Republic - Export Building, 1st Floor
2 Clove Crescent
London E14 2BE
Tel: +44 (0)20 7654 5500
Fax: +44 (0)20 7654 5555
E-mail: water@iwahq.org
www.iwa-network.org

IWA Webinar – Traditional and molecular indicators to characterise sewage in wastewater-based epidemiology

https://iwa-network.org/learn/traditional-and-molecular-indicators-to-characterise-sewage-in-wastewater-based-epidemiology/

Q&A report

Question	Answer
How frequent the sampling should be done to give a reliable data?	The answer depends on the question. For wastewater monitoring you should do 24h composite samples, if you need just a "snapshot" a grab sample is also good. To monitor a WWTP over a year, samples on a monthly or weekly basis should give reliable results. (according to composite samples for chemophysical analysis)
The geographical location is a huge issue here, depending on the human activities in that location gives varying data. How will it be DATA refined and obtain the specific targets?	Unfortunately, there is not a clear answer to this question. The potential for variable MST target shedding based on geographic location is real. I think there are multiple ways to address, but it will depend on the data interpretation goal. For example, if one seeks to compare measurements overtime from a local community, then conducting *a priori* tests to determine the most suitable MST target is a good idea. However, if one seeks to compare results across a broad range of communities where geography could be influential, a different strategy may be necessary focusing on an approach that is not subject to spatial variability.
How about protists? Can they be part of MST Toolbox set?	If you are referring to the use of Protist in MST, I guess you are referring to protozoa or algae. Please note that in MST we are looking at the source of the fecal contamination, and the markers of MST should be associated with this contamination. Therefore, commensal microorganisms of the intestinal microbiota are usually best used. Pathogens usually have limitations in being associated with the epidemiological situation of human or animal populations and this causes them to vary over time. The use of some species of Cryptosporidium or Giardia that may be associated with a host has been evaluated a few years ago, but they have not provided the specificity that other markers of MST have.

Republic - Export Building, 1st Floor
2 Clove Crescent
London E14 2BE
Tel: +44 (0)20 7654 5500
Fax: +44 (0)20 7654 5555
E-mail: water@iwahq.org
www.iwa-network.org

Should the PMMoV be assessed over time according to the community?	PMMoV can be a very useful tool and a process control to indicate that the concentration workflow of more relevant viruses such as SARS-CoV-2 are consistent (Symonds et al., 2019 - Like all other qPCR assays targeting environmental targets, PMMoV qPCR is prone to inhibition by co-concentrated substances and dilution effects due to precipitation (rain and snowmelt). Due to the benefits that the knowledge of the specific PMMoV concentration in the samples under investigation can bear, assessing it over time is a good idea and should be considered.
what do think about crAssphage as MST and which type of more studies about crAssphage is still required to check it suitability as MST in sewage?	CrAssphage as an MST marker has already been studied and evaluated in different types of water samples with sources of fecal and human contamination demonstrating good specificity and sensitivity in the different qPCR techniques that have been developed by different authors. These different qPCR techniques do not differ from each other in terms of results. In the future, more data should be available on its environmental decay and the resistance of the marker to water treatment, and to assess it in its combined use with other MST markers and indicators in the development of prediction models in MST
After a year of COVID 19 survival, can the stool samples provide some indications of having been infected in SARS virus?	Studies showed that SARS-CoV-2 virus can be shedded into stool samples from infected patients, both asymptomatic and symptomatic, starting from 1 – 6 days before the symptom onset to more than 2 weeks after the symptom onset. The shedding rates also vary from person to person. More information is still needed regarding shedding rates and duration, especially for new variants.
While not advocating that we be spendthrifts, is there any way to estimate the error and/or deviation from norm that might result if a study is short funded? Meaning, can we come up with study scenarios and the minimum number and/or frequency of samples to address that scenario?	Recommendations about the frequency of samples is related to the population that is monitored and how mobile it is. In a recent study from Singapore, Wong et al., (2020) measured SARS-CoV-2 concentrations in the outflowing wastewater of a Highrise in which a local outbreak occurred and which had been put under lockdown by the health service. Virus numbers from a high frequency could be correlated well with nasopharyngeal swabs and quickly

the international water association

Republic - Export Building, 1st Floor
2 Clove Crescent
London E14 2BE
Tel: +44 (0)20 7654 5500
Fax: +44 (0)20 7654 5555
E-mail: water@iwahq.org
www.iwa-network.org

	indicated a reduction of transmission. Others have shown similar trends when monitoring dense living conditions such as student dormitories (Gibas et al., 2021) and bi-weekly samples under those conditions seem to become the go-to frequency for small scale campaigns that mainly aim at identifying outbreaks before they become too big. For larger scale operations (like monitoring WWTP with 100k+ PE), weekly grab samples seem to suffice to get an idea of the virus transmission dynamics in the sewershed.
Medema, how the presence of Sars cov-2 can be normalized without having Flow data?	Without flow data, SARS-CoV-2 concentration measurements can be normalized using a fecal indicator, that provides an index of the 'fecal strength' of the water that is sampled. Ideally, this is a human fecal indicator that can be analysed in the same sample (extract) such as CrAssphage or Pepper Mild Mottle Virus. If these are not feasible, a fecal indicator such as E. coli can be used to get an index of the amount of fecal material in a sample.
How is ARG data correlates with human data and policy level?	ARG and resistand pathogens are likely found in the human body and can be correlated in hospital wastwater (Wang, Q., Wang, P., & Yang, Q. (2018). Occurrence and diversity of antibiotic resistance in untreated hospital wastewater. Science of the Total Environment, 621, 990-999.). Concerning the policy level, releasing opportunities depend on a number of factors such as available techniques.
Any evidence for antibiotics resistance gene transfer in WW?	Microbiome analysis of different compartments of treatment plants showed that anthropogenic integrons (associated with ARG) differ greatly and occur twice in activated sludge (Quintela-Baluja, Marcos, et al. "Dynamics of integron structures across a wastewater network–Implications to resistance gene transfer." Water Research 206 (2021): 117720).
What is the panel's opinion on using lesson learnt from WBE to investigate environmental impacts from wastewater and in the context of One Health applications?	I think that we have learned first that we need to form strong partnerships within the environmental and health communities. The success of the monitoring relies on public health being integral to laboratory program and staying involved with the utilities, really representing the community. Second in future monitoring programs we

Republic - Export Building, 1st Floor
2 Clove Crescent
London E14 2BE
Tel: +44 (0)20 7654 5500
Fax: +44 (0)20 7654 5555
E-mail: water@iwahq.org
www.iwa-network.org

	will also need to connect with the animal health/veterinary medicine fields particularly for issues like ABR. Finally, ways to communicate and visualize the results will be important back to the stakeholders, decision makers, politicians and even the public.
What about the faecal indicators < than 2 degrees?	Thank you for the question. Based on what people reported on surface waters in countries like Finland and parts of Canada, it looks like colder temperatures help stabilize the genome of the MST targets (with enteric viruses remaining infectious for more than 90 days in a French river at around 6°C, so colder water would keep them intact, detectable and even culturable for much longer than in the tropics or temperate climates)
For Marlene: I believe you said you are using N1 as a conserved target. Have you evaluated Omicron sequences for any impact to N1, and has anything you've seen so far been concerning?	We are using an N gene target that is different from N1. We have looked at the sequences, and don't expect any impact on our ability to detect omicron with this assay. It is conserved for all SARS-CoV-2 variants that we anticipate being circulating.
Marlene - does your group have a qPCR method for omicron yet?	We are currently looking at the HV 69-70 deletion to indicate omicron, and also developing other tools. Our protocol for that assay using digital PCR is available here: https://www.protocols.io/view/quantification-of-sars-cov-2-variant-mutations-hv6-bv5bn82n
have you also checked which genetic ESBL variants were related with ciprofloxicin in wastewater?	Yes, we did qPCR screening with some isolates. More details are in the publication of Voigt&Zacharias et al., 2020
Should we need re-evaluate our sample composites depending on the goals of our surveillance system. Applying 24 hour composites in an early warning system may result in a loss of resolution of concentration, have any of your teams employed an "active" sample blend which capture sample volumes relevant to system flow models (i.e the working day or 09:00 21:00). To be analysed alongside regular 24hr blends.	we checked a couple of different "composite strategies" for the student halls during the establishing phase of the monitoring campaign and noticed that -as you said- higher sample frequencies tend to result in lower "hits" as the virus load in the individual sample was below the limit of detection. So switching to either 2x 12h or 3x8h helped us to get more consistency into the monitoring (even though in Singapore, the temperature during noon and early afternoon are so high that the decay rates of the targets should be increased)

Republic - Export Building, 1st Floor
2 Clove Crescent
London E14 2BE
Tel: +44 (0)20 7654 5500
Fax: +44 (0)20 7654 5555
E-mail: water@iwahq.org
www.iwa-network.org

Live answered questions (please check recording for the answer)

- You have shown an association between ESBL and ciprofloxacin. Which methods gave the most useful results out of the ones you used? Is maldi-tof useful over qPCR or selective cultures for example?
- In the variable flow, when there is too much rain that enters sewer lines, what will be the composition of sewage?
- No mention of ECOLI in sewage surveillance, why?
- Any explanation for the low and high shedders of CrAss phage?
- When Toxic metal contaminants gets mixed with sewage and wastewater network, how will the measurements vary?

References

Symonds EM, Rosario K, Breitbart M (2019) Pepper mild mottle virus: Agricultural menace turned effective tool for microbial water quality monitoring and assessing (waste)water treatment technologies. PLoS Pathog 15(4): e1007639. https://doi.org/10.1371/journal.ppat.1007639

https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1007639

Wong J, Tan J, Lim Y, Arivalan S, Hapuarachchi H, Mailepessov D, et al. Non-intrusive wastewater surveillance for monitoring of a residential building for COVID-19 cases. Science of The Total Environment. 2021;786:147419.

https://www.sciencedirect.com/science/article/pii/S0048969721024906?via%3Dihub

Gibas C, Lambirth K, Mittal N, Juel MAI, Barua VB, Roppolo Brazell L, et al. Implementing building-level SARS-CoV-2 wastewater surveillance on a university campus. Science of The Total Environment. 2021;782:146749.

https://www.sciencedirect.com/science/article/pii/S0048969721018179