Title: 5(d) SARS-CoV-2 wastewater surveillance in Ireland

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Organisation(s): UCD School of Biomolecular and Biomedical Science, UCD School of Veterinary Medicine, UCD School of Civil Engineering

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Action required:

☐ For noting

☐ For discussion

☐ For decision

Approved for future publication: YES/NO (remove as applicable)
SARS-CoV-2 wastewater surveillance in Ireland

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Background

During the summer months, Ireland managed to successfully suppress the circulation of SARS-CoV-2 in the community. In addition, as testing numbers increased, the positivity rate declined, suggesting that not a lot of viral infections were being missed. However, since early August, the level of virus transmission increased over 100 fold, leading to the requirement for enhanced public health restrictions in October. Thankfully, the enhanced public health measures at the time of writing appear to be successful, but there is concern that the level of transmission of virus in the community will increase again when the restrictions are lifted. We need enhanced surveillance when SARS-CoV-2 levels are low, and ideally, an early warning system that will tell us when SARS-CoV-2 levels are increasing in the community.

Wastewater Surveillance

Recent studies have shown that 27 to 89% of infected individuals shed SARS-CoV-2 in their stool, with \(10^7\) RNA/g faeces one week after symptoms, dropping to \(10^3\) g/faeces after three weeks (1, 2). Viral RNA remains present in faeces after respiratory tract samples are negative, suggesting gastrointestinal replication (3-5). Viable virus particles have been detected in some studies in the first week after symptoms (6). The presence of SARS-CoV-2 RNA in stool samples of infected individuals implies that surveillance of wastewater is a viable means to monitor the circulation of SARS-CoV-2 in the population.

Composite 24-hour sewage samples represent an aggregate of an entire population within the sewage network. This means that analysis of SARS-CoV-2 genetic material in sewage samples will capture both symptomatic and asymptomatic individuals in the community.

Proof of Concept

An increasing number of studies have reported the presence of SARS-CoV-2 RNA in the influent of wastewater treatment plants (7-11). Indeed, surveillance of wastewater for the presence of SARS-CoV-2 RNA has commenced in many countries around the globe (12). Furthermore, several longitudinal studies have shown that the trend in SARS-CoV-2 RNA titres in wastewater is closely correlated with clinically diagnosed new COVID-19 cases (8, 13-15).

Analysis of 24-hour twice weekly composite samples of the influent of three wastewater treatment plants in Ireland has commenced in UCD, funded by the SFI COVID-19 Rapid Response Funding Call. This demonstrated the presence of SARS-CoV-2 genetic material in the influent of three waste water treatment plants. The project focuses on a large (Ringsend, Dublin), medium (Shanganagh, Bray) and small (Enniskerry) WWTP. The Ringsend WWTP serves the greater Dublin area and currently operates above capacity at 1.9 million PE, while the Shanganagh and Enniskerry plants have capacities of 186,000 and 6,000 PE, respectively.

The UCD team has validated SARS-CoV-2 ultrafiltration concentration efficiency and RT-qPCR assays using inactivated SARS-CoV-2 and determined the limits of detection and quantification of the qPCR assays. The team has been reporting on the SARS-CoV-2 RNA in the influent of these plants since 20
June, providing weekly reports to the Irish Epidemiology Modelling Advisory Group (IEMAG). The virus is currently present in all three WWTP (Figure 1).

**Figure 1:** Shown are the total amounts of SARS-CoV-2 RNA (gc/day) in the influent of the Ringsend (top), Shanganagh-Bray (middle) and Enniskerry (bottom) wastewater treatment plants based on the flow rates (m$^3$/d) and the measured concentrations of the N1 marker. The last values (28 October) are calculated on the average influent volumes of the previous dates as their actual values are not yet available. The black symbols represent the data based on the concentrations listed in the rings end table, the blue line is a smoothing curve to visualize trends better.
Wastewater Treatment in Ireland

Treatment of wastewater in Ireland occurs in either domestic wastewater treatment systems treating single households, e.g., septic tanks, or in public wastewater treatment plants with wide ranging capacities serving communities of various sizes. Approximately 500,000 households are connected to domestic wastewater treatment systems and not to public sewerage systems.

Ireland has 1,111 wastewater treatment plants, which vary very significantly in size. The largest of these is the Ringsend treatment plant in Dublin, which has a capacity of 2,327,680 PE (population equivalent), whereas the smallest plant in Sligo has a capacity of just 5 PE. The total capacity of all 1,111 wastewater treatment plants in the country is 5,457,053 PE. Of these, only 59 plants have a capacity over 10,000 PE.

Selection of Wastewater treatment plants

The aim of wastewater surveillance is to capture a large part of the population connected to a sewerage system in an efficient manner, and in addition, to provide country wide surveillance. We therefore propose to include two wastewater treatment plants in each of the 26 counties. In addition, we propose to include all treatment plants that have a capacity of more than 10,000 PE, which increases the total number of plants under surveillance to sixty-nine. These sixty-nine plants have a capacity of 4,559,414 PE, which provides 84% national PE coverage (Fig 1, Table 1). The number of plants can of course be changed to increase coverage in certain counties, depending on available resources.

Figure 2: Population Equivalent (PE) coverage per county with selection of two wastewater treatment plants per county and inclusion of all plants with a capacity greater than 10,000 PE. The details of this graph are presented in Table 1. Red columns: capacity of selected plants, black columns: capacity of all treatment plants per county.
Table 2: Population Equivalent (PE) coverage per county with a selection of two wastewater treatment plants per county and inclusion of all plants with a capacity greater than 10,000 PE. Using these criteria 69 plants are selected for inclusion in wastewater surveillance. Ireland has a total of 1,111 plants.

Proposal

That NPHET would instruct the HSE/HPSC to implement a robust national wastewater surveillance network for SARS-CoV-2 that could be expanded to incorporate surveillance of other pathogens e.g. polio & non-polio enteroviruses (a WHO requirement) in the months and years ahead.

1. Formation of a HPSC/HSE/Public Health oversight group
2. Work to be carried out at UCD for a period of 12 months (costings in appendix 1)
3. Protocols to be shared with regional laboratories so work can ultimately be decentralised
4. Engagement with Irish Water will be required
5. Logistics support to collect and transport specimens to Dublin
6. Weekly reporting from UCD via NVRL to the HPSC and regional DPHs
7. Decisions required on metrics that will stimulate further action e.g. pop-up community swabbing or testing centres in areas with evidence of increased SARS-CoV-2 RNA in wastewater
8. Specimens to be shared on request with other laboratories for surveillance of other pathogens as indicated
Appendix 1

Costings

The current proposal has been costed for the analysis of weekly samples of the influents of two wastewater treatment plants per county (26 in total) for one year (52 weeks) to be carried out in UCD. As mentioned in the above, a desk study will have to be carried out to determine which plants need to be included, and whether more wastewater treatment plants in a particular county need to be included in the surveillance programme.

At present wastewater samples are concentrated using ultrafiltration, which is a convenient methodology for a limited number of samples. However, there is a world-wide shortage of ultrafiltration units. Furthermore, the procedure is time consuming, expensive and requires additional centrifuges. We therefore propose to concentrate SARS-CoV-2 in sewage samples using electronegative membranes, which are 18-times less expensive than ultrafiltration units, are in ready supply and have a higher throughput than ultrafiltration.

Budget

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<td>Transportation of samples to laboratory</td>
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| Total costs of proposal       | €308,934   |

*Please note that the costs of transportation is an estimate only based on €25 per sample. The actual costs may be higher or lower.
Justification of resources

Staff
Funds for two research assistants with expertise in molecular biology are requested. The research assistants will be supervised by Dr Laura Sala-Comorera and Dr Liam Reynolds, two experienced post-doctoral fellows who are currently carrying out wastewater surveillance as part of the SFI project (20/COV/0159) and are funded by the EU Interreg Va Ireland Wales Acclimatize project. They will remain in post until June 2023.

Two research assistants are required to process sewage samples, carry out qPCR assays and compile the results.

Equipment
Additional filtration manifolds and pumps are required to concentrate 52 weekly samples prior to RNA extraction. Funds for a Qiacube (Qiagen) are requested. This instrument facilitates fully automated purification of nucleic acids (RNA) and ensures sample uniformity and high throughput. Rapid manual processing of the number of samples proposed here is not practical.

Consumables
Consumables for RNA extraction and qPCR are need to isolate RNA from concentrated sewage samples, followed by qPCR assays. The SARS-CoV-2 assay is costed for the assay of three viral markers, carried out in triplicate. In addition, the costs for RNA qPCR standards are included. Plastics and chemicals are for laboratory disposables (e.g., qPCR plates, pipette tips) and standard laboratory chemicals.

Logistics
These are costs associated with transportation of sewage samples to the UCD laboratory. The costs are an estimate based on €25 per sample. However, the actual costs may differ.

References


