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DEVELOPMENT OF A METHODOLOGY FOR MONITORING SARS-COV-2 RNA IN WASTEWATER

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Abstract

Coronavirus disease 2019 is a contagious disease that occurs in humans caused by infection with the SARS-CoV-2 virus. Originally emerged in Wuhan, from Hubei Province, China, SARS-CoV-2 has spread rapidly around the world. In order to effectively combat this pandemic, a methodology must be found to be able to predict, early detect and monitor the extent of infections, which is vital for reducing the risk of transmission. Monitoring of SARS-CoV-2 in wastewater to detect and quantify the virus, to estimate the number of infected subjects in a population in a given area has proven to be very promising. Wastewater monitoring has already been implemented in several European countries, as well as in Australia, China and the United States. Although the fact that SARS-CoV-2 may be present in wastewater has been reported in several studies and is being given special attention, a standard procedure for monitoring SARS-CoV-2 in wastewater is still missing. Our goal is to design and propose, based on information reported already in the literature, an efficient methodology for detecting and quantifying SARS-CoV-2 RNA in wastewater. In addition, we also performed a comparison based on performance characteristics of the two methods used to detect and quantify SARS-CoV-2 RNA in wastewater, reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and digital PCR (dPCR).

Keywords*: Covid-19; SARS-CoV-2's RNA; Wastewater surveillance; RT-qPCR; RT-dPCR;*

Introduction

SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) is a highly infectious enveloped virus responsible for coronavirus disease 2019 (COVID-19). Among the measures that have been implemented to stop the spread of the disease are: clinical testing and isolation of infected individuals, follow-up of contacts, social distancing and with the increase in the number of cases, the imposition of traffic restrictions [1]. The environment's transmission pathways include soil, air, water and wastewater environments. The wastewater-based epidemiology (WBE) proved that it can surveilles viral outbreaks and provide a complementary clinical testing method [2]. The SARS-CoV-2 virus is a respiratory virus and can be excreted in saliva, sputum, but also in urine and feces, which has led to monitoring the spread of this virus inside communities by measuring SARS-CoV-2 RNA in wastewater [3, 4]. Numerous studies upon the monitoring of SARS-CoV-2 in wastewater systems [4–13] of different countries, including Spain [14], France [15], Italy [16] and others have been reported since the beginning of the Covid-19 pandemic.

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Since SARS-CoV-2 RNA detection in wastewater samples it is a rather difficult process that depends on a lot of factors, it is necessary to find an optimized and clear methodological framework for the detection and quantification of SARS-CoV-2 RNA in wastewater. In this review, we attempt to establish a methodology for the extraction and detection of SARS-CoV-2 RNA in wastewater samples based on information published in the scientific literature.

Experimental part

Methods

At the moment of conducting this review study, 575 articles have been identified in the PubMed database between the 1st of January 2020 to the 25th February 2022. The "wastewater" and "SARS-CoV-2" keywords were chosen for the search engine.

Results and discussion

As mentioned before in the method section, a total of 575 were obtained from PubMed database. This led us to the conclusion that we are in front of a topic of great interest, which is given special attention with respect to detection of SARS-CoV-2 RNA in wastewater through PCR techniques. In figure 1 we can see the great interest showed for this domain.

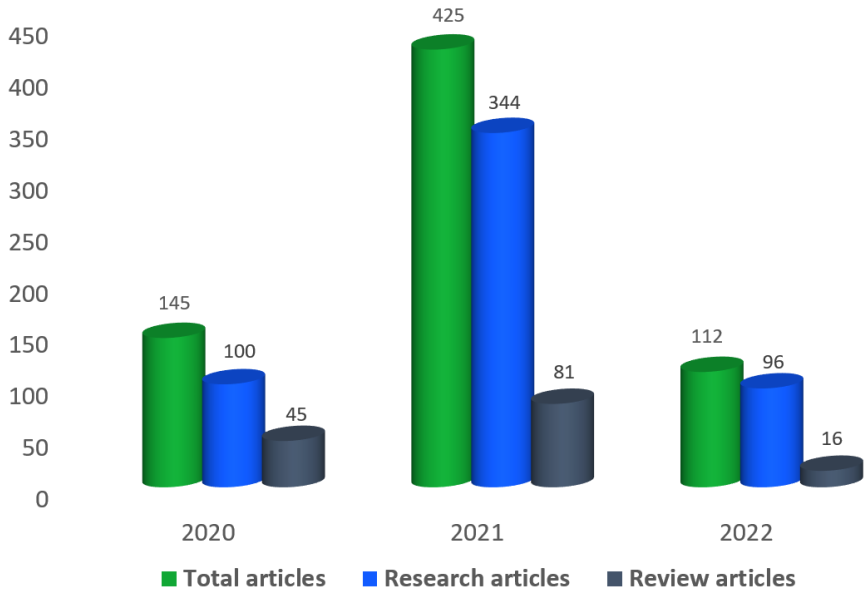

Fig. 1. Available published literature related to SARS-CoV-2 in wastewater (Found in PubMed, between the 1st of January 2020 to the 25th February 2022)

The study of the articles helped us identify several main steps required for the analysis of SARS-CoV-2 in wastewater.

Wastewater sampling

It is the first step in detecting SARS-CoV-2 RNA in wastewater. Its correct realization depends on several variables such as: sampling technique (which can be grab sample or 24-h composite sample), frequency (which can be hourly, multiple days per week daily, weekly, or bi-weekly) and sample type (influential or primary/secondary/treated effluent). Relying on [17], [18] believes, based on defecation frequency and timing (which is is most frequent in the early

morning), that the concentration of SARS-CoV-2 RNA in wastewater, vary diurnally, as well as the sampling technique and frequency. They recommended to take composite wastewater samples in the morning using an autosampler, but if it is not available, it is preferable to take samples using the grab sampling method [5, 18, 19].

Storage of wastewater samples

Storage conditions can influence the detection of SARS-CoV-2 RNA in wastewater samples. It is recommended that wastewater samples be transported on ice from the wastewater treatment plant to the laboratory. After arrival at the laboratory, samples should be kept at 4℃ and should be concentrated as soon as possible. Wastewater samples can be stored at 4℃ 1-5 days. Because the effects of storing wastewater samples at -20 and -80℃ are not yet known and therefore it is recommended to avoid. In order to inactivate bacterial activity several studies recommend that wastewater samples should be stored at 20℃ [20]. This approach may degrade SARS-CoV-2 RNA genetic material because of freezing and de-freezing process. In the opinion of [21] it is better to store the concentrated wastewater at -80℃, where RNAses are missing from the probe rather than the row wastewater. The storage duration is also important. It is preferable that the samples be analyzed as soon as possible, even if SARS-CoV-2 virus may survives for approximate two days at 20℃ and 1-4 days at 4℃ [21].

The concentration of wastewater samples

In general, SARS-CoV-2 concentration levels are very low in wastewater especially at the beginning or at the end of an epidemic. In 2020 study, [22] compared seven concentration methods and estimated the recovery of SARS-CoV-2 RNA in wastewater. This group suggested that absorption-extraction methods can provide rapid and straightforward recovery of SARS-CoV-2 in wastewater. The best recovery in electronegative membrane was observed by the addition of $MgCl_2$ to the wastewater prior to filtration. However, recoveries depended on the sample analysis volume and the filter type [19, 20].

The study of the articles helped us identify several main steps required for the analysis of SARS-CoV-2 in wastewater. These are presented in Figure 2.

Isolation of SARS-CoV-2 from concentrated wastewater samples

SARS-CoV-2 RNA extraction procedures, the concentration of the sample, purity and integrity of the extracted RNA, along with the risk of cross-contamination with another DNA/RNA that may be present in the laboratory, on surfaces or in the air, should be considered in order to obtain high quality SARS-CoV-2 RNA for PCR analysis [19, 22]. SARS-CoV-2 RNA recovery can vary greatly depending on the kits used, and their performance can vary considerably between manufacturers. Some studies used 140-450mL of concentrated samples for RNA extraction and obtained 30-100mL of RNA viral extract. Usually, RNA extraction kits have fewer steps and are equipped with PCR inhibitor (for the RNAses, polysaccharides, metal ions and other substances that are present in wastewaters) removal techniques which makes them more useful to reduce the chance of contamination and inhibition. Inhibitory substances may inhibit PCR amplification, and this can lead to false negative results. To avoid this, it is recommended that each wastewater sample be inoculated with a surrogate virus as a control process. The selected virus is preferable to be morphologically and genetically similar to SARS-CoV-2 but not present in the sample. For SARS-CoV-2, there are some animal coronaviruses, such as murine hepatitis virus, bovine coronavirus, or infectious peritonitis virus, which are ideal controls [19, 22–24].

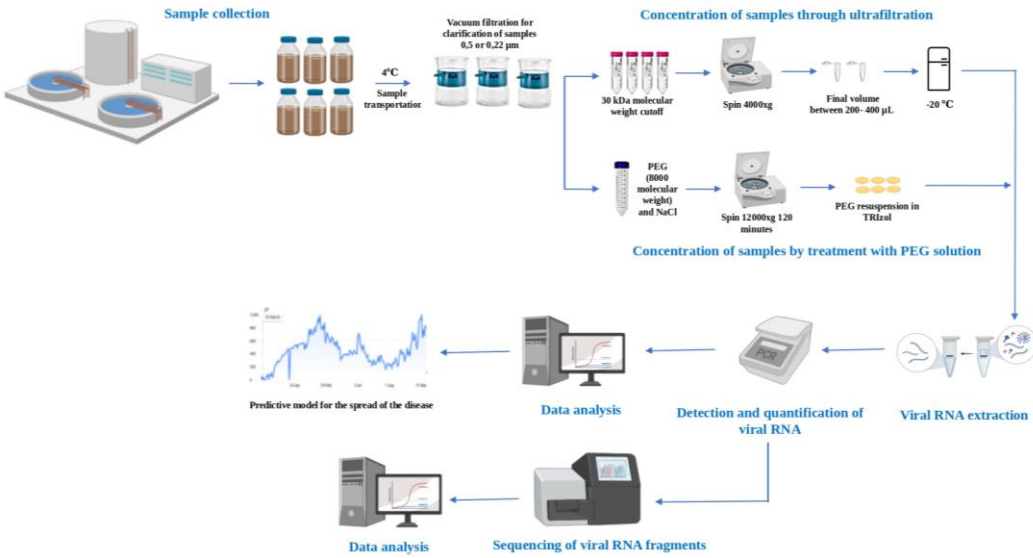

Fig. 2. The most common steps for SARS-CoV-2's RNA analyses in wastewater

Detection and qualification of SARS-CoV-2 of SARS-CoV-2 RNA in wastewater

For the detection and quantification of SARS-CoV-2 RNA in wastewater, the most used methods are RT-PCR, RT-qPCR, nested PCR and digital PCR [25–30]. These PCR technologies use different equipment, protocols, reagents and analysis methods, which leads to a absence of methodological consistency.

RT-qPCR is the most used method for detection of SARS-CoV-2 RNA in wastewater samples, due to its high level of sensitivity, high throughput, laboratory availability and relative low cost. Digital PCR is a rapid and extremely sensitive technique. Also, is more advantageous over qPCR due to executing absolute quantification, without the requirement of any standards. One of the advantages of dPCR vs. qPCR is the direct absolute quantification of SARS-CoV-2 genome copy numbers in a sample without the necessity of external calibration. The disadvantages of dPCR method include expensive instrumentation and high cost reagents [28].

Sequencing

Sequencing methods can be utilized to identify SARS-CoV-2 from wastewater samples and to eliminate false positive results. It could be a powerful tool to complement clinical surveillance. In addition, NGS analysis of wastewater samples infected with SARS-CoV-2 can help in assessing changes in viral diversity, which can indicate the emergence of epidemiologically or clinically relevant mutations and thereby aid public health decision-making. Some disadvantages of sequencing methods are complex setup and high costs, Also, the need for expertise, limit its application in analysis of wastewater [27, 28].

Conclusions

Monitoring of SARS-CoV-2 in wastewater is a very useful approach for detecting viral infections among populations, but there are still several limitations and challenges. In order to obtain accurate results a standardized procedure is required. From sampling to viral quantification, all steps should be evaluated and validated. Wastewater samples should be taken and transported on ice to laboratories where they should be stored at 4°C and processed as soon as possible within 2-3 days. Several sample concentration protocols are available and may be

useful for SARS-CoV-2 recovery. Still their performance can vary between samples and therefore appropriate process controls must be used. qPCR and dPCR methods have been shown to detect SARS-CoV-2. However, a special attention must be given to these methods because can be affected by inhibitors. By following these guidelines, reliable and actionable SARS-CoV-2 RNA concentrations can be obtained in wastewater.

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