23 July 2025

Asia PGI Webinar: Defining priorities for wastewater-based pathogen surveillance – Q&A

1. Can you comment on the importance of quantitative levels of pathogens detected in WWS. Seems that what is important is how levels change over time which can be correlated with the intensity of community transmission. This can be done with qRT-PCR, but what is your advice regarding how to best apply tNGS in this regard?

   Quantitative pathogen detection levels help us understand fluctuations in pathogen load over time and across different locations. This can be further combined with tNGS to identify the variants or subtypes driving these patterns.

2. Could you please elaborate on the detection of vector borne diseases by WES?

   A few studies have detected dengue using WES (https://doi.org/10.1101/2023.10.27.23297694; https://doi.org/10.1016/s2666-5247(24)00150-2); however, it has never been identified in tropical countries like Nepal and India. A reference to share- https://doi.org/10.1016/j.pt.2023.12.005

3. Could you please let us know what sampling methods you usually use, e.g. grab, composite, or passive?
   We used grab sampling method.

4. Can you name the two pipelines which you compared?

   Two bioinformatic pipelines were used for influenza subtypes: the first utilizes NCBI influenza genomes, which include influenza A, B, and C, while the second pipeline focuses solely on influenza A subtypes and employs a conservative approach.

   https://www.medrxiv.org/content/10.1101/2025.01.13.25320458v1

5. When you mentioned Dengue, I didn't quite catch what you thought the main reasons/challenges were with detection?

   Inhibitors in sewage make the virus unstable, and high temperatures degrade it quickly in the environment. We have used both metagenomics and PCR-based approaches to detect dengue in wastewater. A reference to share- https://doi.org/10.1016/j.pt.2023.12.005

6. You have detected salmonella typhi in wastewater, but what about presence of salmonella paratyphi .. what's your experience?

   The panel we are testing also includes paratyphi.

7. What concentration techniques do you primarily use, such as Nanotrap particles, ultrafiltration, or $MgCl_2$ flocculation? Do you apply different concentration methods depending on the target virus, for instance, arboviruses versus respiratory viruses? Also, is it feasible to target both arboviruses and respiratory viruses from a single sample using a common concentration and extraction protocol?

   We use PEG/NaCl protocols that work well for both DNA and RNA viruses. Spiking experiments have been conducted to determine the limit of detection regarding concentrations and extraction efficiency.

8. Do you have any suggestions or experience in non-urban settings?

   Our sampling in non-urban settings, such as agricultural fields, lakes, and tubewell wastewater, has been successful using the same protocols.

9. What about enteric pathogens in wastewater?

   Yes, we have recorded several enteric pathogens, including rotavirus, norovirus, and Aichi virus.

10. What fraction of SARS genome was sequenced out of total samples screened via PCR? What were the assumption/s behind the sequencing approach? Were you able to retrieve whole genome from wastewater, considering all the background and circulating variants in the wastewater?

    We sequenced nearly all positive samples to understand the variants circulating during peak surge versus non-surge periods in the community. Genome coverage is better during the surge period.

11. How do you determine the baseline for detection of pathogens, for example for SARS-CoV-2?

The limit of detection for our kit is 5 copies/ul.
Please check this publication for more details-
https://www.sciencedirect.com/science/article/pii/S2772368225000903#ack0010

12. For a country that is just about to start, what would be the best step in implementing WES, i.e. the initial steps? Can you please explain again about the site selection?

    Yes, site selection is crucial for understanding the sewage network, population of the catchment area, and other factors.
    Here is the link for guidelines for site selection-
    https://data.ccmb.res.in/apsi/protocol/

13. Which NGS platform (e.g. Illumina, Nanopore) does your team find to give better output for metagenomics of wastewater?

    We used Illumina because short reads work well with fragmented RNA/DNA in wastewater samples.

14. In selecting collection sites, do you need to screen them for fecal contamination, e.g. PMMoV, first?

    Yes, we used PMMoV as internal control.

15. What challenges did you face for interpreting the results of wastewater surveillance from the perspective of public health risk, especially to convince policymakers to take any required actions and to consider this as a sustainable surveillance tool?

    The main challenge is who will do the work, availability of labs, and funding.

16. What were your learnings in the development of the assays? What prevents including bacterial detection in the panels?

    Wastewater samples have a high concentration of inhibitors, so we often need to dilute the sample while simultaneously increasing the concentration of primers and probes.

17. How do you determine sample size from an area/wastewater systems, and do you think there's a certain threshold that we should target to be statistically coherent and confirm presence of the pathogen of interest?
Sample size depends on the study design, population size and sampling frequency depending on the pathogen of interest.