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IWA Webinar

"Early detection of Omicron variant with wastewater surveillance" Post Webinar Report and Q&A report

Webinar available at: https://iwa-network.org/learn/early-detection-of-omicron-variant-with-wastewater-surveillance/

Q&A report

Questions to be answered

#	Question	Panelist	Answer
1	Question to Gertjan: what is your estimate, how many covid-19 cases / 100 000 persons are needed before you can differentiate targets from wastewater using ddPCR? How many ml's of wastewater is used for the assay?	Gertjan Medema	
2	For the same number of active cases, might the concentration and load of viral RNA in the wastewater be different because of the variants?	Alexandria Boehm	If variants have differential duration, magnitudes, and times courses of shedding in stool or other excreta that is deposited in the sewer system, then its possible that the relationship between SARS-CoV-2 RNA concentrations in wastewater and reported cases could vary. Also this relationship can be affected by changes in test seeking behavior and testing availability which also may vary as time goes on or perceived threats of diferente variants.
3	In the Netherlands it seems that the clinical positive tests in humans is increasing, while the SARS-CoV detected in sewage is decreasing or at least not	-	

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	increasing in numbers. Is this the same in other countries? Is there an explanation for this phenomenon (more people (Booster)vaccinated, less shedding of omicron, analysismethod?)		
4	Our approach is to sequence and then look for signature mutations. the question is how to identify new mutations? from bioinformrics prospecrtive?	-	
5	Ali - do you know what Omicron sub-types you are detecting?	Alexandria Boehm	Del143-145 is in BA.1 and not BA.2. As of Jan 10, 2022, this mutation is present in 95% of all genomes from all locations globally in GISAID classified as Omicron
6	How long after collecting wastewater samples will the analysis be valid/reliable? i.e. must it be within 1 day or a few days?	Alexandria Boehm	We provide a turn around of 1 day usually from sample retrival to results
7	Who else is looking at borders - airports, ports, transit hubs? We are intersted in origins and onward movement of variants.	-	
8	Does anyone have examples of action taken based on finding omicron in the wastewater? Or was it all just for nothing	-	We found that public health in CA and in the USA was very interested in our omicron data to illustrate that the variant was presente and also watching to see when, if ever, it subsides
9	In terms of detecting SARS-CoV-2 in wastewater, does RT-qPCR or RT-ddPCR have a disadvantage or limitations.	Warish Ahmed	In our lab, we found RT-dPCR is more senstive than RT-qPCR. RT-dPCR can also be more accurate due to the fact that it is free of calibration curve bias
10	How were the samples collected from the aircraft to be representative of the whole airplane or rather the total toilet contents?	Warish Ahmed	Aircraft lavatory is a dry system and therefore detecting pathoegns can be relatively easy. It is highly likely that in a long haul flight people will defecate, if so then we will pick

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			up the signal. We also have data on the % of tank full with wastewater after arrival.
11	Do panelists hypothesize that delta will stick around or be replaced by omicron? Do you see another major variant emerging soon?	Warish Ahmed	Its pretty obvious that Omicron will take over and it is likley that other variants will emereger and take over omicron.
12	Any concerns around privacy on this WBE breakthrough so far?	-	
13	What recommendations to panelists have for how variant data is presented and shared? What are best practices so more comarable if possilbe?	-	
14	@Drs. Bivins and Ahmed, would you recommend more widespread use of wastewater testing on aircraft or is there still more research needed first?	Warish Ahmed and Aaron Bivins	Yes we recommended more widesspread use of wasteewater testing not only for variants bit also for other viroses such as RSVs, influenza etc. We are undertaking further research to fill the knowldege gaps.
15	Analyzing wastewater in public places such as schools, universities, public and private companies, could be a help. Is it ethically feasible?	-	
16	Artic sequencing can reliably demonstrate the prevalanece of different VOCs in wastewater. In Canada the Public Health Agency of Canada has routinely determined the prevalence of different VOCs in wastewater and has nicely shown the progression of the VOCs in samples from across Caanda.	-	
17	Have researchers observed that Omicron is giving a reduced signal of N1 compared with N2? Thanks	-	We measure a custom N gene target so we cannot comment on this, but we know that our target sequence is conserved in

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			variants of concern so far identified
18	Do the airplanes have disinfectants/chemicals in it that interfere with the wastewater signal? Do we know relative percent of people on the plane contribute a sample? (may depend on flight length).	Warish Ahmed	Disinfectant is used in aircraft but our data suggest no degradation of RNA in the presence of disinfectant at least 48 h. A flight is max 18-20 h long.
19	Were you able to Identify any Inhibition from chemicals added to Aircraft wastewater?	Warish Ahmed	Inhibition is not a problem for aircraft wastewater samples. All our samples were inhibitors free
20	What is the residence time of the sewage before it reaches the wastewater treatment plants ? Is there a testing of the influent into the wastewater plant as well as the outflow ? Is there degradation noticed ? Finally are there infectivity tests for the samples (if wastewater is to be reused say for drinking)	-	
21	Do you think ddPCR would still be a viable method for variant detection in a situation where multiple, highly-similar variants circulate without any being truly dominant, or is such a situation unlikely in your opinion?	-	
21	From last one six months our four persons operating the sewage treatment plant had never been effected with Covid or Omicron . All of the operators are fully Vaccinated now. Do we require advance pre caustions for the operators ?	Warish Ahmed	So far, there is no evidence that SARS-CoV-2 is transmitted via exposure to wastewater or stool.

Answered questions

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#	Question	Panelist	Answer
1	Question to Dr. Medema: Do you have any success of converting the RT-ddPCR assays targeting single mutations to RT-qPCR assays? Not all the labs have RT-ddPCR machines, and RT-ddPCR takes a much longer time to finish comparing with RT-qPCR.	Gertjan Medema	Dear Anda Quintero. We have not gone through such steps. Other research teams have been using RT-qPCR (https://pubs.acs.org/doi/10.1021/acs.estlett.1c00375 or https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8262398/)
2	Did the Netherlands take any action based on the omicron findings in the wastewater?	-	Not 'only' on the wastewater data. Both clinical and wastewater surveillance indicated emergence, and a lockdown (schools, restaurants, non-essential shops closed etc.) was called by our govt in mid-december
3	What does N501Y stand for? Is that the test used for testing for the presence of the variants? or a feature of the virus itself?	-	N501Y is a mutation that is present in the spike gene of the alpha variant and also of the omicron variant
4	Gertjan - when you say "%" in your analysis can you eloborate on what this means please. Is it the % of drops in which you find your target?	Gertjan Medema	Dear David, it is the percentage of positive variant drops. So if 1000 drops (of the 10-20000) are positive in total and 100 of those are positive for N501Y, we report 10% of the targets detected in the sample contains the N501Y mutation
5	What platform for ddPCR are you using?	-	Biorad QX200. Published in https://www.sciencedirect.com/science/article/pii/S0048969721045307
6	Detection of omicron variant through wastewater testing could be effective in developed countries because of the central system of wastewater collection in cities. How about countries like Nigeria and majority alike in Western Africa where wastewater collection	-	Dear Chukwuemeka Uyo. There are initiatives in several countries (Ghana, Malawi, South Africa, Nepal and maybe others) that attempt to conduct this surveillance in non-sewered settings. Single households will be a challenge, what is used in these studies

the international water association

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	and treatment is mostly done by individual households?		are streams/drains in urn=ban areas, tanker trucks, public toilets).
7	Question to G.Medema: Have you tested other dd-PCR tests, such as the test for del69/70, also present in omicron?	Gertjan Medema	We did test del69/70 in November, then still negative. We will test is further
8	Congratulations on this great presentation and initiatives. Specially when facing low viral loads (low concentration on ww samples) and when it comes to VoC screening what is the importance of a sample concentration step?	-	Dear Rui. Yes, low viral loads are more challenging. In our dd-PCR less drops will turn positive and hence the sensitivity of the assay and the establishment of the % of omicron is reduced. Good methods for concentration of the virus, without concentration of PCR inhibitors, woud be very valuable
9	Hi E. Leenan - we have organised a discussion with Austria, Switzerland and Ontario (and us, England) about these observations - would you like an invite?	-	Hi E. Leenan - we have organised a discussion with Austria, Switzerland and Ontario (and us, England) about these observations - would you like an invite?
10	We rely to know signature mutations for detction of VOC? how can WW sequencing be used to idenify new mutations/ variants in the future?	-	Dear Mukhlid Yousif. Very true. Of course, if we use NGS and sequence what is seen in wastewater, we may be able to pick up the emergence of new variants in the population via environmental surveillance. But this is yet to be demonstrated.
11	Did the WBE techniques allow to trace back the earliest possible days when Omicron emerged? (possibly even earlier than the first day Omicron was discovered)	-	yes…we definitely saw omicron before clinical case reporting did
12	Question to Dr. Boehm, which RT-PCR methods are you using, RT-qPCR and RT-ddPCR?	Alexandria Boehm	rt-ddPCR
13	Dear Alexandra. I'm interested in your units of cp/g. Is this just the weight of the dried sludge sample?	Alexandria Boehm	Yes… we calculate the dry weight and normalize per gram solids

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14	Ali - do you know what Omicron sub-types you are detecting?	Alexandria Boehm	Yes, see response to comment above.
15	Gertjan - Ali: Do you have any data on the comparison of the sensitivity of RT-qPCR versus RT-ddPCR, for the same assay. I have some concern about the sensitivity of the RT-ddPCR.	Gertjan Medema	Hi Leslie. Yes, the sensitivity of our ddPCR assays is less (about half) than of our RT-qPCR assay on the N1-gene fragment.
16	Wastewater solids and direct extraction, Which approach or kit are you using for RNA extraction?	Alexandria Boehm	You can find the detailed methods on protocols.io or in our papers. You can look on my website or email me for those
17	A question for Ali similar to the above, is how was the solid sample collected to be representative ?	Alexandria Boehm	the solid sample is representative as collected from the primary clarifier - represents a mixture of solids settled over the last few hours. Also at some of our plants, we settle solids from composite influent samples using imhof cones. All these samples are highly representative based on the data we have collected and how well it relates to contributing population health
18	Follow up for Alexander - have you made any progress on linking a cp/g to a population, or cp/litre of wastewater?	Alexandria Boehm	There is no reason to link it to cp per liter wastewater. the concentration in cp/g correlates strongly to incidence rates
19	'@Drs. Bivins and Ahmed, would you recommend more widespread use of wastewater testing on aircraft or is there still more research needed first?	Warish Ahmed and Aaron Bivins	
20	For routine testing of wastewater, from the discussion and presentations it seems that RT-qPCR or ddPCR based targeted assays maybe ideal vs NGS if time was of the essence. Can the panelists	-	For routine testing of wastewater, from the discussion and presentations it seems that RT-qPCR or ddPCR based targeted assays maybe ideal vs NGS if time was of the essence. Can the panelists

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	comment on these two technologies for a routine testing program?		comment on these two technologies for a routine testing program?
21	In the Netherlands and the californa samples can you clarify these were from primary settlement tanks or a solids concentrating device in the influent?	-	in CA - some of the plants settle solids from the influent. and others are from the primary clarifier. both methods provide excellent associations with COVID-19 incidence rates
22	On the 50:50% example in ddPCR: What do we know about different shedding behavior of Omicron vs. Delta?	-	no data yet on that!
23	Follow up to Ali: In California what kind of device were you using to concentrate the soilds from the influent - is there a devise available commercially? Would this be feasible in nearer source settings?	Alexandria Boehm	Imhof cones are used for settling solids from influent. the sample from the primary clarifier is already thickened