Monitoring the presence and infection risk of SARS-CoV-2 in the environment: approaches, limitations and interpretation

By: Transmission of Covid-19 in the Wider Environment Group (TWEG), reporting to SAGE

Summary

- Environmental monitoring for SARS-CoV-2 has been undertaken to (1) provide epidemiological evidence on the extent of outbreaks, and (2) provide evidence on the hazard posed by contaminated surfaces, water and air.
- The main analytical approaches to quantify virus in environmental samples include molecular methods (e.g. qPCR) and cell culture. Molecular methods are particularly widely used due to their speed, relatively low cost and lower biosafety requirements.
- RNA is generally more stable in the environment than infectious virus. qPCR may therefore overestimate the presence of infectious virus and data need to be interpreted carefully.
- Where possible, risk assessment of transmission via water, surfaces, food or air should draw on studies using cell culture (to demonstrate the presence of infectious virus) and epidemiological evidence (demonstrating that infection has taken place).
- In the absence of such evidence, qPCR can provide useful evidence to support decision-making on transmission risk if key factors are taken into account (Box 1).
- Viral particles are often distributed heterogeneously in the environment so robust sampling design is crucial for any environmental sampling programme. Key considerations for sampling water, air and surfaces are set out in sections 3-5.

1 Introduction

This paper provides an overview of the key principles and approaches to monitor SARS-CoV-2 in the environment. It covers sampling from water (freshwater, wastewater and marine), air and surfaces (including surfaces of food), and discusses the strengths and limitations of key detection methods. It is intended as a primer for Government officials, managers of organisations and those without technical expertise in the subject who may be considering the need for assessing Covid-19 risk in a setting for which they are responsible. While it is not an in-depth review or a methods manual, it provides some guidance on how to interpret research results and environmental monitoring data. References to more detailed specialist literature are provided.

1.1 Objectives for environmental monitoring

Environmental monitoring of SARS-CoV-2 often has one of two main objectives:

1. To detect presence, infection trends and/or variants of the virus in a population, or
2. To inform the assessment of infection risk to people from contaminated water, air, objects or surfaces.

In either case, sampling can be undertaken either as part of a one-off study or through an ongoing programme of surveillance. Work to assess risk in a particular setting, for example, may not require ongoing monitoring once it has been established whether infectious virus can be present.

Detecting presence/ prevalence: Monitoring viral RNA in the environment can be used to complement clinical surveillance by providing information on the prevalence and spread of disease in a population. For example, wastewater monitoring is being used in the UK and several other countries to track outbreaks of Covid-19. Wastewater-based epidemiology can be used either at large scale (e.g. sampling at a sewage treatment works serving thousands of people), at sub-catchment scales, or at an institutional level (e.g. in a school or business).

Sampling air filters from enclosed spaces, including buildings or aircraft[1] could potentially be used in the same way but has not yet been proven useful in any reported cases for SARS-CoV-2, and samples pertain to individual buildings rather than providing data at a community or organisational level.

One important potential application of environmental surveillance is to track the prevalence of different viral variants and the emergence of new ones. Viral genome sequencing of wastewater samples in particular can be used to analyse the genetic diversity of variants circulating in a whole population[2]. Wastewater samples have the advantage that they could contain all the variants of SARS-CoV-2 circulating within a discrete population, including those from infected people who do not present clinical symptoms. This information might help track the geographical spread of the virus as the pandemic proceeds. It can also potentially help detect the emergence of new zoonoses or less pathogenic forms.

Assessing transmission risk: To assess whether there is an infection risk, environmental samples ideally need to demonstrate that the virus is infectious (viable) and present in adequate quantity for transmission to take place. In addition, an assessment needs to be made of whether people are likely to be exposed to an infectious dose in the particular location that has been monitored or sampled, or in the environment where the virus would have originated (e.g. virus in wastewater could indicate potential risk in the originating toilet environments). The approaches discussed in this paper can be used to build up evidence of the presence of infectious virus in the environment and potential infection hazard.

Most environmental monitoring of SARS-CoV-2 to date uses molecular methods to detect viral RNA. Detection of RNA alone, however, does not indicate the presence of infectious virus or a risk of infection. RNA is more stable in the environment than infectious virus, and there is no clear correlation between RNA abundance and infectiousness. Emerging data show that 90% of infectious SARS-CoV-2 in filtered fresh water is lost after approximately 2 days at 20°C whereas SARS-CoV-2 RNA is effectively stable under similar (laboratory) conditions (Wim Meijer, pers. comm.[3]).

Despite these limitations, it is feasible, with attention to a number of limitations, to use high counts of RNA (low C_t values) as evidence of a potential hazard. RNA is relatively easy to measure and must be present if infectious virus is present. The caveats to this approach are set out in section 2, Box 1.

Direct assessment of infectivity and transmission is more challenging. One approach to detect infectious SARS-CoV-2 is through cell culture in the laboratory. Studies pursuing this approach are currently few in number[4]. Even culture techniques, which may show that infectious virus is present, do not necessarily show that it is present at a sufficiently high dose to infect a person. Determining this infection risk requires knowledge of the minimum infectious dose of the virus, which for SARS-CoV-2, is currently not known with certainty. Ultimately, epidemiological evidence that people have actually been infected in that environment is the clearest indicator of risk of transmission, although attributing transmission to a specific environment with a high level of certainty is generally challenging (Figure 1).

Detection of viral RNA	Detection of infectious virus	Evidence of transmission
• **Approaches:** Molecular techniques (e.g. RT-qPCR). • **Advantages:** rapid, relatively cost effective, does not require cat 3 labs. qPCR can be used to monitor how much virus is circulating in the population • **Limitations:** Does not provide evidence on the presence of infective virus.	• **Approaches:** Microbiological methods (cell culture) • **Advantages:** Can detect presence of infective virus. • **Limitations:** Presence of infective virus does not mean that infection is necessarily possible through that route. Requires cat. 3 containment facilities.	• **Approaches:** Direct epidemiological evidence of transmission to humans. Studies showing infection of animal models. • **Advantages:** Shows that infection is possible. • **Limitations:** Can be difficult to identify exact route of infection (e.g. from surfaces, airborne droplets etc).

Figure 1. Advantages and limitations of approaches to detect viral presence, infectivity and transmission.

One reason that relatively few studies seek to measure infectious virus is due to the hazards such approaches pose. The virus is classed as a hazard group (HG) 3 pathogen by the Advisory Committee on Dangerous Pathogens (ACDP), which means any work involving growth or propagation of live virus requires the use of high containment biosafety level 3 (BSL3) laboratories with appropriate controls to reduce risk. Detection of RNA can be carried out using BSL2 laboratories which are more widely available and cost less to access.

2 Monitoring and enumeration of SARS-CoV-2

2.1 Steps in environmental monitoring

Detecting and enumerating a virus in the environment requires a number of steps (figure 2).

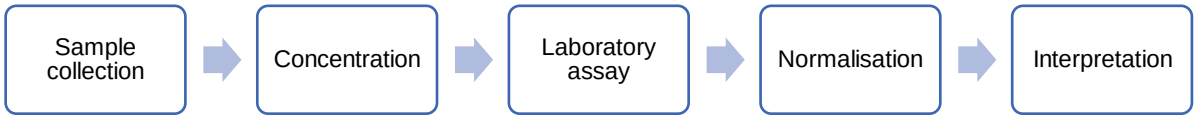

Figure 2: Steps in environmental monitoring for SARS-CoV-2 and other viruses.

Each step in this process presents challenges and multiple techniques have been developed to overcome them.

- **Sample collection:** Standardised protocols are needed to ensure that environmental samples are collected in a consistent way. Methods need to avoid the risk of sample contamination (e.g. from field staff undertaking sampling). Viruses may degrade rapidly so sample storage and transport needs to be carefully controlled – usually at low temperature. Storage before analysis should be minimised. Viruses can also be inactivated by the presence of chemicals including detergents and cleaning products. Vessels used to collect and transport samples therefore need to be free of such contaminants.
- **Concentration:** Whether in water, air or on surfaces, viral particles are likely to be extremely dilute and need to be concentrated before they can be detected. In molecular approaches, this can include a stage to concentrate the virus or its RNA (e.g. through filtration or precipitation), and an amplification stage (repeated replication of RNA to allow its detection).
- **Laboratory assays:** Molecular or cell culture approaches can be used, see section 2.2 below. Most measurements of SARS-CoV-2 in the environment use molecular methods.
- **Normalisation:** Various approaches can be applied to control for dilution (e.g. by rainwater in sewage systems) and sample degradation, including the use of chemical and biological population markers.

- **Interpretation:** Data must be analysed, contextualised, and interpreted. This needs to consider inherent variability in the detection and concertation of virus through the previous steps.

Laboratory assays are discussed in section 2.2 below. Other steps are discussed in sections 3, 4 and 5 with respect to water, air and surfaces (including surfaces of food).

2.2 Laboratory assays

Analysis of environmental samples for viruses can follow two broad approaches, detecting either (i) specific components of the virus (e.g. nucleic acid sequences, antigens or other complex polypeptides) or (ii) intact infectious virus particles. In the first, molecular methods such as Polymerase Chain Reaction (PCR) detect the presence of short unique sequences of RNA present in the genome of the target virus. Results are usually expressed as a cycle threshold (C_t) value or as a number of genome copies (GC) per unit of volume, mass or area (see section 2.2.1). In the second, intact (and infectious) virus can be detected by cell culture. In this case, virus levels are usually expressed as plaque-forming units (pfu) or tissue culture infectious doses ($TCID_{50}$), again commonly normalised by volume, mass, or area (see section 2.2.2).

2.2.1 Molecular methods

Molecular methods focus on detecting virus nucleic acids: in SARS-CoV-2, this is viral RNA. Although present in minuscule amounts, even in a concentrated sample, the nucleic acid can be amplified in a quantitative fashion and, since the reaction is highly specific, the RNA can be detected preferentially over other similar molecules in the sample. The principal technique used is the *polymerase chain reaction* (PCR), whereby a specific part of the viral RNA is targeted using enzymes (reverse transcriptase to generate a DNA strand complementary to the RNA and polymerase to replicate the strand repeatedly until it can be detected by fluorimetry[5]). The process is quantitative, hence the term *reverse transcription quantitative PCR* (RT-qPCR).

Results of qPCR can be expressed as a cycle threshold (C_t) value – the number of replication cycles (repeated PCR reactions) before a positive signal of the RNA is seen. The lower the C_t value, the higher the concentration of RNA. C_t values can alternatively be converted to a number of gene copies (GC) per unit of volume, area or mass using a suitable calibration curve. The very low copy numbers found in environmental samples are often close to the limits of detection. In such cases, small performance differences between individual PCR machines can become significant. Tailored calibration curves for individual machines, methodologies and labs are therefore often used to derive GC.

As with any analytical technique, RT-qPCR methods are subject to performance limits. Typically, analytical methods are characterised with a limit of detection (LOD, the lowest level of analyte that can be resolved) and a limit of quantification (LOQ, the lowest level that can be reliably enumerated, typically 2- 5 times higher than the LOD). In wastewater, early estimates are for LODs of a few thousand gene copies per litre with LOQ perhaps 10,000 - 20,000 GC l^{-1}. In comparison, faecal shedding rates range from 10^8 to 10^{12} genome copies per day from an infected individual.

Advantages[6] of PCR approaches include their potential for use by non-virologists, the possibility for automation, cost-effectiveness, speed, and there being less need for sophisticated containment facilities and high levels of expertise.

The disadvantage of molecular approaches is that they do not detect infectious virus. This has long been recognised in detecting (for example) noroviruses in aquatic matrices. It must therefore be recognised that the detection of SARS-CoV-2 RNA in a sample does not

necessarily mean the sample contains infectious virus. Box 1 sets out some of the considerations and significant reasons for imprecision that need to be understood when interpreting infection risk based on RT-qPCR results.

Box 1: Interpreting infection risk from RNA detection.

Although it does not measure the presence of infectious virus, RT-qPCR of environmental samples can provide decision makers with valuable information from which to infer infection risk. Caution must be taken in the interpretation of results, although this approach is routinely used to assess risk in some environments.

Various studies have examined the relationship between PCR measurement of RNA presence and cell culture results for the same samples.

From clinical studies it has been shown that live virus cannot be recovered from fresh samples with C_t values of greater than 34[7] [8]. At lower C_t values, the chance of viral isolation increases and, at a C_t value of 25 or below, viral isolation is successful from most samples. Because RNA is relatively stable, a high C_t value does not necessarily correlate to the presence of live virus in environmental samples (Figure 3).

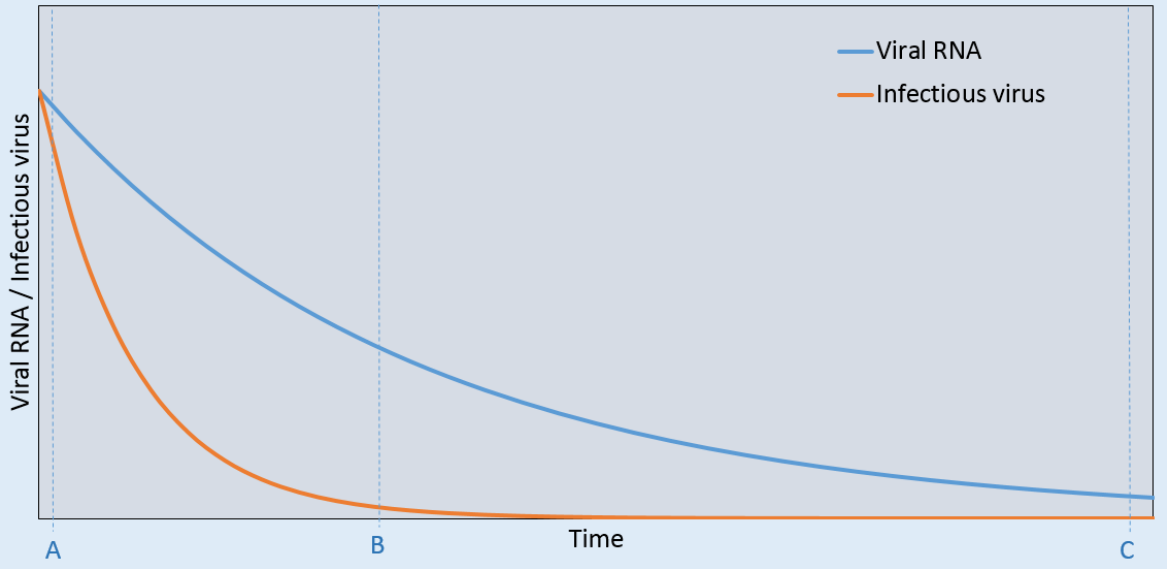

Figure 3: A schematic illustration of the different lifetimes of RNA and infectious virus in the environment. Decision makers interpreting risks from environmental sampling at time points A, B and C must consider the time since the virus was added to the environment, and the different decay rates of infectious virus and viral RNA. Decay rates, and the relative difference between them, are affected by a wide range of environmental factors, including surface type and cleanliness, water composition, presence of oxygen, temperature, etc.

Conclusions can be tentatively drawn as follows[Error! Bookmark not defined.] [9]:

- A negative PCR may indicate that no infectious virus is present at the specific location and time where the sample was taken if the factors below are taken into account.
- A positive PCR indicates presence of SARS-CoV-2 RNA. This does not necessarily mean that infectious virus is present.
    - A C_t value >35 indicates presence of SARS-CoV-2 RNA but probably not infectious virus. Ct values over 35 could indicate laboratory contamination.
    - A C_t value of about 25-34 may indicate possible presence of infectious virus. The lower the C_t value the higher the probability of presence of infectious virus and the higher the potential concentration.
    - A C_t-value <25 indicates possible presence of infectious virus if sample is taken from recently touched surfaces or close to infected people. However

it is possible to have a C_t value < 25 with no infectious virus (see Figure 3) if sampling is some time after the virus was first introduced to the environment.

The main factors to consider when making inferences on infectivity from PCR results include:

(1) **Degradation rate of infectious virus:** The relationships between C_t values and cell culture results have been derived from fresh clinical samples in which viral decay is likely to be minimal. The rate at which infectious virus degrades depends on ambient conditions. At high temperature, high relative humidity, and in the presence of chemicals, contaminants or microorganisms, PCR is likely to overestimate the presence of infectious virus, especially if it has been in the environment for some time.
(2) **Degradation rate of RNA:** In the environment, RNA is much more persistent than infectious virus (see section 1.1). Temperature, microbial activity and chemicals also affect the rate at which RNA breaks down. In the majority of cases, however, RNA can be expected to degrade more slowly than infectious virus as illustrated in Figure 3.
(3) **Inhibition of PCR:** The presence of some chemicals and biosolids inhibit the PCR process which can differentially inhibit virus RNA recovery efficiency and gene amplification. The relationship between genetic signal strength (copy number) and the presence of infectious virus will be highly dependent on the matrix type, and will vary more in environmental than clinical samples.
(4) **Extraction efficiency:** There is widespread acknowledgment that the techniques used to concentrate RNA prior to PCR analysis can produce different efficiencies between laboratories. Inter-laboratory trials are needed to test the robustness of (especially) the concentration or detection stages. Trials for SARS-CoV-2 RT qPCR have not yet been undertaken under rigorous conditions (such as those governed by the United Kingdom Accreditation Service), although informal lab inter-comparisons have been undertaken.
(5) **Sampling design:** It is difficult to sample the environment in a repeatable way. Viral particles are often distributed heterogeneously in the environment so the variance between samples is often high.
(6) **Virus shedding patterns:** SARS-CoV-2 virus isolation has not yet been successful from people more that after 8 days after becoming symptomatic, regardless of C_t value[10]. In contrast, RNA can be shed for up to 83 days from the upper respiratory tract and 126 days in stools[10].
(7) **Infectious dose:** The median infectious dose at which 50% of those exposed to SARS-CoV-2 become infected (ID_{50}) has not been yet determined. Even in cases where RNA is an indicator of presence of infectious virus, the virus may not be present at sufficient levels to infect a person in that environment.

Genome sequencing

In recent years, high throughput sequencing methods have been developed that allow direct elucidation of nucleic acid sequences in an environmental sample without the need for culturing of target organisms. Large numbers of relatively short base pair sequences (typically 500-700 bp) can be read in this way and combined using specialist software to reconstruct entire genomes. As a result, it would be possible to identify any changes in virus genomes over time and the emergence of new strains or variants.

Other methods

Beyond PCR, other methods exist to detect viral components but are not currently widely used in environmental monitoring. These include reverse transcriptase loop-mediated isothermal amplification (RT-LAMP), aptamer methods, and laser-based detection. Immunoassays such as lateral flow antigen tests can detect specific viral proteins. In this

case, antibodies in a test kit bind to the virus creating a coloured band. However, this approach is generally not sensitive enough to detect virus levels at the low concentrations typically found in environmental samples.

2.2.2 Cell culture

To detect infectious virus, cell cultures (mammalian cells grown in flasks and maintained in normal physiological conditions) are inoculated with concentrate from the environmental sample and observed for specific changes caused by the virus (usually the death of the cells). Infectious virus can be enumerated using a variety of cell culture-based assays.

The important advantage of such an approach is that it detects infectious virus, capable of initiating disease in a susceptible individual. Drawbacks are that it is time-consuming (4-5 days for results), labour-intensive, and requires suitable containment facilities (BSL 3 in the case of SARS-CoV-2). It also needs considerable expertise and experience. Other pathogenic viruses may also be present in the samples, so assays should ensure that SARS-CoV-2 is the main cause of cell death (by measuring the increase in virus in the external medium).

In environmental virology, the choice of cell culture is usually determined by experiment to find which is most sensitive to the target virus. The origin of the cells (e.g. monkey, human) is largely immaterial. In cell lines such as Vero-E6 (derived from monkey kidney cell lines), which are currently used in most SARS-CoV-2 infectivity studies, the cells have become transformed through repeated use over many years and have lost many of their original characteristics. Detection in cell culture is sometimes less sensitive than detection by molecular methods. This is because it takes more than one virus particle to initiate infection in a cell culture. For example, using influenza virus, Fabian *et al*.[11] found that one $TCID_{50}$ (a statistical measure of virus infectivity) represents approximately 300 genome copies (other studies have suggested values from 100-650 copies[12 13]). Emerging evidence suggests that one plaque-forming unit (pfu) is equivalent to c. 300-10000 gene copies of SARS-CoV-2. For this reason, cell lines overexpressing the ACE2 receptor (the binding site for SARS-CoV-2) and transmembrane protease, serine 2 (TMPRSS2) should ideally be used to improve the sensitivity of the assay.

Lack of sensitivity can also sometimes be compensated for using cell culture to grow up the virus, increasing its numbers, followed by PCR where the cell culture harbouring infectious growing virus is analysed by PCR (ICC-PCR). This technique combines the advantages of both approaches.

3 Water

There is no evidence that SARS-CoV-2 is present in drinking water from either a chlorinated mains or non-mains supply. However, it is now clear that SARS-CoV-2 RNA is shed in faeces from many infected individuals and therefore enters building wastewater systems and the sewage network[14]. The theoretical potential exists for viral material to subsequently enter freshwater or marine water bodies, though inactivation rates and dilution are likely to be high. The ambient conditions in sewers and natural waterbodies mean that any infectious SARS-CoV-2 is likely to degrade rapidly, whereas RNA is relatively persistent. The likelihood of detecting infectious virus therefore decreases further from its source. Most measurement in water so far has focused on sampling wastewater in the sewage network to monitor prevalence of Covid19 in the population.

The approaches set out below broadly describe the process used in current wastewater monitoring for SARS-CoV-2 RNA 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 there is little or no evidence of SARS-CoV-2 RNA in such surface waters[15].

3.1 Sample collection

Sampling in the water environment can be performed at different locations including:

- Immediately at the sewer exit from a building or location or within a small facility such as a septic tank.
- Within a major sewer, such as at a manhole.
- At intermediate sewer infrastructure points such as pumping stations or holding tanks.
- At the initial influent to a treatment works.
- At the point at which treated effluent is discharged from a treatment works.
- In the wider environment, e.g. rivers, estuaries or seawater.

Each location comes with its own challenges, including practical issues such as access, safety of personnel, sample variability, likelihood of blockages etc.

Sample collection from water or wastewater can take the form either of a grab sample, whereby a sample of water is collected in a clean vessel, providing a snapshot at a point in time. Alternatively, an autosampler can be used to collect regular samples over a set period (e.g. 24 hours). Such devices can be programmed either to take samples at regular intervals 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 better when concentrations are highly variable or dependent on diurnal human behaviour, as is the case with wastewater.

Other considerations include:

- The matrix–wastewater can be a complex mixture of municipal and industrial effluents (and is quite different from clinical samples).
- Differential distribution of virus between solid and liquid phases in different sample types.
- Biosafety considerations when handling samples.
- Data must be collected for each sampling or analytical step to ensure that the appropriate context can be given to subsequent interpretation of results.
- It is important to report on all of the factors in the study that could impact the result (i.e. detection results need to be related to water quality and other metadata).

3.2 Sample transport, storage, handling and preparation

Once taken, samples need to be kept cold (4-6 ^{o}C) until they can be analysed. Samples of wastewater must be handled in secure BSL 2 laboratories with suitable safety cabinets. Samples should be analysed as soon as they arrive at the laboratory. If not possible, long-term storage of samples should be undertaken at -80°C.

3.3 Virus Concentration

RNA from SARS-CoV-2 cannot typically be detected directly in aquatic matrices as it will be too dilute. Hence, the virus must first be concentrated. There are many methods for concentrating viruses in water[16], including those that depend on the size of the virus (entrapment filtration), its electrostatic charge (adsorption/elution), its molecular mass (ultracentrifugation), and reactions with chemicals such as polyethylene glycol[17] and different salt solutions. Some methods suit some viruses better than others. SARS-CoV-2 bears a lipid envelope on its surface which is necessary for the virus to be infectious, and it is a virus of moderate sensitivity to physico-chemical changes. Procedures involving organic solvents or marked pH changes are therefore not suitable for concentration of infectious virus[18]. In the

UK wastewater monitoring programmes for SARS-CoV-2, 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 (see Ahmed et al for more details[19]).

3.4 Laboratory assays

Once concentrated, the concentrate can be analysed by a variety of techniques to demonstrate virus presence. These are discussed in section 2.1 above. Detection methods should be optimised for environmental samples, not clinical samples. Molecular approaches to detect the presence of RNA are now well established[20 21]. Ongoing attempts to culture SARS-CoV-2 from contaminated sewage or from the wider water environment have so far failed.

3.5 Normalisation

The main application of wastewater monitoring for SARS-CoV-2 is to infer infection rates in the population served by that sewer system. However, once the virus is known to be present it perhaps becomes more important to know how prevalent the virus is and how the prevalence of infection is changing. This implies that we need to know how many people are contributing to the wastewater so that detected amounts of RNA can be compared or "normalised" to faecal load. Various options exist which may allow estimation of relative human faecal load in different samples. Most of these compare SARS-CoV-2 RNA concentrations with the concentration of a marker that is assumed to be generated at a set rate per person. If the concentration of the marker decreases, the sample may be more dilute, e.g. due to rainwater ingress. Such markers may include biomarkers like human mitochondrial DNA, human RNA, or crAssphage (a benign bacteriophage that is common in the human gut[22 23]). Alternatively, organic or inorganic compounds (for example ammonium or contaminants such as pharmaceutical products) can be used in the same way. Flow volume can also be used to control for dilution. It remains unclear which of these are effective and work is ongoing to understand this aspect better.

3.6 Interpretation and limitations

The use of wastewater analysis for detection of SARS-CoV-2 in a population is potentially a sensitive technique capable of providing a leading indicator in advance of clinical testing, particularly where significant numbers of otherwise asymptomatic infections occur. The approach can support public health decision making in various ways:

- To provide early warning of new disease outbreaks in the population in a specific sewage catchment area.
- To identify outbreaks that might not be detected by clinical testing, due to asymptomatic infection or reticence to testing, inform the allocation of testing resourcing or non-pharmaceutical interventions.
- To estimate the prevalence and trend in infections at population level. Once Covid-19 is established in a population then wastewater data may provide a reliable confirmation of when infection rates fall.
- To follow the progress of infections in remote or disengaged communities, or those lacking access to formal healthcare[24].
- As a long term, low cost surveillance option which is capable of detecting low numbers of infected individuals that might not be detected via random testing.
- Genetic sequencing of virus in wastewater samples can give an indication of the prevalence of different strains or variants circulating within a population, helping understand the origin, evolution and spread of the disease.

Linking C_t values or gene counts per litre to a specific number of people infected in a population is difficult. A variety of variables can affect correlation, such as rates of dilution, target RNA degradation, and/or PCR inhibition (see section on normalisation above). These

issues are often specific to a particular sewage network or waterbody. At present we lack a good understanding of the fate of virus in sewers including how virus particles may disintegrate or be consumed by bacteria or protozoa, nor how any freed RNA behaves as it is transported in the water. It is known that colder temperatures favour preservation and virus particle survival, but precise degradation rates are lacking.

The use of wastewater-based epidemiology to inform real-time public health policy decisions in response to a pandemic is still very new. Work is ongoing around the world on how to make best deploy it and use the data it generates. However, more work is needed to refine techniques[25].

Current studies have demonstrated little success in recovering infectious SARS-CoV-2 virus from faecal samples in clinical cases. Therefore, although there is a genetic signal for the presence of RNA, there may not be infectious virus present in wastewater.

4 Air

4.1 Sample collection

Sampling the air for the presence of viruses is reliant on the selection of an air sampler to collect particles containing the agent of interest. Air samplers operate in a range of methods including the following:

- Impingement – collection in a fluid.
- Impaction – inertial collection on to a surface.
- Cyclonic separation – collection onto a swirling film of liquid.
- Filtration – collection onto a filter.
- Electrostatic precipitation.
- Condensation based inertial deposition.

The process of sampling can damage and inactivate microorganisms and thus reduce their viability making them non-culturable, even if they were so prior to sampling. Some samplers are designed to reduce stress on microorganisms and allow them to be cultured to assess infectivity. If PCR-based measurement of RNA is to be used then most types of air sampler can be used as RNA is much more stable than infectious virus, as discussed above. For example, pre-existing filtration systems intended to monitor PM_{10} pollution could be analysed for the presence of SARS CoV-2 RNA[26]. One paper has reported detection of SARS-CoV-2 RNA from such samplers, but its methods have been criticised[26]. The Environment Agency is currently carrying out a limited study using the filters from VOC monitors for analysis for SARS-CoV-2 RNA. There are also large volume biological collectors used by the military which could be used for such purposes.

The main samplers being used in published studies of SARS CoV-2 aerosols in indoor environments are the Coriolos sampler and the Sartorius MD8 gelatine membrane filtration sampler (for example Zhou et al[27] and Moore et al[28]). Personal samplers have also been used over longer periods. A novel Biospot Vivas condensation sampler was the sampler used in the only publication claiming to have recovered infectious virus from air[29]. Various other groups have unsuccessfully attempted to recover infectious virus. Other studies have used personal samplers operating for longer periods of time such as the NIOSH sampler[30].

Novel techniques are currently being investigated to establish the presence of viral RNA in faecal aerosols generated as a result of turbulent wastewater flows in building sanitary plumbing systems. There is currently no evidence that viral RNA can be extracted from this method, however, the technique has been successfully applied to detect bacteria in faecal aerosols[31] [32].

4.2 Interpretation and limitations

SARS-CoV-2 RNA can be detected from air samples if a large volume of air has been sampled over a considerable amount of time. For example, from a pollution sampler or if the sampler is situated in a crowded area. This information, however, is of limited value as it is impossible to link the presence of RNA to a cause and any infectious virus sampled would be inactivated. Detection of RNA from such long-term sampling of ambient air does not indicate the presence of infectious virus nor provide much insight on the risk of infection. The approach may be more useful in indoor settings where detection of the virus can be linked to a specific group of people. The use of high volume samplers in crowded areas could potentially detect the virus and give information about potential exposure risk in such a setting.

Failure to detect infectious viruses in air samples does not necessarily demonstrate the absence of live virus in samples where viral RNA was detected by molecular methods.

In contrast to outdoor air monitoring, two different research groups have recently demonstrated the presence of infectious SARS-CoV-2 viruses in aerosol samples from patient rooms in quarantine and isolation care [33] [34]. These studies focus on high risk environments where virus levels can be expected to be high. They are likely to underestimate the amount of viable airborne virus available for inhalation by others[35].

5 Surfaces

5.1 Sample collection

Surfaces can be easily sampled using swab or sponge-based systems to detect the presence of SARS-CoV-2. Many of these methods have been validated for biological agents[36] and more recently for SARS-CoV-2[37]. Surface sampling has mainly been carried out in hospital environments but publications and pre-prints are being generated which have found SARS-CoV-2 RNA in other settings, including outdoors. Harvey et al found that 8% of swab samples taken in outdoor public spaces in Massachusetts USA were PCR positive and related positivity to population incidence[38]. However only three of the PCR positives could be quantified at between 2.54 and 102.43 gene copies per cm^2 suggesting that contamination was very low level[38]. Although the authors suggest that surface sampling could be used for surveillance, it would be difficult to sample with the right time and spatial frequency to be confident that samples were a reliable predictor of virus in the community. Two ongoing UKRI projects examining risk on public transport (TRACK and VIRAL) are conducting regular surface sampling on trains, tube and buses and will be able to report on the usefulness of this type of surveillance in a few months' time.

Swab samples can be analysed for viral presence by PCR or tissue culture and results presented as gene copies or plaque forming units per cm^2 or per swab. Viral isolation can also be attempted from areas with low C_t values. Kampf suggests that under laboratory conditions at C_t value of lower than 30 is generally required for virus isolation[39]. SARS-CoV-2 RNA can be found on inanimate surfaces up to 28 days after discharge of patients with COVID-19, which further limits the relevance of RNA detection on surfaces[39].

The method used to recover virus from surfaces is very important. Most work to date has focused on clinical detection in lung and oral specimens. The effectiveness of approaches to sample surfaces in the wider environment is much less clear. We know that surface swabbing recovery rates vary widely, depending on factors such as surface roughness, surface hydrophobicity, surface organic load, type of swab used, recovery medium affecting RNA stability and the presence of compounds that inhibit or affect RT-PCR or LAMP assays[40] [41].

If surfaces can be returned to the lab then there are alternatives to swabbing depending on composition and surface area including sonication and improved methods such as pulsification and vortexing with glass beads.

5.2 Surface sampling of food

Recovering either RNA or infectious virus from food presents specific challenges, and appropriate methods depend on the type of food. Methods for the recovery of SARS-CoV-2 or coronavirus RNA from most types of food have not been validated or optimised and interpretation is therefore uncertain, although the Food Standards Agency is in the process of commissioning research to address this gap. In addition, current international consensus is that it is highly unlikely that the ingestion of SARS-CoV-2 will result in illness; there is currently no documented evidence that food or food packaging are likely to be a source and/or vehicle for the transmission of SARS-CoV-2. Given the lack of evidence associating food or food packaging with the transmission of SARS-CoV-2, the testing of food or food packaging for SARS-CoV-2 for reasons of food safety is not advised and is not considered to add value for food safety purposes[42].

5.3 Interpretation and limitations

Interpretation of the sample data using PCR is difficult. Environmental factors such as temperature and relative humidity affect rate of degradation. It has been shown from the Diamond Princess that RNA positives can still be obtained 17 days after a cabin was inhabited by a known case[43] and 9 days after a surface was treated with bleach[44]. PHE has unpublished data showing that RNA can be detected in surfaces three weeks after drying with only minor losses. Therefore, even a low C_t value PCR positive can reflect historical contamination and will not contain any viable virus. It can be argued that surface sampling for SARS-CoV-2 RNA is of little value use unless combined with observational studies. However, SARS-CoV-2 presence on surfaces could be used as an indication of potential infectiousness of a space.

6 Using Environmental samples to assess risk

Quantifying transmission risk is a challenge, however models such as Quantitative Microbial Risk Assessment (QMRA) offers one possible approach[45]. This is a widely used approach in environmental engineering to assess risks from pathogens, particularly in water and food. The approach has been used in assessing transmission of viruses, including a number of early models that have attempted to analyse the transmission of SARS-CoV-2[38 46 47]. Such models require data on the dose-response as well as data on the amount of virus that people are exposed too.

Alongside laboratory data, environmental samples provide a valuable data source for such models. However environmental data must be treated with care for the reasons discussed above. Models require quantitative data, and as highlighted in this paper this can be difficult to determine and may be uncertain, with values dependent on the specific time and location of sampling as well as the sampling method. The challenge in relating viral RNA to infectious virus means that data is difficult to interpret and use in an appropriate way in a model. It is also not clear whether it is appropriate to compare risks calculated using data from surfaces with those from viral samples in the air. For example, the study by Harvey et al, used RNA surface sample data to calculate the transmission risk via a hand to nose contact[38], while Wilson et al[47] used data from RNA concentrations in air to assess the risk of infection via inhalation with different types of masks. Both studies showed low risks, however it is not possible to determine whether the analysis is comparable for the two routes of exposure due to differences in the sampling methods and ability to detect RNA. Ideally transmission models need probability distributions for sampled data to give a realistic understanding of the uncertainty in risk; such distributions require significant data for confidence.

The challenges with evaluating risk are also compounded by the lack of dose-response data for the virus. Data are available for a number of other coronaviruses[48], however most of these are derived from animal studies. It is not clear how the infectious dose from animal studies will correlate to viral RNA or infectious virus sampled from the environment.

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