Understanding and managing uncertainty and variability for wastewater monitoring beyond the pandemic: Lessons learned from the United Kingdom National COVID-19 Surveillance Programmes

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Abstract

The COVID-19 pandemic has put unprecedented pressure on public health resources around the world. From adversity, opportunities have arisen to measure the state and dynamics of human disease at a scale not seen before. Early in the COVID-19 epidemic, scientists and engineers demonstrated the use of wastewater as a medium by which the virus could be monitored, both temporally and spatially. In the United Kingdom, this evidence prompted the development of National wastewater surveillance programmes involving UK Government agencies, academics and private companies. In terms of speed and scale, the programmes have proven to be unique in its efforts to deliver measures of virus dynamics across a large proportion of the populations in all four regions of the country. This success has demonstrated that wastewater-based epidemiology (WBE) can be a critical component in public health protection at regional and national levels, and looking beyond COVID-19, is likely to be a core tool in monitoring and informing on a range of biological and chemical markers of human health; some established (e.g. pharmaceutical usage) and some emerging (e.g. metabolites of stress). We present here a discussion of uncertainty and variation associated with surveillance of wastewater, focusing on lessons-learned from the UK programmes monitoring COVID-19, but addressing the areas that can broadly be applied to WBE more generally. Through discussion and the use of case studies, we highlight that sources of uncertainty and variability that can impact measurement quality and, importantly, interpretation of data for public health decision-making, are varied and complex. While some factors remain poorly understood and require dedicated research, we present approaches taken by the UK programmes to manage and mitigate the more tractable components. This work provides a platform to integrate uncertainty management, through data analysis, quality assurance, and modelling, into the inevitable expansion of WBE activities as part of One Health initiatives.

Key words: COVID-19, Wastewater-based epidemiology, Measurement variability, Uncertainty

Introduction 1

Environmental surveillance for public health 2

The acquisition of data and extraction of information from environmental 3 samples to manage and improve public health has been a cornerstone of soci- 4 etal development for nearly 200 years [1]. However, in comparison with medical 5 and pharmaceutical innovation, much of the work in the field of environmen- 6 tal public health is largely unrecognised outside of the professional commu- 7 nities invested in its use. This is evident with wastewater, a conduit for an 8 array of bio- and chemical markers that can be analysed to provide information 9 on human activities, behaviours, and health status in populations [2, 3, 4, 5], 10 but which has remained a relatively untapped resource given its known poten- 11 tial [6, 7, 8, 9, 10]. However, the negative perception of wastewater as solely a 12 polluting substance, to be removed (from the human and natural environments) 13 and cleaned (often by energy intensive processes), has undergone re-evaluation 14 in recent years. The focus on sewage as a resource rather than a waste prod- 15 uct is driving innovation in the water industry. Accordingly, the diversity of 16 biotic and abiotic features within the sewage matrix presents an opportunity to 17 acquire actionable insights through routine monitoring and analysis of its com- 18 ponents. An increasing technological and computational capacity for deriving 19 knowledge from measurements and data has manifested in efforts to 'smarten' 20 the water industry [11], fusing data science with fundamental science and engi- 21 neering principles. This provides opportunities for greater utilisation of sewage 22 for the common good, be it in the production of resources such as energy and 23 high value chemicals [12], or as a proxy of human health and behaviours, which 24 will have transformative impacts for society. 25

Wastewater-based epidemiology in a time of crisis 26

The nature and extent of the COVID-19 pandemic has driven an unprece- 27 dented response from a diverse array of stakeholders, internationally. The efforts 28 to tackle both the spread of the disease and its impact on populations have high- 29 lighted the need for disparate communities of scientists, government agencies, 30 decision makers and the public to work together and collectively address the 31 multiplicity of public health, economic and social challenges that have emerged 32 over the course of the pandemic [13, 14]. This is also the case with the de- 33 velopment of wastewater-based epidemiology (WBE) as an important tool to 34 facilitate the detection and spatiotemporal monitoring of SARS-CoV-2 virus 35 dynamics in the environment being undertaken in many countries [15, 16, 17]. 36

Several studies have shown that the risk of infection by active SARS-CoV-2 37 virus in pre- or post-treated wastewater is low, particularly in modern sanitation 38 systems [18, 19, 20, 21]. Nevertheless, inactive fragments of the virus RNA have 39 been shown to persist longer in water than infectious virus [22]. Subsequently, 40 most reports on SARS-CoV-2 detection and quantification in wastewater have 41 focused on monitoring of the inactive virus, more specifically, the targeting of 42 small regions of the virus genome using an array of analytical methods, such as 43 reverse transcription-quantitative polymerase chain reaction (RT-qPCR) [23], 44

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genomic sequencing [24, 25, 26], and, more recently, mass spectrometry [27], to detect and identify the emergence and spread of novel variants in the population.

Beyond COVID-19

Although the COVID-19 pandemic has highlighted the benefits of *ex-situ* monitoring human-associated disease in the environment, WBE has also been successfully applied in other public health contexts, such as tracking pharmaceuticals, such as self-prescribed drug usage in cities [28, 29], antimicrobial resistance [30], and assessment of human exposure to environmental pollution [6, 31, 10]. The severity of the current pandemic is a strong motivation for increased and integrated public health and environmental surveillance at national and supra-national scales [32, 33]. Whether it is future-proofing for potential new pandemics [34] or *water fingerprinting* to determine factors impacting both physical and mental health in communities [35], wastewater surveillance will become a vital tool at the disposal of governments and public health authorities at the nexus of public and environmental health beyond COVID-19.

Wastewater and Public Health, an uncertain relationship

The manuscript is focused on the understanding and management of uncertainty in WBE, framed by, but not limited to, lessons-learned from wastewater surveillance during the COVID-19 pandemic. For broader discussion of WBE and its implementation as a tool for informing decision-making and policy, there are a plethora of excellent review articles that may be referred to [15, 36, 37]. The data rich, technologically diverse and computationally powerful resources available for WBE present an opportunity to deliver next-generation public health solutions in combination with targeted or passive environmental monitoring. Deriving an understanding of sources of uncertainty and implementing methods to estimate and account for measurement error is therefore critical for the design and implementation of wastewater surveillance to support public health decision-making. We posit that the insights presented here have wider consequences for WBE efforts beyond the pandemic.

Here we provide insights from our broad and extensive experiences gained while working with the United Kingdom (UK) WBE surveillance programmes and share this collective knowledge to support public health initiatives beyond the COVID-19 pandemic. The proceeding sections are then organised according to uncertainty and variability derived from source (population, shedding), in-network characteristics, and sampling and sample analysis. We finish by providing four case-studies related to separate aspects of applied WBE in which uncertainty and measurement variability are addressed and managed.

Uncertainty and its impact on wastewater surveillance

The perceived benefits of using measurements from wastewater samples for epidemiology include the relatively unbiased signal response to the determinant of focus (e.g. disease) and the provision of near real-time insights obtained from

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changes in the magnitude or direction of this response [38]. With respect to COVID-19, clinical sampling of individuals in the community, via nasopharyngeal swabs, saliva or serological tests, is subject to biases associated with factors common to sub-sampling of heterogeneous populations [39], and mass-testing to obtain a representative sample size is costly. Wastewater, on the other hand, provides an aggregated picture of community disease state through the measurement of virus RNA excreted by, theoretically, all viable shedders with the disease in the sewer catchment [40], and can be implemented at a relatively low-cost in comparison with clinical sampling [41]. In reality, however, the accuracy and representativeness of any measurement acquired from wastewater is subject to a number of influencing factors, which can be classed as observable (e.g. sample dilution by exogenous hydrological flows), or partially observable (e.g. in-network analyte decay/degradation). Two recent reviews of wastewater-based SARS-CoV-2 detection have focused on factors contributing to uncertainty in disease prevalence estimation [42] and, more specifically, errors associated with laboratory quantification using RT-qPCR [43].

It is well understood that environmental measurements are subject to extraneous factors that account for differing degrees of variability and uncertainty in the signal (see [44], for example), and surveillance for WBE is particularly impacted by the complexity of the media being sampled [35, 45, 42]. Figure 1 presents an overview of the known and potential sources of uncertainty in WBE for COVID-19, grouped into spatiotemporal classes (i.e., where and when the uncertainty is likely to impact the measurement). For COVID-19, variability manifests as a significant problem when different measured virus RNA concentrations are observed for, theoretically, the same proportion of infected individuals in the population. More precisely, it is the uncertainty arising between the target analyte (RNA) and its representation of the stressor of concern (disease prevalence or incidence). Unwanted variability can occur over time at a given sample site due, for example, to rainfall or snow melt entering into a combined sewer network during or after wet weather events, and diluting the analyte concentrations relative to a dry weather baseline. With target analytes such as virus particles, which can attach to solids in the network, the impact of increased flow in the sewer is likely non-linear due to the effects of turbulence and scouring on settled solids resuspension. Although, to our knowledge, no evidence of this currently exists for SARS-CoV-2.

Variation between sites is also a problem when using WBE measurements for comparison across geographies, or when aggregating to provide supra-catchment perspectives of target analyte dynamics. For example, a large catchment having a long residence time may systematically produce lower concentration measurements than a smaller site, even though the disease prevalence could be the same in both catchments. As shown in Figure 1, factors causing uncertainty or unwanted variability can range from large-scale processes, such as highly transient populations, to those at a smaller scale including laboratory specific methods [43]. In each case, strategies are needed to account for the variability in a way that is appropriate for the intended use of the data.

Uncertainty is unexplained variability and, importantly, imposes a lower level
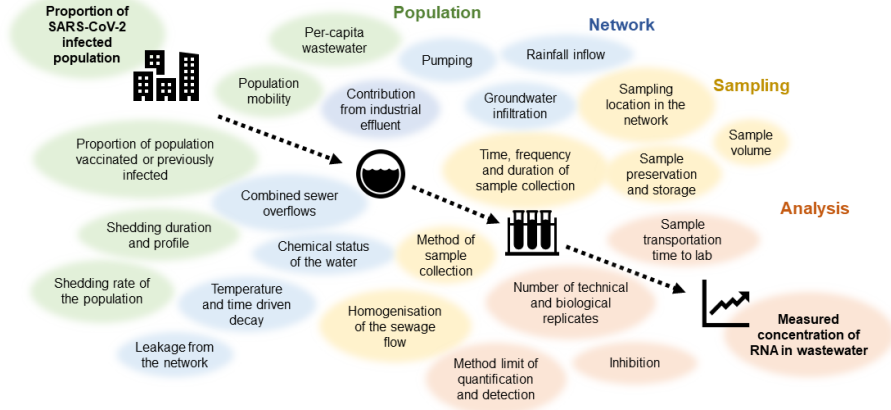
Figure 1: Summary of known and suspected sources of uncertainty for WBE; a perspective specific to UK wastewater surveillance of SARS-CoV-2

of confidence on a measurement than accountable and manageable (signal) variation [46]. Evaluation of uncertainty is necessary for WBE as quantifying the error bounds (and understanding the limits) of sample measurements is critical for capturing the inherent risk associated with public health decision-making processes. These risks are similar for likely all applications of WBE, i.e. incorrect estimation of target analyte(s); inability to compare measurements from different environments or under different conditions; loss of confidence in ability to detect or quantify the target analyte(s). The risks associated with uncertainty to wastewater surveillance of COVID-19 are wide-ranging and depend on its use-case. For example, recent attention focused on how using measurements from wastewater in epidemiological models [47, 48] could increase parametric uncertainty and error bounds on model estimates [49]. This, in turn, will affect the suitability of the model for tracking and predicting the dynamics of the disease [50]. Using raw wastewater measurements without accounting for factors that can bias interpretation, such as wastewater dilution or signal decay, may have a significant impact for decision-making when used to complement other sources of disease prevalence.

COVID-19 Wastewater Surveillance in the United Kingdom

England

Sampling of wastewater in England is being carried out by the Environment Agency, UK Water utilities, and the Environmental Monitoring for Health Protection team, part of the Joint Biosecurity Centre created to support government response to the COVID-19 pandemic. Sample collection started in June 2020 at the inlets to 44 sewage treatment works (STWs). The sites were selected to provide good population coverage and geographical representation across the

country. In total, the original sites covered 17.7 million people (over 31% of the population of England). The sampling capacity was increased considerably at the start of 2021 and, as of July 2021, comprised 556 sites, including 263 STWs, 238 network sites (manholes or pumping stations in the sewer catchment), and 55 near-source sites (single or groups of buildings). The sites are distributed across the networks of the nine water utilities in England. By July 2021, wastewater sampling covered 39.4 million people (70% of the population of England). Recent sites were selected according to multiple criteria including demographic disease risk, population coverage and, for network sites, access points (e.g. manholes, pumping stations) that ensure safe access and well-mixed samples. STWs are sampled four times per week, using either autosamplers (composite) or by grab, or spot, sampling, post influent screening. The method of sampling is typically dependent on infrastructure at the STWs. An example of the potential for large variability in wastewater concentration between samples taken, either as grab or composite, is shown in Figure S3. In-network samples, collected upstream of the STWs, are used to constrain areas of concern in nine 'core' cities representing the largest conurbations in England, and three smaller strategic cities (based on historical COVID-19 trends). Samples from network sites are collected daily, mostly as grab samples, while near-source samples are largely taken using autosamplers at a fixed sub-sampling frequency (See Figure S1 for an overview, as of July 2021).

Scotland

Sampling and testing of wastewater across Scotland has been performed by Scottish Water and the Scottish Environmental Protection Agency (SEPA). In total there are over 1,800 sewage and wastewater treatment works in the Scottish Network, serving from fewer than 100 people to populations over 600,000. Initial testing of the premise that SARS-CoV-2 virus fragments were detectable in wastewater began in April 2020 with the development of a national monitoring programme operationalised by late May 2020. A network of 28 sites were initially prioritised, designed to maximise the coverage of population across Scotland's 14 National Health Service (NHS) Health Regions, while also ensuring that laboratory capacity was available at that time. The 28 sites covered a total of 2.6 million people (just over half of the five million sewered population in Scotland), with the goal of achieving a coverage of 40% in each of the 14 regions. As the need for wastewater monitoring has increased, so too has the monitoring network, which has expanded to 108 sites covering 4.2 million people. Autosamplers are used to obtain composite samples from the influent at each sewage works over a 24-hour period, which are then sent to SEPA for analysis. Results are then published via data visualisation dashboards. One dashboard, designed for the general public, holds the raw virus concentrations for each site (https://informatics.sepa.org.uk/RNAmonitoring), while a second dashboard, designed for public health officials, has additional metrics and comparisons to reported case numbers.

Additionally, Scottish Water, SEPA and a variety of NHS and Public Health professionals from across Scotland have been working together to collect and

analyse samples from within the sewer network itself (via manholes). These ₂₀₂ were sited at the request of health professionals in order to better understand ₂₀₃ the virus prevalence in areas of concern within larger sewer networks. These ₂₀₄ samples are taken by means of a grab sample. In total, 28 such sites have been ₂₀₅ monitored at one time with 14 still being active as of July 2021. ₂₀₆

In total, over 5,000 samples have been tested and recorded as of July 2021. ₂₀₇ The sampling frequency varies between sites depending on several factors and ₂₀₈ has changed at different times as the needs of stakeholders has changed over ₂₀₉ time. Sampling at treatment work inlets has been variable, with the majority ₂₁₀ sampled at a frequency of once or twice a week, but some as much as 4 times ₂₁₁ per week, a trade-off between lab capacity and data density. All in-network ₂₁₂ samples are monitored five times per week in their initial week to establish a ₂₁₃ baseline before being sampled twice a week thereafter. ₂₁₄

Wales ₂₁₅

The Wales wastewater monitoring programme started as a pilot in March ₂₁₆ 2020 as the first wave of COVID-19 spread across the UK [51]. This early ₂₁₇ work highlighted the potential for tracking SARS-CoV-2 and also led to the ₂₁₈ development of robust methodologies for extracting and quantifying the virus ₂₁₉ in wastewater [52]. This pilot phase was then expanded in September 2020 to ₂₂₀ 20 sites across the country. These sites were initially sampled three times per ₂₂₁ week, increasing to five weekdays by June 2021, to try and reduce the variabil- ₂₂₂ ity in the wastewater SARS-CoV-2 RNA signal and, thus, improve its usability. ₂₂₃ One of the major challenges in Wales has been the lack of on-site infrastructure ₂₂₄ needed to take composite wastewater samples. Therefore, all samples are cur- ₂₂₅ rently taken as grab samples, targeted at the early morning wastewater peak ₂₂₆ (between 08:00 and 11:00 h). However, it is now known from deploying the ₂₂₇ enveloped *Pseudomonas* virus, phi6, into the sewer network that this approach ₂₂₈ may miss the effluent peak, leading to an underestimation of viral abundance. ₂₂₉ Another challenge has been the poor geographical coverage in Wales. The coun- ₂₃₀ try has two urban corridors centred around the northern and southern coasts ₂₃₁ in which 80% of the population resides. Consequently, wastewater surveillance ₂₃₂ has focused in these areas, leaving ca. 20% of the country, mainly in central and ₂₃₃ western Wales unmonitored, resulting in uncaptured localised outbreaks in small ₂₃₄ urban centres. Another major issue is that the capital city, Cardiff, is served ₂₃₅ by a very large centralised STW (930,000 people). Although this captures 30% ₂₃₆ of the Welsh population in one sample, the lack of granularity prevents the po- ₂₃₇ tential for using wastewater surveillance to target regions of the city to control ₂₃₈ localised COVID-19 outbreaks (e.g. implementation of surge testing and walk-in ₂₃₉ vaccination centres). The lack of sampling at weekends also prevents capturing ₂₄₀ of the large migration of tourists from North West England into North Wales. ₂₄₁ The wastewater samples taken in Wales were also used to pilot their potential to ₂₄₂ track other viruses of public health interest (e.g. influenza A and B, norovirus, ₂₄₃ respiratory syncytial virus, enterovirus D68). Analysis showed that wastewa- ₂₄₄ ter contained all these viruses with the exception of Enterovirus D68. Looking ₂₄₅ forward, the Wales wastewater surveillance programme is now being expanded ₂₄₆

to many more sites with the aim to capture 90% of the population on mains 247
sewerage and with analysis of a greater number of public health indicators. 248

Northern Ireland 249

Wastewater surveillance in Northern Ireland (NI) has several unique chal- 250
lenges compared to other parts of the UK, which are related to the urban and 251
rural distribution of population. NI has an extensive wastewater treatment net- 252
work operated by Northern Ireland Water (NI Water). In total, there are 1114 253
STWs in the NI Water network, serving just under 80% of the NI population. 254
Each STW serves a wastewater drainage catchment area of variable sizes. Up 255
to 68% of the NI Population is served by the 40 largest STWs. However, these 256
larger STWs serve predominantly urban, as opposed to rural communities, and 257
tend to be disproportionately located in eastern parts of NI. The integrated 258
wastewater testing and geographic surveillance programme for SARS-CoV-2 in 259
NI is led by Queen's University Belfast, funded by the Department of Agricul- 260
ture, Environment and Rural Affairs (DAERA) in collaboration with the Public 261
Health Agency NI (PHA-NI). 262

Currently there are SARS-CoV-2 wastewater samples being taken at 14 sam- 263
pling sites at STWs covering 35.3% of the NI population. The current sampling 264
strategy was based on several key factors, including population coverage, geo- 265
graphic distribution of wastewater surveillance and a close alignment and agile 266
response to PHA-NI test and trace results. Consideration is being given to sig- 267
nificantly expanding the sampling sites, allowing for the wastewater surveillance 268
of a significant portion of the NI population. 269

An important aspect of the approach in NI has been the use of Geographical 270
Information Systems (GIS) to develop spatial GIS-based wastewater monitor- 271
ing and reporting system integrating public health data to model population 272
geographies and align with wastewater drainage catchment areas. Modelling 273
population across NI using GIS provides an approach to estimate populations 274
covered by the wastewater network, the population within individual wastew- 275
ater drainage catchment areas, and an estimate of how to balance capturing 276
the maximum percentage of the population from a relatively limited number 277
of sample sites, while ensuring an adequate geographic spread across NI. This 278
has been achieved through the development of an interactive wastewater SARS- 279
CoV-2 Surveillance Dashboard. The Dashboard provides a display of the anal- 280
ysis results of sampling at various locations in NI and enables users to see and 281
understand population distribution modelling across NI wastewater network. 282
This offers the most efficient and informative sampling strategy for the pro- 283
gramme, and an approach to contextualise wastewater test results in terms of 284
socio-economic deprivation 285

An indication of the extent of wastewater surveillance across all four regions, 286
indicating the geospatial locations of sampling sites (as of July 2021), is shown 287
in Figure S2. 288

Drivers of variability and sources of uncertainty

Population factors

Knowledge of the contributing population size upstream of the sampling lo- 291
cation is important for calculation of per capita concentrations and to facilitate 292
comparison between sample sites. It is important to have an accurate esti- 293
mate of population size, a) to ensure that inter-site comparisons are made on 294
an equivalent basis, and b) to account for the effects of intra-site population 295
change on the loads of measured target(s) in the wastewater. Population size is, 296
however, uncertain and variable. The mean population size may be estimated 297
based on census data and additional demographic statistics, but such estimates 298
cannot be easily updated to account for changes resulting from births, deaths 299
and migration, and can quickly become outdated [53]. Fluctuations in popu- 300
lation during the sampling period can contribute further uncertainty. These 301
include, for example, weekly and seasons variations due to the flux of com- 302
muters and tourism or student populations, respectively. Dynamic population 303
estimates may be obtained using water quality parameters such as ammonia 304
and orthophosphate; however, this is subject to bias due to the contribution of 305
additional sources such as industrial discharges [54]. The use of mobile device 306
data [55] and biomarkers present in urine (e.g. caffeine, pharmaceuticals) [56], 307
are alternative metrics that have been shown to significantly reduce measure- 308
ment uncertainty when used to estimate population size for normalisation of 309
target analyte concentrations. 310

To illustrate the potential effects of population variability, Figure 2 shows 311
the impact of reporting per capita SARS-CoV-2 loads instead of SARS-CoV-2 312
concentrations on trends identified at a STW site in England. In this case, a 313
site-specific mean daily ammonia discharge per capita ($\overline{x}$) is estimated using 314
Equation 1, based on measured ammonia concentrations (X_d) and wastewater 315
flow rates (Q_d) for the entire sampling period and the Office for National Statis- 316
tics population estimate (P) for the catchment. SARS-CoV-2 gene copies per 317
capita per day (L_d) are then calculated on a daily basis using Equation 2, based 318
on this value and the measured SARS-CoV-2 (S_d) and ammonia concentrations 319
for the current day. 320

$$\overline{x} = \frac{\overline{X_d Q_d}}{P} \tag{1}$$

$$L_d = \frac{S_d \overline{x}}{X_d} \tag{2}$$

Error bars are included in Figure 2 to indicate standard deviation resulting 321
from variability in the site-specific ammoniacal nitrogen discharge per capita; 322
these do not capture any other sources of uncertainty. 323

An important and poorly understood source of uncertainty related to pro- 324
portion of contributing population is the quantity and rate of analyte released 325
into the network through faecal or urinary shedding. Faecal shedding of SARS- 326
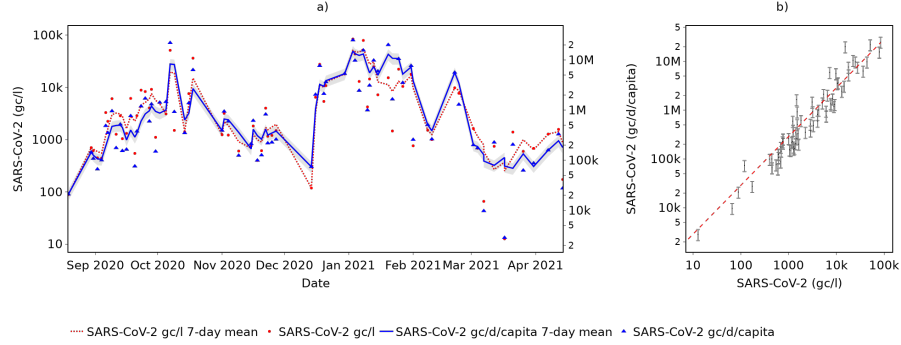CoV-2 RNA varies both between individuals and over the infection course of 327

Figure 2: Example comparison of wastewater SARS-CoV-2 concentrations and SARS-CoV-2 loads per capita at an English sewage treatment works.

any given individual [40]. Indeed, a recent study has indicated, from near- ₃₂₈
source data, that faecal shedding peaks on average 6 days post-infection (95% ₃₂₉
Uncertainty Interval 4 - 8 days) [57]. The impact of shedding variability be- ₃₃₀
tween individuals is attenuated for large catchments and during high prevalence ₃₃₁
periods because the sewerage system naturally averages the signal from many ₃₃₂
people [58]. Due to the greater variability in the wastewater measurements ₃₃₃
compared with clinical data sources, the power of WBE surveillance, at least ₃₃₄
for COVID-19, is expected to be greatest when transmission (and prevalence) ₃₃₅
or clinical testing is low; i.e. capturing (re)emergence of disease in a commu- ₃₃₆
nity. However, quantitative estimates of the number of individuals infected are ₃₃₇
likely to remain elusive when infection prevalence is low or the sampled popu- ₃₃₈
lation is small, such as for near-source sampling. Temporal variability of viral ₃₃₉
RNA shedding over the infection course implies that the concentration of SARS- ₃₄₀
CoV-2 gene copies in wastewater is a convolution of disease incidence with the ₃₄₁
shedding profile [59]. Consequently, techniques for relating epidemiological in- ₃₄₂
dicators to wastewater-based signals need to consider multiple time lags, for ₃₄₃
example by employing distributed lag models [60]. Studies to investigate viral ₃₄₄
shedding prior to symptom onset are urgently required because existing data ₃₄₅
have been collected from hospitalised patients [61, 62, 63]. Similarly, the im- ₃₄₆
pact of vaccination on faecal shedding of viral RNA is unknown, although data ₃₄₇
from nasal swabs suggest that viral loads are likely to be reduced [64]. Given ₃₄₈
this, quantifying virus at near-source with any precision remains elusive, and ₃₄₉
further work to understand faecal shedding distribution is critical for adoption ₃₅₀
of wastewater measurements in epidemiological models for estimating transmis- ₃₅₁
sion rates (i.e. effective reproduction number, R_{eff}) [65]. This information ₃₅₂
can be applied broadly to other analytes routinely shed in the urine and faeces ₃₅₃
that correlate to public health indicators, although shedding profiles could be ₃₅₄
markedly different from those for viruses. ₃₅₅

11

Characteristics of the sewage network (proportion of gravity-fed or pres-   357
surised pipes; size of the network; retention capacity; location and triggering of   358
combined sewer overflows (CSOs), and use of sustainable urban drainage, in-   359
frastructure to separate stormwater flow in the catchment) may impact both the   360
quantity of analytes of interest within the water and their distribution within   361
the sewage volumes.   362

The daily flow patterns in most wastewater systems are oscillatory, driven   363
by multiple factors such as sewer network design, industrial discharge events   364
and prevailing weather conditions. However, the flow signal, under dry weather   365
flow conditions, is governed by household water usage, which often presents as   366
morning and evening 'peak flow' pulses, especially in small catchments. These   367
daily oscillations are damped in catchments with a wide network or large storage   368
capacity where peak flow can be retained and processed later, leading to a   369
homogenisation of the signal [66]. Pumps across the network or at the inlet of   370
STWs can also homogenise analyte concentrations within the flow, with sumps   371
or wet wells acting as small retention tanks. Ingress of non-human derived flow,   372
e.g. from rainfall or snow melt, in combined sewers, or groundwater infiltration   373
in all sewers, can bias measurements by signal dilution.   374

Sewer network size, sewer gradient, pipe friction, and presence of retention   375
tanks can impact the time-of-travel of wastewater 'packets' (typically < 1 to   376
24-hours in the UK, dependent on catchment size), and may reduce target con-   377
centrations that are prone to degradation [67] (i.e. those with a short T90,   378
the time for one log unit reduction in concentration). Moreover, the type of   379
sewage system (gravity-fed or pressurised pipes) can directly impact the decay   380
rate of analytes of interests due to differences in biofilm composition within   381
these two environments [68] (fully anaerobic for rising mains, and mixed anaer-   382
obic/aerobic in gravity sewers). Further, the sheer stress created by cycling   383
between pressurised and unpressurised pipes might further hasten the decay   384
of labile analytes. Finally, retention tanks may also increase the binding of   385
hydrophobic targets, such as SARS-CoV-2 virions, with suspended solids to   386
form complex matrices [69], which may obfuscate their subsequent detection by   387
laboratory analysis, or result in settling-resuspension phenomena in the sewer   388
pipes [70], decoupling the temporal dynamics of the virus RNA from the dis-   389
charge event. Significant sewer pipe leakages may also influence the fate of   390
virus, and consequently its downstream detection and quantification, especially   391
in older networks.   392

Adjusting for the impact of network characteristics across a national pro-   393
gramme is challenging due to the need for quantitative, comparable information   394
for individual site networks. In England, this data is typically owned by private   395
water utilities and, in many cases, the precise configuration of the network is   396
not known, unless access is provided by the companies. However, the impact of   397
some site characteristics can be mitigated by taking into account co-dependent,   398
measurable parameters. For example, ammonia concentration can be used as a   399
proxy for the dilution effects in combined sewers, and catchment area is a rough   400

approximation for network size. While proxy variables are useful in the absence   401
of true measurements, their use in management of target measurement uncer-   402
tainty may be limited by how representative it is of the variable of interest. The   403
use of multi-biomarkers to better represent human wastewater contribution (See   404
Case Study 4), or GIS-based modelling and public health information to better   405
characterise catchment population are currently employed methods to mitigate   406
this limitation.   407

Sampling strategy   408

In the context of WBE and public health surveillance, acquiring a repre-   409
sentative sample that captures the analyte of interest is fundamental to sup-   410
port actions that have the potential to impact the well-being of individuals and   411
communities [66]. The source of the analyte(s) targeted, through urine (e.g.   412
metabolites of pharmaceuticals) or faeces (e.g. viruses), can impose additional   413
variability in measurements, and beyond the COVID-19 pandemic, wastewater   414
surveillance programmes will need to build in sampling flexibility to account for   415
this uncertainty [71].   416

Broadly, there are two ways to take a sample: (i) a 'grab' or 'spot' sample   417
where a single sample of wastewater is taken using a small container, and (ii) a   418
'composite' sample where samples are taken regularly throughout the day using   419
an automated device (autosampler) and the samples mixed together in a sin-   420
gle container. Several autosampling modes may be used to create a composite   421
sample: *time-proportional*, where a constant sample volume is taken at regular   422
time intervals; *flow-proportional*, where the time interval is kept constant but   423
the sample volume adjusted to the flow in the sewers; and *volume-proportional*   424
sampling, where a constant volume sample is taken each time a fixed volume   425
passes through the sewer. Measurement uncertainties are heavily impacted by   426
the type, mode and timing of sampling depending on variability of flow and an-   427
alyte concentration over time [66]. The difference in the probability of detection   428
between sampling methods becomes greater as prevalence (of the target analyte)   429
decreases. Specifically, when concentration is low, detection likelihood via grab   430
sampling would be much lower than with composites, while as concentration   431
increases, the probability of detection using grabs becomes comparable. This   432
would suggest that composite sampling is preferable during periods of low tar-   433
get analyte concentration. However, if the daily signal is concentrated in time,   434
well-timed grab samples could capture higher concentrations than is possible   435
with composite samples (as shown in Figure 3). The nature of composite sam-   436
ples means that it dilutes a 'sharp' signal, which can be a disadvantage at low   437
prevalence times. Areas with a more temporally constant signal would be less   438
sensitive to the choice of sampling method. This risk can be mitigated somewhat   439
by the appropriate design and use of autosamplers. However, the autosampling   440
method can also impact measurement confidence. Time-proportional sampling   441
can lead to under- or over-weighting of sample during periods of high or low   442
flow, respectively, resulting in loss of representativeness. A flow-proportional   443
sampler extracts a fixed volume of sewage when a predetermined volume of flow   444
has accumulated. The resulting daily sample will be weighted by flow and could   445

13

be argued to be more representative of the conditions of that day, assuming that   446
the substance of interest is distributed uniformly through the day. However, on   447
low flow days the sample volume may be too low for effective analysis, while   448
on wet days the full volume may have been taken long before the end of the   449
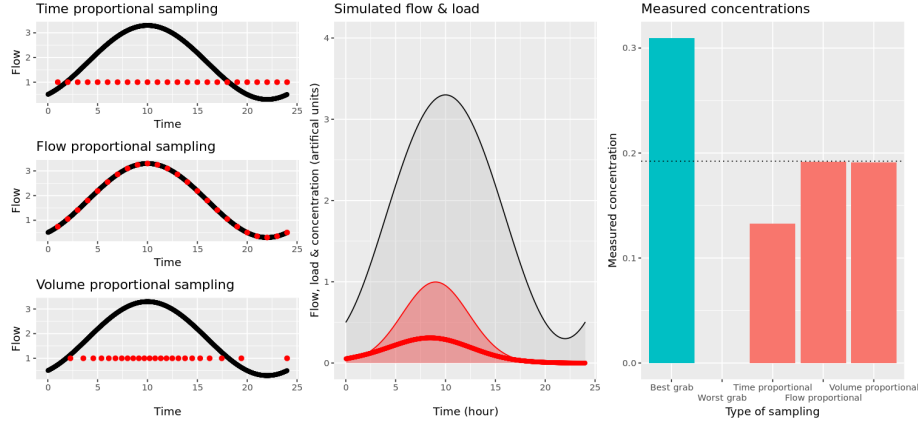sampling period.   450

Figure 3: Simulated sample concentrations using different sampling methods. Left: Representation of composite sampling methods; the flow is represented in black and sampling time and volume in red. Middle: Simulated flow (black), load (red), and resulting concentration (thick red line). Right: Resulting concentrations based on the sampling method. Highest and lowest values represent the maximal and minimal concentration that can be obtained with grab sampling from this simulated flow/load.

As samples are not always collected daily, sampling cadence must be con-   451
sidered when determining WBE sampling strategies. Aliasing effects may result   452
in incorrect interpretation of signal dynamics, or produce artefacts in mod-   453
els used for back-calculation of target stressors, for example [72]. A sampling   454
frequency as close to the daily cadence will reduce uncertainty arising from tem-   455
poral variability. This has been quantified through a data ablation experiment   456
for 186 network sites monitoring SARS-CoV-2 in England, for which a number   457
of samples were artificially removed to compute the relative bias introduced by   458
reducing the sampling cadence, as shown in Figure 4. Consideration of sampling   459
frequency in relation to sample location in the network is necessary. In small   460
catchments, or near-source applications (e.g. monitoring of critical infrastruc-   461
ture such as prisons, care homes and schools), high-rate composite sampling   462
may not be enough when all discharge events should be captured. Technolo-   463
gies that can provide continuous active sampling or passive samplers are more   464
suitable in this context [73, 74].   465

Variability due to differences within and between site sampling deployment   466
have a potential be a significant influence on the measurement, particularly   467
when establishing a national surveillance system with a large number of sites   468
and different site personnel involved. Detailed and clear sampling protocols and   469
ongoing training of staff are essential to minimise some of the sources of this   470
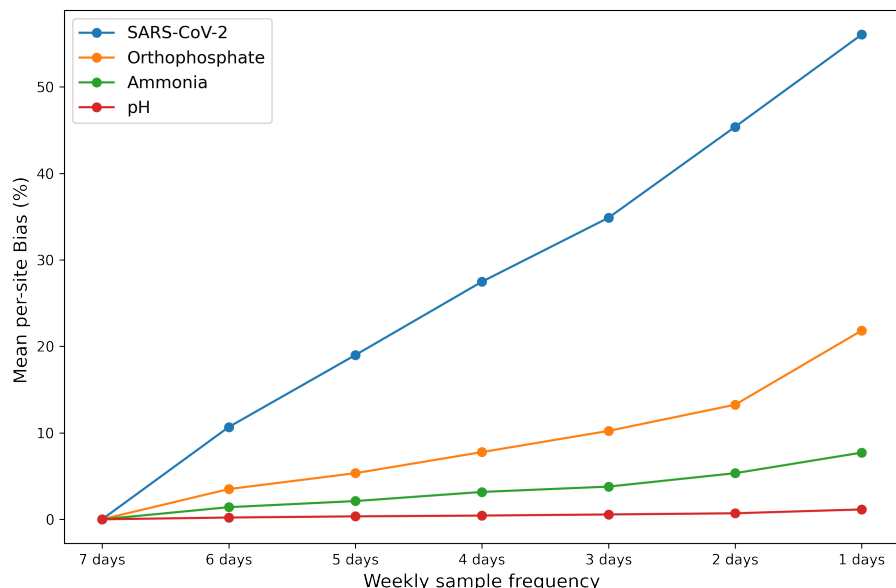
Figure 4: Per-site mean percent bias, compared to the 7-day baseline, in SARS-CoV-2 and other marker measurements when reducing the sampling frequency artificially for 186 network sites across England. Whilst the bias for ammonia, orthophosphate and pH is limited to $\sim 10\%$, a bias of up to $\sim 65\%$ can be introduced in the mean estimate of SARS-CoV-2 when decreasing the cadence frequency.

variation.   471

Sample analysis   472

Wastewater is a highly complex and variable media, containing compounds   473
that can decrease detection sensitivity, which results in false-negative results,   474
whilst also compromising the ability to quantify the analyte of interest, such   475
as genetic fragments, accurately. As significant knowledge performing sample   476
analysis has been gained while monitoring the COVID-19 pandemic, insights   477
relating to the uncertainty arising from SARS-CoV-2 quantification have been   478
addressed, leading to a consolidated application of wastewater lab-analysis for   479
WBE.   480

Due to the low concentrations of SARS-CoV-2 in wastewater, methods are   481
required to pre-concentrate the virus prior to analysis. The most commonly used   482
methods include precipitation with salt or polyethene glycol (PEG [52]), electro-   483
statically charged membrane filtration [75], ultrafiltration [76], or adsorption-   484
precipitation with aluminium chloride or silica [77]. Due to the expense and   485
poor availability of ultrafiltration devices, the English wastewater surveillance   486
programme initially adopted the PEG precipitation method. This was based   487
on previous success at recovering viruses from wastewater [78]. However, the   488

overnight precipitation step in the method increased the time from sample col- 489
lection to reporting. A decision was then made to switch from PEG to salt (am- 490
monium sulphate) precipitation as the latter only requires a 1-hour incubation 491
step. Parallel studies with duplicate wastewater samples showed no significant 492
differences in recovery between the two methods for SARS-CoV-2 RNA (data 493
not shown). This ammonium sulphate workflow now allows viral RNA to be 494
concentrated, extracted and quantified within a 24-hour window. 495

Another key step in SARS-CoV-2 determination from wastewater is to pro- 496
duce RNA extracts that ensure consistency in the quantity, quality, and purity 497
of extracted nucleic acids for their applicability in downstream processes (e.g. 498
detection, quantification, sequencing). SARS-CoV-2 determination is generally 499
carried out with a nucleic acid-based PCR assay. However, given the wide-range 500
of PCR inhibitors in wastewater and the options available for handling them, 501
no single method serves all applications; a multifaceted approach being the best 502
solution to avoid amplification failure. Therefore, efficient extraction methods 503
are required to purify inhibitor-free RNA, together with the use of inhibitor- 504
tolerant quantitative reverse transcription PCR (RT-qPCR) mixes containing 505
enhancers/additives to help reduce inhibition (e.g. gp32 and BSA). On the other 506
hand, the low levels of SARS-CoV-2 in wastewater means that sample dilution 507
to alleviate inhibition is not recommended or should be limited [43]. Alter- 508
natively, the samples can be analysed by one-step digital-PCR (dPCR) rather 509
than RT-qPCR. To estimate the efficiency of viral RNA recovery, all samples 510
in the English programme are spiked with phi6 virus. Typically, the recovery 511
of phi6 ranges from 1 to 50%, indicating that the viral recovery methods still 512
need to be optimised for some wastewater types. This is supported by studies 513
from England where wastewater has been spiked with heat-treated SARS-CoV- 514
2 and where recovery is often incomplete (ca. 30 - 50% recovery; Kevill *et al.*, 515
2021, unpublished). Alongside SARS-CoV-2, a range of other faecal-marker 516
viruses (e.g. crAssphage, pepper mild mottle virus) have been measured in 517
wastewater [36]. In the English programme, crAssphage was initially used to 518
help normalise the SARS-CoV-2 results to account for dilution by industrial 519
wastewater and rainfall, however, this created extra workload and delayed the 520
workflow, and was subsequently dropped in favour of other indicators of faecal 521
load (e.g. ammonia). Case Study 3 presents some specific results from our 522
management of laboratory analysis uncertainty and variability across the UK 523
wastewater surveillance programmes. 524

Management of variability and mitigation of uncertainty 525

Population normalisation and measurement correction 526

Measurement correction is key to addressing variation resulting from sam- 527
pling, sample transport and storage, as well as possible errors linked with sample 528
processing (including sample preparation: biomarker extraction from wastew- 529
ater, concentration, and analysis). Normalisation of data is important to re- 530
duce uncertainties related to changing wastewater flows (resulting from diur- 531

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nal changes and seasonal variability in rainfall patterns), movement of popula- 532
tion, biomarker sources (e.g. intake vs. environmental occurrence) as well as 533
biomarker stability and its transformation (e.g. human metabolism or metabolic 534
degradation of sewer microorganisms). WBE in chemical exposure studies (e.g. 535
illicit drugs, pesticides, industrial chemicals, pharmaceuticals) has been subject 536
to comprehensive evaluation of uncertainties due to its application requiring 537
a reliable quantitative measurement (e.g. per capita drug consumption). In 538
chemistry-based WBE, 24-hour composite sampling is strongly advised, as well 539
as having labelled internal standards (analogues of biomarkers that do not exist 540
in nature, e.g. benzoylecgonine D8, which is used as an internal standard to 541
benzoylecgonine) used to compensate for errors occurring throughout sample 542
storage, processing and analysis. Flow measurements of wastewater are re- 543
quired, as well as an understanding of stability of biomarkers in wastewater and 544
their extraction efficiency/matrix effects (e.g. interfering chemicals during anal- 545
ysis). However, with biology- or pathogen-based WBE, grab sampling is still 546
the norm and there is an inherent lack of flow measurements in large national 547
campaigns, as well as in near-source applications, which skew the results and 548
make the studies more qualitative in nature. Biomarker selection in chemistry- 549
based WBE requires its pre-use validation including the following requirements: 550
(1) originating in human (with no other sources), (2) accounting for human 551
metabolism, (3) stable in sewers, and (4) with excellent analytical performance 552
in biomarker quantification (the latter is usually followed by inter-lab studies, or 553
'ring trials'). These factors are yet to be fully evaluated in biology-based WBE 554
(and indeed in new chemistry WBE applications), where biomarkers are stres- 555
sors themselves, and there is limited (albeit rapidly increasing) understanding of 556
analytical method performance and stability of biomarkers. Most importantly, 557
it is currently impossible to differentiate between different sources of stressor 558
release to the sewerage systems. 559

Chemical analysis of certain biomarker groups, especially metabolites of 560
high-usage, prescription only pharmaceuticals (e.g. antidepressants, antidiabet- 561
ics, and antiepileptics) with well-defined consumption patterns, can provide im- 562
portant insights into diurnal changes in population size contributing to wastew- 563
ater. Antidepressants are shown in Figure 5 as an example. Measurements were 564
undertaken over seven consecutive days in five English towns/cities as discussed 565
in Case Study 4. A significant positive relationship between the daily loads of 566
antidepressants, their metabolites and the population size served by respective 567
wastewater treatment plants was observed (Pearson coefficient, $r \geq 0.997$, $p <$ 568
0.0002). As expected, metabolites showed the lowest spatiotemporal variability 569
in the studied intercity catchment ($< 16\%$ for desmethylvenlafaxine and $<12\%$ 570
for desmethylcitalopram), when compared to their respective parent antidepres- 571
sants (venlafaxine and citalopram), which can be directly disposed-off into the 572
drain. This indicates their suitability as population markers. Figure 5 also indi- 573
cates the benefit of normalisation in trying to understand consumption patterns. 574
In the figure, double normalisation was applied to account for variable flows and 575
population. As a result, per capita change in consumption patterns can be ob- 576
served and conclusions drawn regarding the variable consumption patterns in 577

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cities with different socioeconomic status. Further discussion on how certain   578
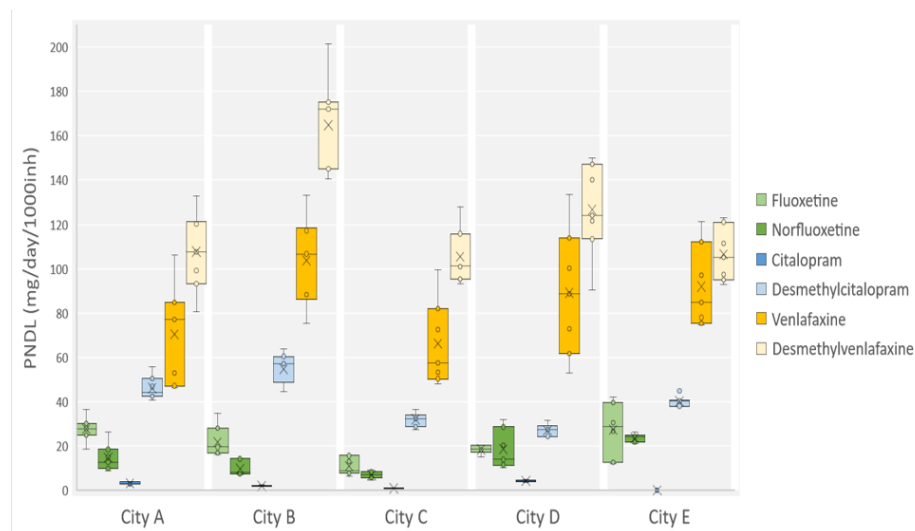variables affect back-calculations of chemical intake can be found in Case Study   579
4.   580

Figure 5: Population normalised daily loads (PNDL) of antidepressants (mg/day/1000 inhabitants) and their metabolites.

Design and implementation of sampling   581

The sampling strategies employed for SARS-CoV-2 surveillance across the   582
UK have aimed primarily to address two key factors: percentage of the pop-   583
ulation covered and geographic representation, which includes both urban and   584
rural area coverage. In addition, the sampling strategies have needed to allow   585
for an agile sampling response to assist with surveillance of COVID-19 inci-   586
dence clusters, as highlighted by governmental public health testing strategies.   587
For sampling at STWs, these factors need to be facilitated by the regionally di-   588
verse privately and publicly owned sewage networks. Uncertainties arise in the   589
actual population represented by the sampling strategies due to a mismatch be-   590
tween census administration geographies and population equivalents calculated   591
for STWs. These may include estimates of the number of actual residents within   592
a STW catchment, transient populations (i.e. those at workplaces, educational   593
facilities, or communal gatherings such as sports or entertainment events), and   594
the load placed upon each STW by industrial activity. Spatial data analysis   595
approaches can be used to characterise the contribution of STW catchments to   596
administrative geographies, which enable greater integration with public health   597
case data [79].   598

For sampling at STWs, the use of composite samples can help mitigate un-   599
certainty associated with diurnal flow variations (see Figure 3) but such samples   600
may underestimate the magnitude of the peak concentration and, therefore, are   601

18

more suited to understanding the average daily load within the sewer network 602
catchment. Consideration of the ideal sampling site at each STW needs to ac- 603
count for the specific configuration of the inlet channels, equalisation storage, 604
and mixing characteristics. In many cases, it is not possible to get a well-mixed 605
sample with equal representation of all parts of the sewer catchment because 606
of the design of the STW inlet piping. Several studies have sampled primary 607
sewage sludge for SARS-CoV-2, with generally higher detection than from liquid 608
influent samples [80, 81], although data on STW flows and process operation 609
dynamics is required to fully characterise the period of time that each sam- 610
ple would represent. Sampling of solids has not been extensively performed in 611
the UK. Mixed or combined sewerage systems (e.g. those receiving stormwater 612
or industrial effluent) can also have a significant impact on sampling perfor- 613
mance as dilution from additional flow and a more complex, or inhibitory, mix 614
of wastewater constituents may obfuscate the ability to detect the signal (See 615
Case Study 1 and Figure S4). 616

For network and near-source sampling, the large size and spatiotemporal 617
complexity of urban water networks means that it is not economically or lo- 618
gistically feasible to collect a sufficient number of samples to ensure statistical 619
significance of sampling results for estimating system wide average concentra- 620
tions [82]. Consideration of the diurnal variation of flows from both domestic 621
and industrial sources and impact of rainfall can help to select sampling locations 622
that are less vulnerable to influence by these factors. Well-calibrated hydraulic 623
models of the sewer networks can be a useful tool to understand dry weather 624
and wet weather dynamics. For example, Figure 6 illustrates the modelled dry 625
weather contribution to wastewater flow for one of the core cities sampled by the 626
surveillance programme in England, showing that some locations are dominated 627
by infiltration flows with less than 40% of total daily flow derived from domestic 628
wastewater. Many of the network sites initially sampled in the UK consistently 629
showed non-detectable levels of SARS-CoV-2 and ammonia, consistent with the 630
model results, and these locations were subsequently removed from the sampling 631
programme. Grab samples in sewer networks require precise timing to capture 632
flows because many manholes are dry for large portions of the day, including 633
near-source locations and upstream ends of the network. Many individual grab 634
samples from network locations had non-detectable levels of SARS-CoV-2 in 635
the core cities (see Figure S5). Sampling these locations daily, ideally with a 636
slight variation in the time of sample collection, does not mitigate the underly- 637
ing uncertainty associated with grab sampling but can assist with visualisation 638
of trends and patterns despite the variability in individual sample results. 639

Case studies from the UK Wastewater Surveillance Programmes 640

Case study 1: Flow normalisation 641

Several approaches have been developed within the UK wastewater surveil- 642
lance programmes to account for rainfall dilution of SARS-CoV-2 RNA mea- 643
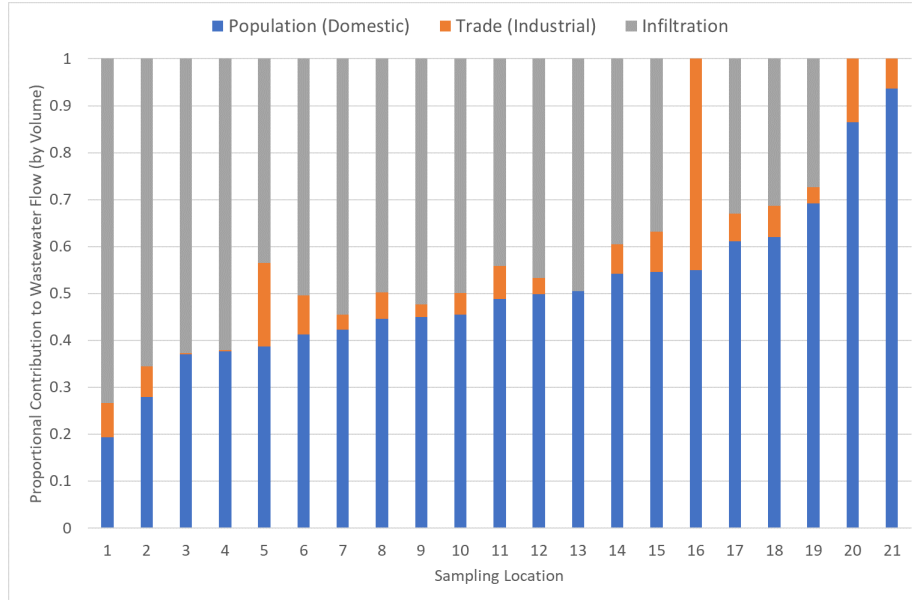surements in wastewater. Given that flow data is only partially available across 644

Figure 6: Contribution to wastewater flow by volume over a 24-hour dry weather day for sampling locations across one of the core cities, derived from the hydraulic model of the sewer network.

all the monitored sites, indirect normalisation techniques using other biochem- 645
ical markers as a proxy for the real flow can be used, with an assumption that 646
the majority of markers originated from a source with a constant load. Note 647
that these corrections account for the temporal variability, but are not suffi- 648
cient to estimate the average flow level, which can vary significantly between 649
sites (as shown in the population normalisation section). Different techniques, 650
based on a similar premise, are presented below for flow variability correction 651
in (a) Scotland and (b) England. Dilution effects appear to only have a minor 652
impact on SARS-CoV-2 concentrations in wastewater, with significant changes 653
occurring only during heavy rainfall events or discharges from other sources. 654
That is, variation due to dilution effects with sample-by-sample variability from 655
other sources of noise (e.g. faecal shedding), appears to be minimal. However, 656
from an epidemiological perspective, highly diluted measurements caused by 657
storm events, for example, may result in the need to correct values by factors as 658
much as 0.6 (results not shown), which would significantly skew interpretation 659
of disease prevalence if ignored when interpreting the data. 660

a) Flow normalisation, as applied by the Scottish COVID-19 surveillance pro- 661
gramme 662

In Scotland, in addition to detecting and quantifying SARS-CoV-2, chemical 663
analytes, in particular ammonia, have been collected and processed from the 664
wastewater. These are available up to two weeks prior to flow measurements - 665

20

with flow, at some sites, not measured at all. As a result, a cross-site model is used to relate ammonia concentrations with flow measurements, taking into account population size as a proxy for faecal shedding in the catchments.

A linear mixed model (LMM), with flow related to ammonia and population (on log10 scales), was developed and random intercepts and slopes were included for each site. This model was shown to fit the Scottish data better than a simpler linear regression model with the slopes for log10(ammonia concentration) and for log10(population) fixed at -1 and +1, respectively. Model performances were compared using Akaike Information Criterion (AIC) and a Kenward-Roger approximation of the Wald test for LMMs. A review of the Scottish data at each site, using a generalised additive model (GAM) with the Tweedie distribution, showed that unnormalised data was equally or more noisy than normalised (but scaled) data once trends were taken into account. A graph of example sites with fitted ammonia/flow curves is shown in Figure S7.

Current practice in Scotland is to normalise by flow rate if available, then ammonia concentration. If neither are available, then an estimate of flow based on a spline function using recent ammonia trends is used (fitted on overall national trends over time plus site specific effects). If 'capping' is an issue, where CSOs prevent sewer overloading by discharging to natural water bodies, then normalising against ammonia would be preferential as a more representative measure of true flow. Anecdotally, it is not thought that capping is a major issue in Scottish wastewater networks, based on communication with water sector professionals.

b) Flow normalisation, as applied by the English COVID-19 surveillance programme

The approach assumes that the flow F_t at time t is not directly observable. Therefore, information about the flow can be obtained by observing the correlation of concentrations ρ_{ti} of different markers i and that a dilution estimate based on a single marker is not robust enough as it is not possible to distinguish between a decrease in flow and an increase in marker load, e.g. due to a one-off industrial or agricultural discharge. The model assumes

$$\log F_t \sim \text{Normal}\left(0, \lambda^2\right)$$
$$\log x_{ti} \sim \text{Normal}\left(\mu_i, \sigma_i^2\right)$$
$$\therefore \log \rho_{ti} = \log x_{ti} - \log F_t,$$

where λ^2 is the flow variance, μ_i and σ_i^2 are the mean and variance of the load of marker i (all in log space). $\langle \log F_t \rangle$ is fixed at 0 to identify the model.

Accounting for variable dilution using multiple markers relies on the same basic premise as the approach presented for Scotland's case study, although with three key differences: first, using multiple markers (such as ammoniacal nitrogen and orthophosphate) jointly to estimate flow variability improves the accuracy of estimates. It also allows us to identify outliers (such as one-off discharges),

21

and estimate flow variability as long as at least one marker is quantified (al- 704
though with larger error bars). If no marker is quantified, the model predicts 705
average flows with substantial error bars. Secondly, rather than assuming total 706
marker loads are constant, they are assumed to be constant *in expectation*. In 707
other words, natural variability of biomarker loads is accounted for. This al- 708
lows to assign variable importance to different markers in a data-driven fashion. 709
For example, crAssphage gene copy concentrations exhibit more natural vari- 710
ability than ammonia-nitrogen concentrations, and more importance should be 711
assigned to the latter – although both can inform our dilution estimates. Fi- 712
nally, a generative modelling approach is used to test hypotheses *in silico*, and 713
any inferences in the form of posterior distributions over parameter values in- 714
clude principled estimates of uncertainty. The model also handles missing data 715
gracefully and can incorporate limits of detection where appropriate (not further 716
considered here). Unfortunately, the model needs to be fit whenever new data 717
become available, and it is more computationally expensive than other meth- 718
ods. Any combination of two or more markers can be used to estimate flows 719
using the multi-marker method provided that their total loads are constant in 720
expectation. An example of the correction for an English STW is presented on 721
Figure 7. 722
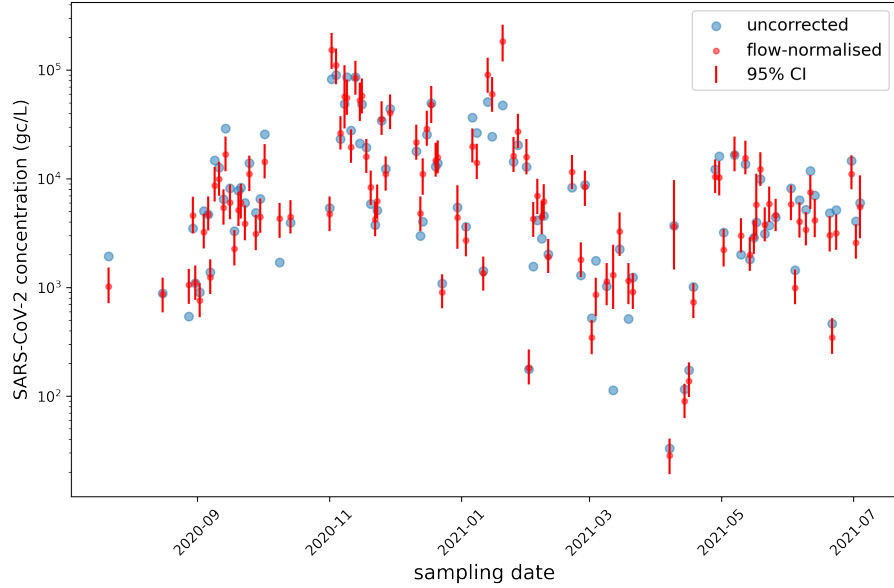
Figure 7: SARS-CoV-2 concentration (gc/L) over time with flow variability correction: ex-
ample with Bolton sewage treatment works. Blue points represent the original uncorrected
SARS-CoV-2 concentrations and red points and associated 95% confidence intervals are the
estimates after accounting for flow variability.

22

Case study 2: Handling data anomalies

Identifying and reconciling anomalously low measurements in England

As discussed, the devolved administration programmes for COVID-19 wastewater surveillance have generated a large number of SARS-CoV-2 virus RNA measurements. As with all environmental measurements, the signal recovered will be subject to anomalies, or outliers, that diverge from the expected data trends, with some defined statistical significance. Measurements can vary by several orders of magnitude, with extrema possibly representing unaccountable occurrences such as ' 'super-spreader' events, single release of highly concentrated sewage (e.g. transported from non-networked sites, in-network holding tanks or wet wells), or due to sample capture of a highly aggregated, unmixed load. Alternatively, anomalies may represent measurement error or uncertainty due, for example, to inappropriate sampling frequency, miscalculation or unknown peak flow (for grab samples), or sample/laboratory contamination. Such data anomalies can cause many problems for further analysis or visualisation, and depending on context, different interventions are typically needed when they are detected.

In the English programme, post-laboratory analyses were conducted to attempt to identify measurements that may be anomalously low, by defining the likelihood that a measurement falls within some expectation criteria. In particular, a machine learning approach, using a Gradient Boosting for regression model, was trained with a quantile loss function to predict 90% SARS-CoV-2 concentration intervals at the sampled sites. These predictions were used to explore unexpectedly low data points (below the 5th percentile prediction interval) where similar sites in terms of geography and collection method exhibited relatively high measurements.

The analysis identified 762 samples as anomalous out of 25,957 that did not report a quantified value. In particular, the model highlighted low measurements during January and February 2021 despite infection rates across the country were high. The analysis could be extended to explore any recorded values that do not fall within the predicted range, whether low or high. Figure 8 illustrates the frequency of anomalous data points when compared to ammonia concentrations, suggesting that lower concentrations of ammonia are associated with a higher proportion of unquantifiable samples. This suggests that flow dilution has the impact of reducing SARS-CoV-2 concentrations below the 5th percentile prediction interval.

Tracking measurement outliers in England

On a weekly basis, sites with rapid and sudden increase of SARS-CoV-2 are identified using parametric confidence bands around a linear regression model fit to the data. The model is used to predict the SARS-CoV-2 concentrations 7-days in advance and a 80% confidence band is calculated for this extended linear regression. This accounts for the uncertainty of the mean virus RNA concentrations over time. As new data is acquired, if the latest measurement falls outside the upper limit it indicates that the sample has exceeded the predicted concentration and needs further investigation. Outliers identified with
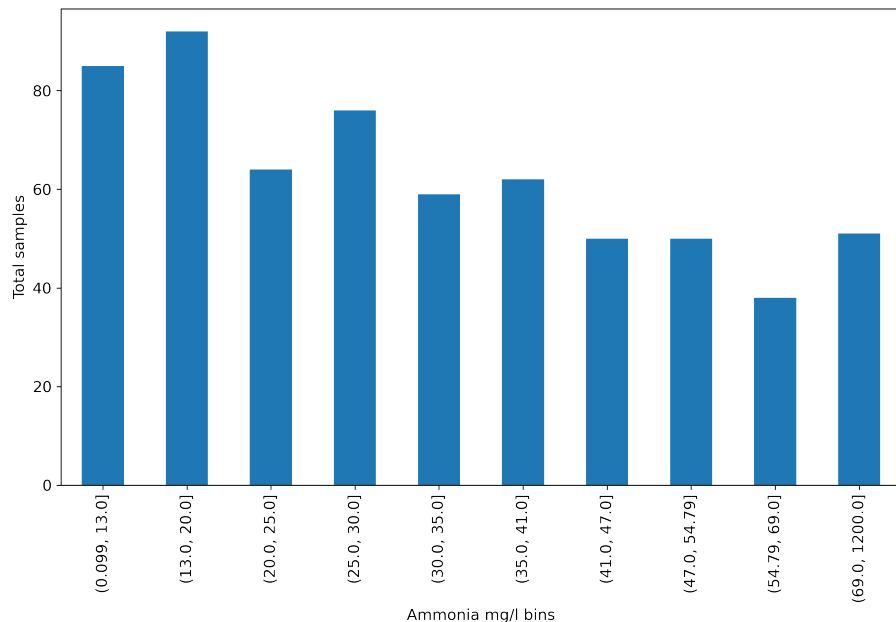
23

Figure 8: Frequency of anomalous SARS-CoV-2 RNA concentrations, identified using a gradient boosted regression and binned by the associated ammonia concentration. The data suggests that lower ammonia concentration and, by proxy, higher dilution, results in lower confidence in detection of viral RNA.

this method are visualised on a map of England and assessed alongside appropriate meta-data, such as the inorganics (e.g., ammonia, orthophosphate), see Figure S8. Weekly maximums that lie above pre-defined threshold values are also flagged as outliers. After following this process, sites of concern are reported to the National Laboratory Service (NLS) who conducts further quality assurance.

Identifying and reconciling anomalously high measurements in Scotland

Under the Scottish programme, Biomathematics and Statistics Scotland (BioSS) conducted a similar procedure, though instead the focus was on anomalously high values (e.g. spikes), with the aim of flagging and potentially removing anomalies as soon as they are recorded. A Generalised Additive Model (GAM) was used to identify when high amounts of wastewater COVID-19 (relative to case rates, or relative to the previous variability of the site) is indicative of the wastewater measure not corresponding to future cases. With a suitable threshold, this was used to remove these measurements from aggregates, and/or trigger further investigation [83].

24

Case Study 3: Uncertainty arising from laboratory analysis of SARS-CoV-2, 784
and its mitigation within the UK wastewater surveillance programme 785

The analytical variability, in terms of both replicability and reproducibility), 786
for the estimation of SARS-CoV-2 in wastewater has been a major focus of the 787
UK wastewater surveillance programmes. In England, the use of two main lab- 788
oratories (required due to the need for high throughput analysis capacity) pro- 789
vided significant challenges, but also opportunities to assess the reproducibility 790
of sample analysis. A single protocol was adopted at both sites, and dupli- 791
cate samples were analysed and found to be comparable. In addition, a short 792
inter-laboratory ring trial was carried out involving these laboratories plus two 793
others in Northern Ireland and Scotland that used different methods (Walker 794
et al., 2021, unpublished). Significant differences were found in the absolute 795
SARS-CoV-2 concentrations measured by all laboratories. However, these dif- 796
ferences (less than one log between labs) were much lower than reported in other 797
ring trials [84]. Further, the variability between the laboratories was similar to 798
previous inter-laboratory trials for quantifying viruses (e.g. Norovirus, Hepati- 799
tis A) in shellfish [85]. The differences in the SARS-CoV-2 recovery between 800
laboratories is likely due in part to the differences in the initial virus concentra- 801
tion method (e.g. ultrafiltration versus ammonium sulphate precipitation) and 802
the use of different RT-qPCR standards. The UK is now leading the develop- 803
ment of an ISO standard for quantifying SARS-CoV-2 in wastewater to allow 804
greater comparability and traceability in data generated, both within the UK 805
and internationally. 806

The efficiency of downstream applications depends strongly on the purity 807
of the RNA sample used. In this regard, MIQE guidelines stipulate that a 808
measurement of a nucleic acid quantity is essential, while an assessment of purity 809
is desirable [86]. This is particularly important to avoid false negatives when 810
SARS-CoV-2 concentrations are too low to be quantified after dilution, requiring 811
the use of internal controls, such as RNA/DNA spikes, to detect inhibitors and 812
verify several other parameters of the workflow (See Figure S9). Furthermore, 813
the effect of wastewater properties has been assessed in a range of mesocosm- 814
based wastewater studies. These found that the presence of suspended solids 815
(turbidity range 10 - 400 NTU) or surfactants (0 - 100 mg/l) had minimal impact 816
on RNA recovery using PEG or ammonium sulphate precipitation methods 817
unless present at very high concentrations atypical of UK wastewater. 818

RT-qPCR can introduce additional variability at different steps during the 819
quantification of SARS-CoV-2. Firstly, the reverse transcription can vary with 820
the same samples by two to threefold depending on the amount and quality of 821
RNA [87]. On the other hand, sample variability increases when the target com- 822
plementary DNA (cDNA) is diluted, mainly when the quantitative cycle (CQ) 823
values are greater than 30. This is due to stochastic amplification, measurement 824
uncertainty, and subsampling error [88]. The RT-qPCR variability can easily 825
range between 10% to 200% of the coefficient of variation (CV) and can only 826
be minimised by interrogating a larger proportion of the sample using more 827
technical replicates and applying the average CQ [88]. Figure S10 shows the 828

25

variability of SARS-CoV 2 measurements in wastewater at different CQ values 829
from an England pilot study. 830

Case Study 4: Population normalisation and measurement correction: lessons 831
learned from WBE application in exposure studies beyond COVID 832

A study of multi-group chemical profiling in five contrasting urban popu- 833
lations, each served by a major STW contributing to one river catchment in 834
South-West England and covering an area of approximately 2000 km^2 and a 835
population of approximately 1.5 million (this constitutes >75% of the overall 836
population in the catchment) was undertaken to understand measurement vari- 837
ability at an inter-city granularity (See Figure S6 for a map of the five study 838
locations, and Table S1 for data on their network characteristics). A detailed 839
discussion of multi-chemical fluxes in urban catchments has been provided by 840
Proctor *et al.* [89] and the methodology used to measure chemicals and back- 841
calculate mass loads and intake are found in recent literature [90, 10]. Key 842
contributing factors to WBE uncertainties are carefully considered and included 843
in the study to enable a fully quantitative measurement of city-wide intake for 844
selected chemicals: 845

- Robust sampling and sample collection involving 24-hour flow proportional 846
  sampling in ice packed or refrigerated autosamplers maintaining biomarker 847
  stability; 848

- 7-day consecutive sampling to allow for temporal (weekday versus week- 849
  end) changes in biomarkers to be observed; 850

- Robust wastewater flow measurement and population size estimates; 851

- Fully validated analytical methods and the highest level of quality as- 852
  surance (e.g. limits of detection and quantification, intra- and inter-day 853
  accuracy and precision, recovery from matrix); 854

- Characteristic biomarker selection for back-calculation of chemical expo- 855
  sure (e.g. metabolite versus parent compound to account for direct dis- 856
  posal of unused chemicals); 857

- Full biomarker mass balance in wastewater that accounts for biomarker 858
  presence in both solid and liquid phases with a full understanding of per- 859
  centage biomarker recovered from the matrix. 860

The aim of the study is to understand and characterise key uncertainties to 861
enable accurate back-calculation of city-wide exposure to chemicals. To validate 862
the developed back-calculation protocol, high-resolution spatiotemporal NHS 863
pharmaceutical prescription databases are used for system calibration, in terms 864
of biomarker selection and its correction factor, as well as for overall spatiotem- 865
poral system performance evaluation. A detailed discussion on multi-chemical 866
exposure can be found in [10]. Here, focus is only given to carbamazepine and 867
citalopram, two model chemicals, and two key variabilities for back-calculation 868

26

of their usage at an inter-city level (that are not currently considered for UK SARS-CoV-2 monitoring): characteristic endogenous biomarker selection and establishment of correction factors accounting for human metabolism.

Carbamazepine intake (Figure 9: red line) is back-calculated using both parent compound (source carbamazepine) and its metabolite (carbamazepine-10,11-epoxide, CBZ10-11). While both biomarkers correlate well with NHS prescription data (Figure 9: blue line), using carbamazepine as a biomarker might lead to an overestimation of intake if direct disposal of unused carbamazepine takes place (see city A, Sunday, Figure 9). Interestingly, this is not the case if CBZ10-11 is used (no spike in city A during Sunday), which indicates its superiority over carbamazepine itself.

27
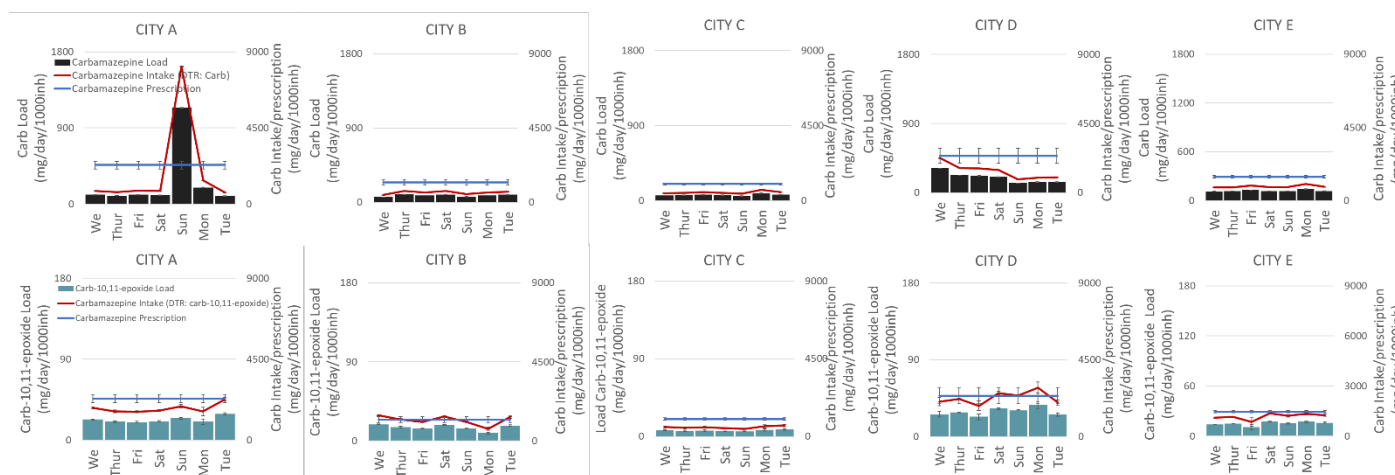
Figure 9: Comparison of carbamazepine daily loads, intake (calculated using both carbamazepine and carbamazepine-10,11-epoxide) and prescribed carbamazepine in five cities over a 7-day sampling week.

28

An understanding of the extent of metabolism of biomarkers or metabolic formation of biomarkers is key in quantitative back-calculation of chemical intake. Figure 10 shows an example of a significant overestimation of citalopram intake observed when using commonly applied weighted average correction factors based on the existing literature. This often include only phase I metabolism of chemical excreted in urine (desmethylcitalopram in this case), as opposed to the focused approach, where metabolism correction factors (mCFs) are calculated using only comprehensive datasets from studies combining phase I and II metabolites (glucuronides) excreted in both urine and faeces. Understanding biomarker excretion in faeces is of critical importance for compounds with a more hydrophobic nature, such as citalopram as it is, to a large extent, excreted in faeces. Additionally, citalopram and its metabolites undergo extensive glucuronide conjugation. Overlooking excretion in faeces and phase II metabolism will lead to incorrect CFs as seen in Figure 10. Having prescription data per 10 - 100 households/postcodes allows for the validation of the correction factors used. Prescription databases (if associated with well-defined regional units such as streets) can therefore serve as internal calibration systems.
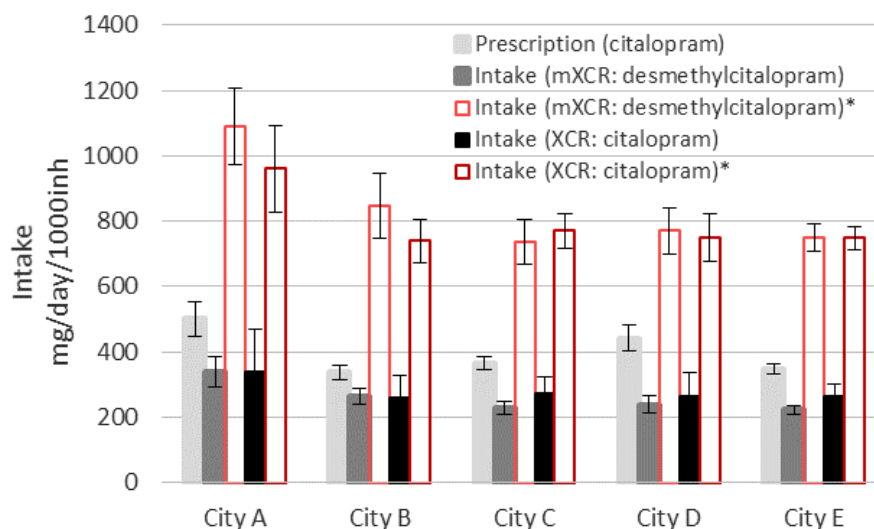
Figure 10: Citalopram intake calculated using citalopram and desmethyl-citalopram, with and without inclusion of phase II metabolites (Note: (*) indicates no inclusion of phase II metabolism, which leads to overestimation of intake).

This case study shows the importance of careful biomarker selection to enable highly accurate 'quantitative' calculation of per capita stressor intake. This is not currently performed with SARS-CoV-2 surveillance, where the stressor itself is used as a biomarker. As a result, various sources of the genetic material present in the wastewater sample are accounted for and, hence, calculation of

the per capita intake (or viral load) is not possible. Further work is required to establish a biomarker suite enabling quantitative measurement of SARS-CoV-2. In the interim, it is likely that WBE can only be used as an early warning system for SARS-CoV-2 surveillance and verification of disease prevalence trends at the community level, and not as a quantitative measure of community infection rates.

Conclusions

The scale of the COVID-19 pandemic has resulted in an unparalleled response from a diverse community of stakeholders, working collaboratively to control and reduce the transmission and impact of the disease. The early demonstration that wastewater was a viable medium for tracking the virus, led to academic and government initiatives to operationalise wastewater-based epidemiology for monitoring its dynamics at local, regional, and national scales. In the UK, COVID-19 surveillance programmes across the four nations (England, Wales, Scotland, and Northern Ireland), have demonstrated, perhaps uniquely, the opportunity for WBE to be used routinely and at unprecedented scale to combat a public health emergency. From their inception, the national wastewater surveillance programmes have delivered insights to support public health decision-making and to guide Government and key stakeholders in interpreting the measurements of SARS-CoV-2 in wastewater to provide a broader understanding of the disease in the populations.

This work has allowed for a broader appreciation of WBE as a tool for monitoring public health in populations at scale, with initiatives likely to focus on a 'beyond COVID' uplift of WBE as part of establishing One Health programmes across the world. However, their effectiveness requires that the data generated to support the function of WBE is meaningful and representative of the target(s) being monitored. Wastewater is a more complex environment than typical media used for monitoring of human health, with multiple factors potentially accounting for greater uncertainty or variability in the measured signal that in, for example, a clinical setting. Managing this uncertainty is one of the key challenges to ensure successful employment of WBE for public health protection.

Here perspectives are provided on the confidence in wastewater-derived measurements by those working across the national programmes, given work performed to understand, quantify and manage measurement uncertainty and variability. The work emphasises that while some sources of uncertainty may not be impactful, or can be adequately accounted for (e.g. extraneous flow dilution, sampling method), other sources are inconsistent or difficult to quantify directly (e.g. shedding distributions, in-network behaviour). While these intractable factors will, with consolidated research efforts, become less opaque, there is unlikely to be a general approach to manage measurement uncertainty for all applications of WBE beyond COVID. Making use of the greatly increased capacity for WBE in the UK, and more widely, will require new methods for

extracting actionable information from wastewater data, but also methods for determining the limits of its application.

Acknowledgments

Disclaimer

Joint Biosecurity Centre [MJW, ALJ, MRB, JTB, JG, TH, CJL, MM, CSo, CSw]: The views expressed in this paper are those of the authors and do not necessarily reflect the views or policies of the Department of Health and Social Care.

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Supporting Information

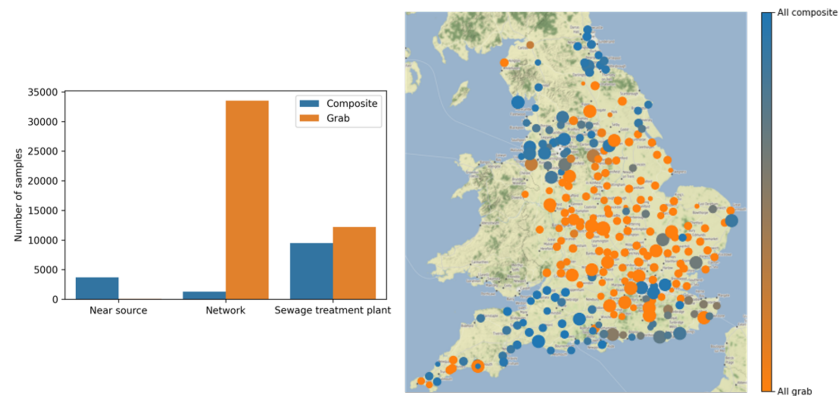*Views from the UK wastewater surveillance programmes*

Figure S1: A view of all wastewater sampling sites in England as of July 2021, indicating relative number of grab versus composite samples across the three site location types (near-source, in-network, and STW).
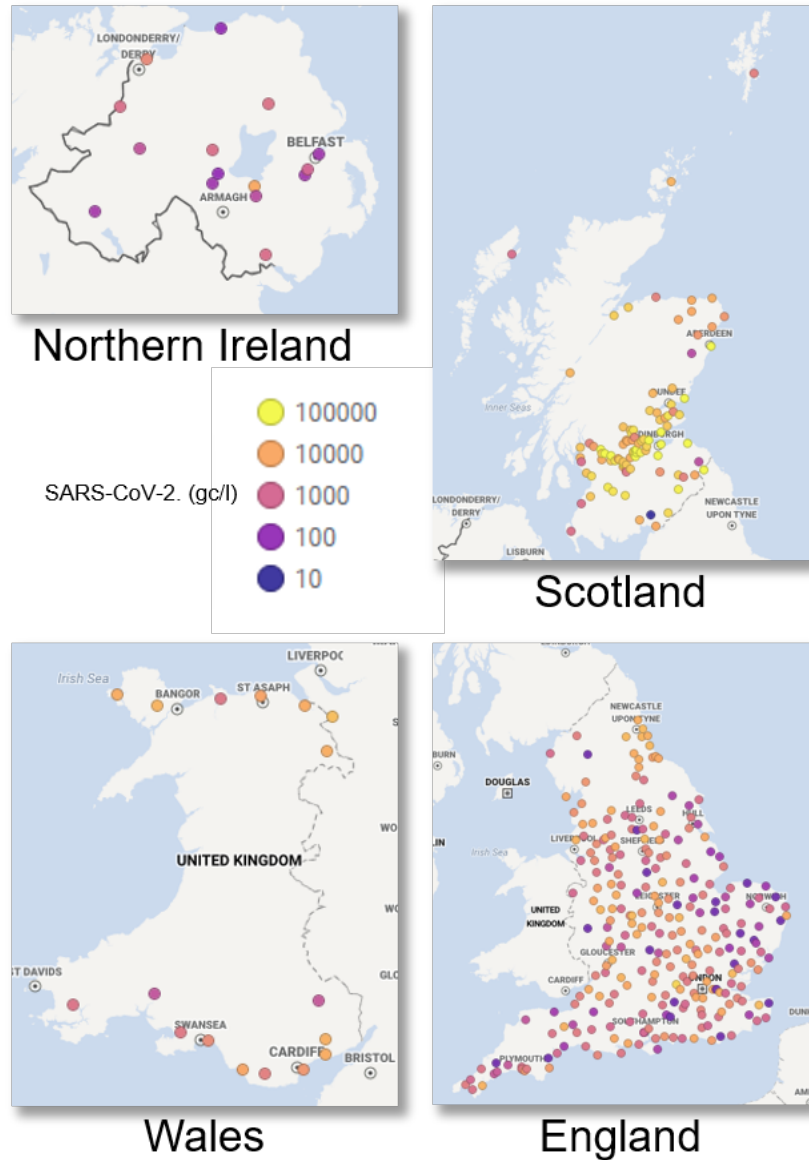
Figure S2: Maps of the four regions of the United Kingdom showing the wastewater sampling locations for the respective national COVID-19 surveillance programmes (as of July 2021). Markers represent centroids of the catchments serving the sample point and shading is the 7-day average SARS-CoV-2 RNA concentration (gene copy per litre) measurements at each site over the last week of June 2021. This is only an example of the spatial distribution of sampling in the UK and comparisons of concentrations between sites should not be made from these figures due to differences in sampling frequency and network characteristics across locations.

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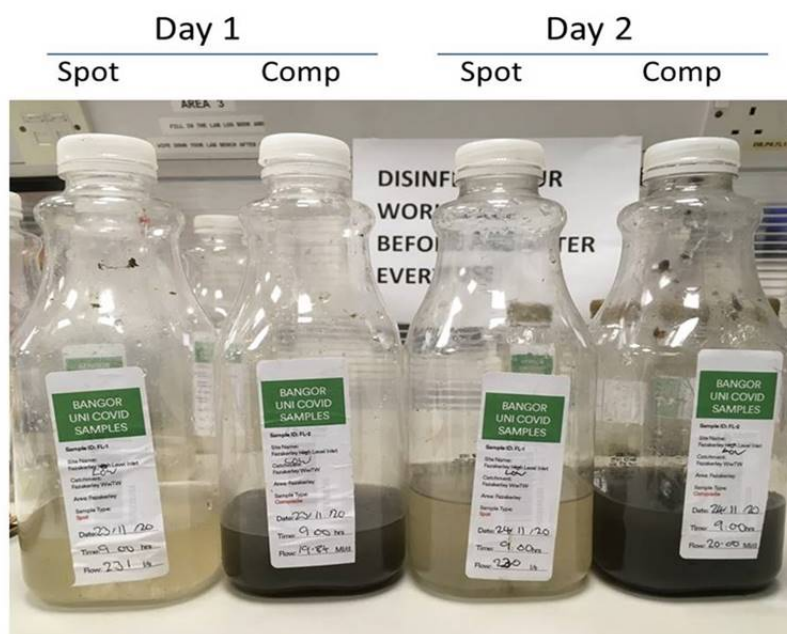
Figure S3: A visual representation of wastewater concentration variability between sampling methods (spot = grab, comp = composite) at a STW site in England. The samples were collected at the same time (09:00 h) on consecutive days for laboratory analysis at Bangor University. The darker the sample, the higher the likelihood of capturing a representative sample, while lighter samples suggest greater flow dilution, or that the sample has missed the peak discharge window.
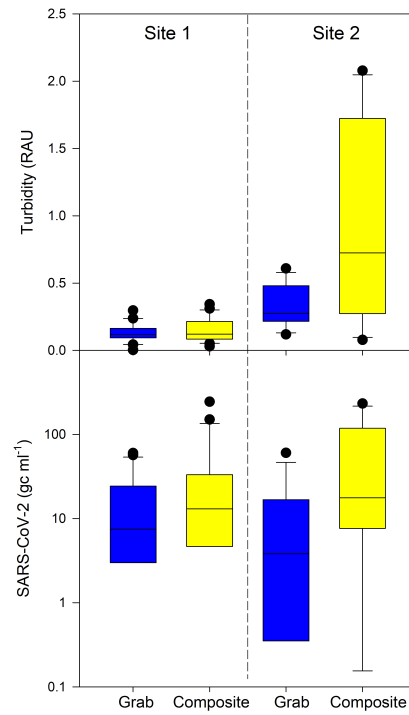
Figure S4: Comparison of grab and composite samples taken from two sites as part of the English wastewater surveillance programme: Site 1 - Domestic source; Site 2 - Domestic + Industrial sources. The boxplots suggest a greater degree of within sample and between-method variability for Site 2 than Site 1, suggesting that combined sewerage systems (i.e. those receiving stormwater or industrial inflow in addition to domestic flow) may impart greater signal variability . Additionally, the lower SARS-CoV-2 measurements for grabs at Site 2, implies that autosampling is more likely to capture the target analyte signal in complex or dilute media.
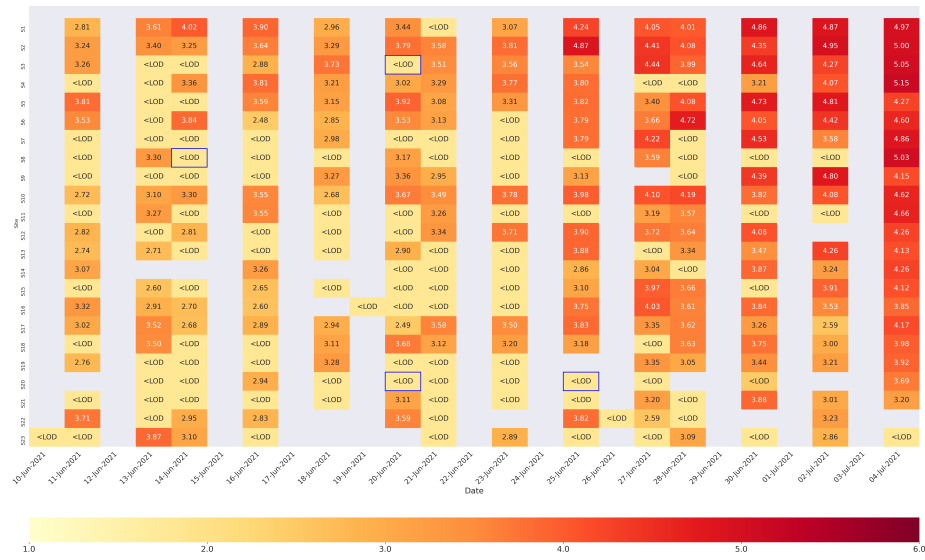
Figure S5: An anonymised heatmap view of 'core city' SARS-CoV-2 RNA concentrations measured in wastewater over a one month period from June - July 2021. Each row is an in-network sample location in the city and each column represents a sample day. Missing values represent a missing sample or no sample taken. Values are the log10 virus RNA concentrations (gene copies per litre). Cells with blue borders are flagged as likely being influenced by high dilution events, and < LOD are measurements below the laboratory limit of detection.
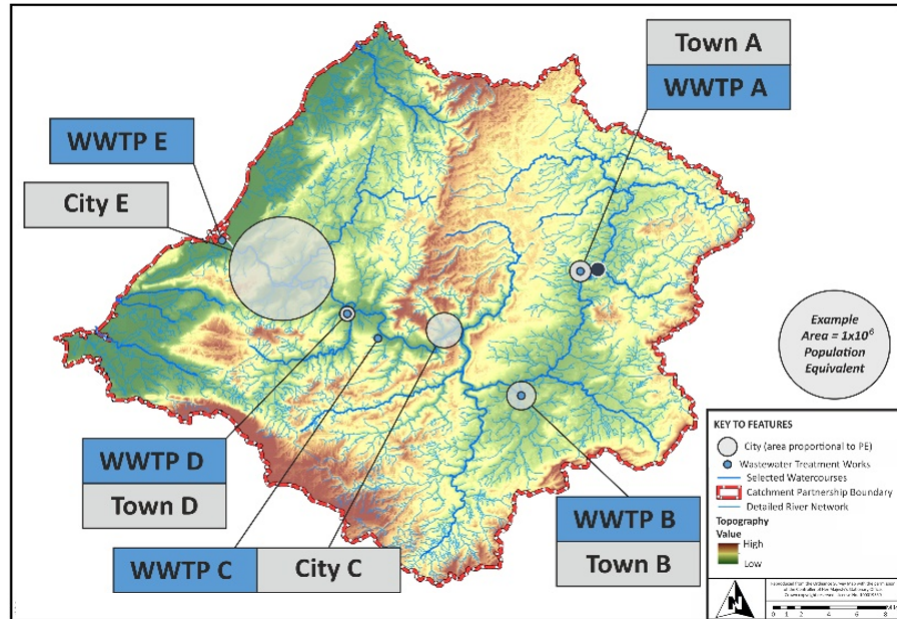
Figure S6: Site locations of studied STWs and corresponding cities and towns.

Table S1: Network characteristics for the studied sites. Residence times are given for typical summer dry-weather flows. P.E. = Population Equivalent.

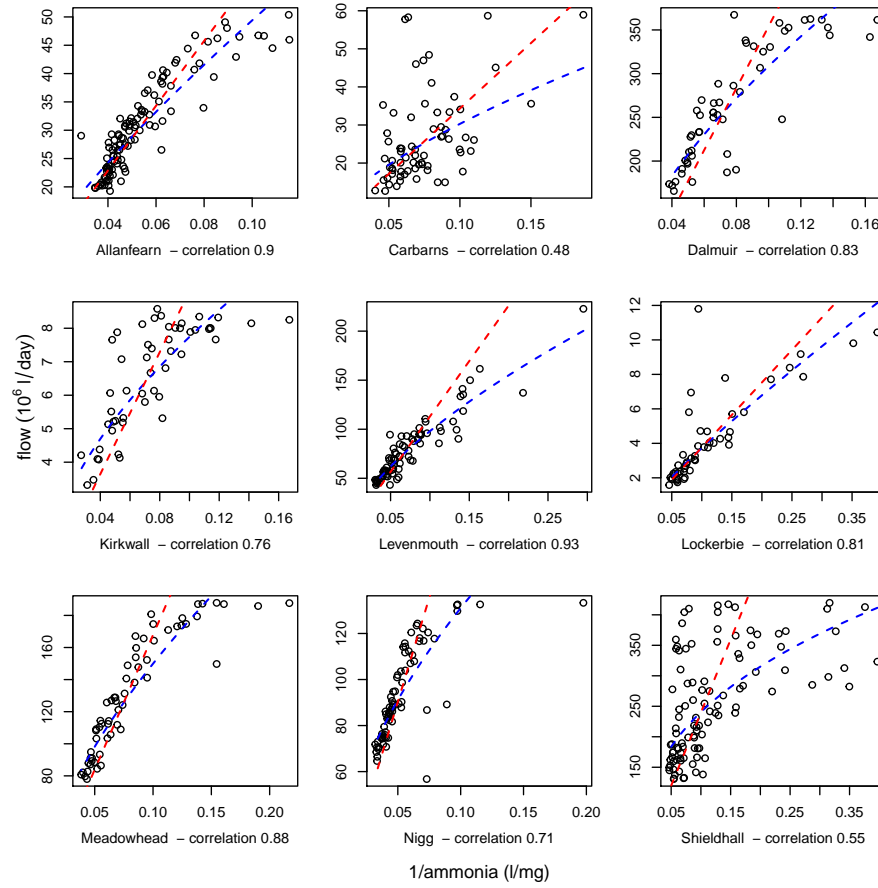| Site	Sewer residence time (h)	Popn. served (P.E.)	Ind. contrib. to P.E.	Mean flow rate (m^3 d^{-1})
A	<0.5 - 4	37,714	0.4 %	8,242 ± 3,085
B	< 0.5 - 4	68.453	30.0 %	11,202 ± 3,202
C	< 0.5 - 9	109,543	1.2 %	24,875 ± 2,167
D	< 0.5 - 2	18,274	0.1 %	2,924 ± 199
E	< 1 - 24	867,244	23.9 %	153,061 ± 12,245

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Figure S7: Relationship between flow rate and 1/ammonia concentration at Scottish wastewater sites with more than 40 coupled observations (up to 25 May 2021). The lines show the fitted regression estimates: blue is for the full random coefficient model and red is for the model with the slope for log ammonia fixed at -1. The strength of the relationship varies between sites, as shown by the correlations given. At some sites (e.g. Lockerbie), the fitted lines are quite close, and in other cases (e.g. Shieldhall), the difference is more marked.
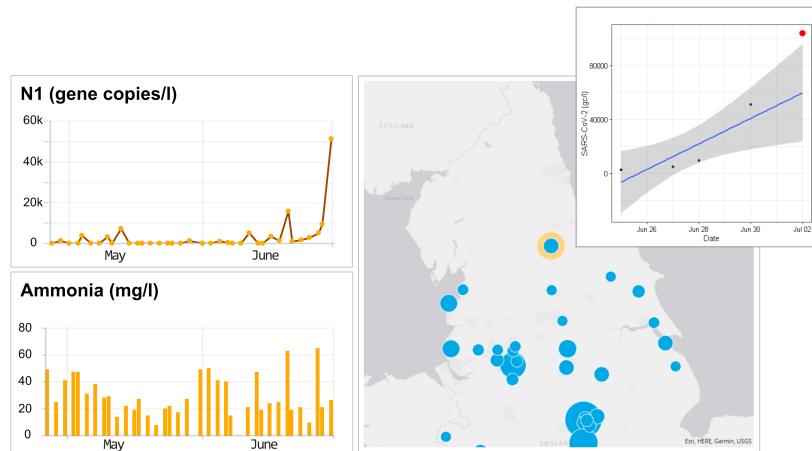
Figure S8: An example output from a tool developed by the Environment Agency to visualise outliers in SARS-CoV-2 time-series measurements collected as part of the wastewater surveillance programme in England. Inset: Outlier detection using an 80% confidence interval around the linear regression; four weekly samples (black points) are used to generate a linear model and parametric confidence interval, a new sample (red point) is assessed against the confidence band and flagged as an outlier if it falls outside the band. This map was created using ArcGIS® software by Esri. ArcGIS® and ArcMap™ are the intellectual property of Esri and are used herein under license. Copyright © Esri and its licensors. All rights reserved.
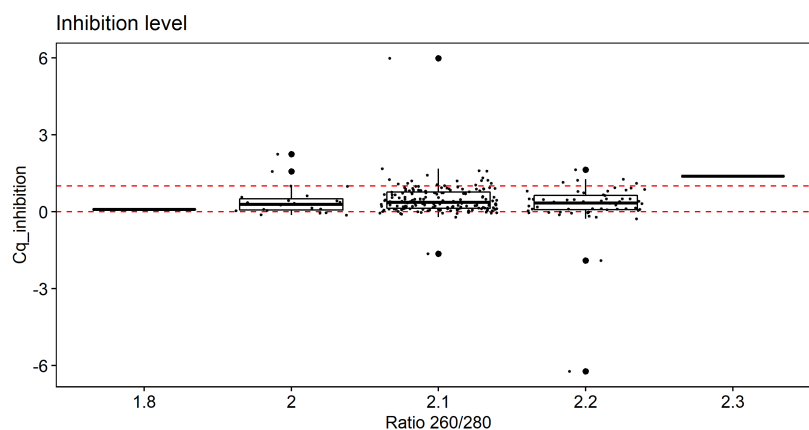
Figure S9: Inhibition level of clean samples spiked with synthetic single-stranded RNA (ss-RNA). The inhibition level was calculated by spiking ssRNA into wastewater extracts and comparing the measured Cq to RNA spiked into molecular negatives (no template controls). The modified PEG method keep the inhibition level below 1.0 Cq and the RNA quality between 2.0 and 2.2.
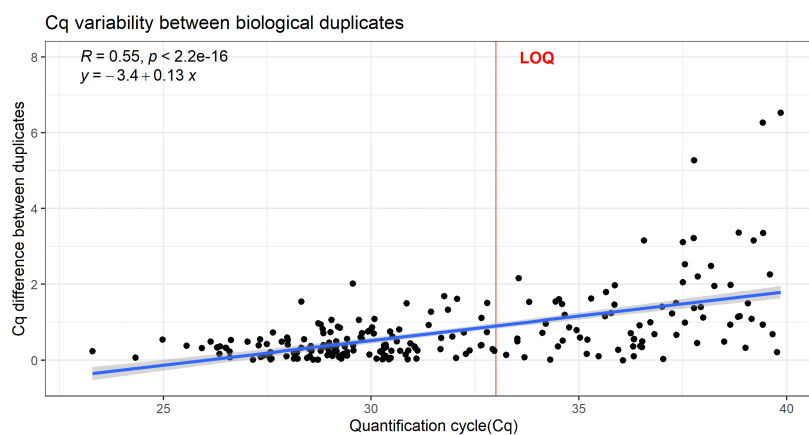

Figure S10: SARS-CoV-2 N1 gene variability between biological duplicates. The Cq variability increased with lower target concentrations (higher Cq). The CV was 1.0 ± 0.9 and 2.6 ± 2.3 for samples with Cq values below and above the LOQ, respectively.