre sampled outside a multi-residency building in a small city in England. Public health officials were advised that increasing RNA load may indicate a low number of positive individuals. Five cases were subsequently identified in the residential complex over the course of three days (orange region). This example suggests that WBE could be used to identify local outbreaks using near-to-source wastewater sampling.