Wastewater monitoring for tracking of COVID-19 infections

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Introduction

Municipal wastewater collected by sewers comprises a heterogenic mixture of pollution introduced by the community. As most significant pollutions, the removal of the organic carbon as well as the nutrient species Nitrogen and Phosphorous is the most important goal of that purification step. Only recently, further constituents in wastewater, such as organic micropollutants, microplastics and antibiotic resistant bacteria, summarized under the term "contaminants of emerging concern", are addressed as significant pollution in wastewater, too. Nowadays, wastewater is considered as an integrating mirror of human society, depicting all substances used in domestic and industrial applications as well as all organisms released via human faeces. This "mirror" subsequently can be used to extract information e.g. on the use of pharmaceutical active compounds or illicit drugs by the population connected to the sewer catchment or reflect diseases spread among the people discharging into the sewer. The assessment of human health aspects in raw wastewater resulted in the term "wastewater epidemiology" that received high attention during the current Covid-19 pandemic.

Starting with scientists from the KWR Water Research Institute in the Netherlands very early in the pandemic, a global effort was undertaken to investigate and apply wastewater epidemiology for SARS-CoV-2 monitoring in the population. Wastewater monitoring integrates all persons in the sewer catchment releasing viral particles, avoids abundance bias resulting from human testing capacities and strategies, and is completely anonymous.

Workflow

The methodological base of any SARS-CoV-2 monitoring in wastewater is the detection and quantification of the viral genome via qPCR, the same molecular biological tool applied like in human testing. In the following section, a brief overview on the methodology and special equipment necessary is given. Within the frame of the given EUWI+ initiative, the focus will be on a simplified approach that could be implemented even without a wastewater treatment plant itself.

1. The first step is the collection of a representative daily composite sample of raw wastewater. Usually this is done by a flow proportional to the integrated sampling (CVVT - Constant Volume Variable Time) implemented at wastewater treatment plants, but e.g. time proportional samples collected by mobile autosamplers would also do it. Samples must not be frozen for storage but can be sent around for further processing by cooled transport e.g. in styrofoam boxes with cooling bags – or be stored for few days at 4°C.
2. Due to the very low abundance of the virus in wastewater (in the rough range of about 100 copies per mL), an enrichment step has to be done. By this step, the particles in about 80 to

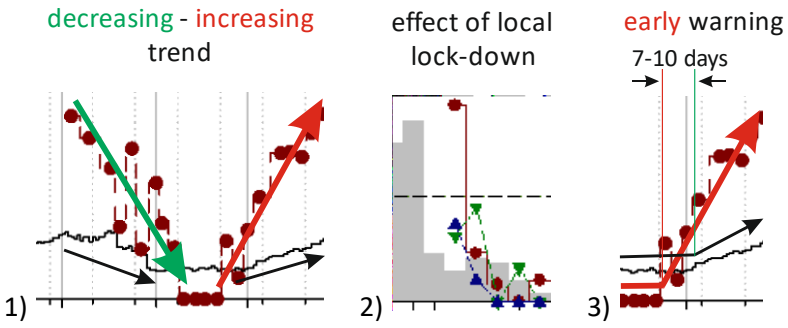
100 mL of wastewater are concentrated in a small volume of about 0.5 mL. Several options are available for that step, but most commonly a PEG (Polyethylene Glycol) precipitation involving high g-force centrifugation at 4°C is used.

3. After concentrating the virus in a small volume, the integrity of the virus is destroyed and the viral RNA genome is extracted by a special multi-step approach. This step is very sensitive, as RNA is degrading easily. Special kits developed and provided by a broad variety of supplies are suitable to perform the RNA extraction from the sample.
4. Before a subsequent quantification step by qPCR, the isolated RNA has to be reverse transcribed to DNA, as PCR is relying on DNA as target molecule. This reverse transcription usually is performed in a first step within the RT PCR reaction, where a quantification of one or more target sequences coding for different parts of the viral genome is quantified. Genes coding for S (spike), E (envelope), M (membrane), and/or N (nucleocapsid) proteins or subunits respectively can be used as target sequences.

Whereas the first two steps (sampling and enrichment) are specific to wastewater samples, the last two steps (RNA extraction and quantification) directly correspond to the approach used in human testing. Sampling and enrichment do not require special education and can be done after a brief training by skilled technicians. For RNA extraction and quantification, specialized laboratories and educated personnel are needed. A feasible alternative could be to transfer the samples into the human testing workflow after step 2.

Possibilities and Potential

Depending on the questions to be addressed and the goal of the monitoring, analysis of the raw wastewater can be done in different temporal resolutions. Despite the huge amount of potential methodological bias and uncertainties, the approach of SARS-Cov-2 monitoring in raw wastewater provides very important and valuable information that can supplement pandemic management:



1. Trend analysis is one of the most obvious results that can be obtained from regular monitoring. For following trends, even weekly samples showed to be sufficient.
2. Even for monitoring, the effect of pandemic management measures as e.g. the decrease of abundances after the implementation of a lock-down or other measures, a weekly sample could be indicative.
3. The approach of using wastewater monitoring as a predictive early warning system with a pre-warning time of about 7 to 10 days is not trivial at all and requires a more frequent sampling (at least 3 times a week), statistical algorithms and a fast processing of samples.

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