Journal of Korean Society on Water Environment, Vol. 40, No. 1, pp. 19-35 (January, 2024)
pISSN 2289-0971 eISSN 2289-098X https://doi.org/10.15681/KSWE.2024.40.1.19

총 설

SARS-CoV-2의 하수조사를 위한 대체 및 신속 검출 방법

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Alternative and Rapid Detection Methods for Wastewater Surveillance of SARS-CoV-2

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(Received 8 September 2023, Revised 4 December 2023, Accepted 27 December 2023)

Abstract

The global pandemic, coronavirus disease caused by Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to the implementation of wastewater surveillance as a means to monitor the spread of SARS-CoV-2 prevalence in the community. The challenging aspect of establishing wastewater surveillance requires a well-equipped laboratory for wastewater sample analysis. According to previous studies, RT-PCR-based molecular tests are the most widely used and popular detection method worldwide. However, this approach for the detection or quantification of SARS-CoV-2 from wastewater demands a specialized laboratory, skilled personnel, expensive instruments, and a workflow that typically takes 6 to 8 hours to provide results for a few samples. Rapid and reliable alternative detection methods are needed to enable less-well-qualified practitioners to set up and provide sensitive detection of SARS-CoV-2 within wastewater at regional laboratories. In some cases, the structural and molecular characteristics of SARS-CoV-2 are unknown, and various strategies for the correct diagnosis of COVID-19 have been proposed by research laboratories. The ongoing research and development of alternative and rapid technologies, namely RT-LAMP, ELISA, Biosensors, and GeneXpert, offer a wide range of potential options not only for SARS-CoV-2 detection but also for other viruses. This study aims to discuss the effective regional rapid detection and quantification methods in community wastewater.

Key words : Rapid detection, RT-LAMP, SARS-CoV-2, Wastewater surveillance

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1. Introduction

Coronavirus disease 2019 (COVID-19) has become a pandemic worldwide, resulting in nearly 6.7 million deaths (World Health Organization, 2022). The SARS-CoV-2 viral particles or associated genetic fragments are excreted in the stool and body fluids of infected individuals (Tran et al., 2021). Therefore, wastewater surveillance for the SARS-CoV-2 pathogen is an effective way to track the health of entire communities. Wastewater surveillance serves as a sensitive indicator to determine the magnitude of SARS-CoV-2 circulation within the population and if its transmission is on the rise or decline. This global approach for addressing COVID-19 emphasizes the potential of wastewater data to complement existing established epidemic control measures. SARS-CoV-2, has already been detected in many wastewater treatment plants (Medema et al., 2020; Randazzo et al., 2020) during the early stage of the pandemic (Ahmed, Angel et al., 2020; Fernández-de-Mera et al., 2021; Haramoto et al., 2020; La Rosa et al., 2020; Medema et al., 2020; Sherchan et al., 2020). A robust population-scale testing strategy for SARS-CoV-2 based on rapid, reliable, decentralized, and inexpensive diagnostic testing is a high priority for clinical testing and wastewater monitoring. Consistent with mask-wearing, frequent hand washing, and social distancing, this testing approach could be sufficient to prevent and contain major outbreaks while COVID-19 immunization programs are underway. Therefore, quantifying SARS-CoV-2 in wastewater treatment plant allows for monitoring the infection among the community via wastewater-based epidemiology (WBE) (Ahmed, Bivins et al., 2020). However, wastewater surveillance is beneficial for early warning and monitoring of disease outbreaks and to inform the effectiveness of public health interventions against enteric viruses such as previously demonstrated norovirus, hepatitis A virus, and poliovirus (Asghar et al., 2014; Hellmer et al., 2014).

Wastewater surveillance for COVID-19 provides many benefits and is a cost-effective way to investigate the transmission dynamics of an entire community (Larsen and Wigginton, 2020).

Particularly in regions lacking access to clinical testing or facing unavailability, as well as in areas with a high volume of patients, wastewater-based surveillance offers an alternative solution to quantify disease trends at the population level (Beattie et al., 2022). At present wastewater surveillance of SARS-CoV-2 RNA has been used in at least 55 countries to monitor the presence and support management of COVID-19 in many Communities (Ahmed, Angel et al., 2020; Bertrand et al., 2021; Carrillo-Reyes et al., 2021; Gibas et al., 2021; Kumar et al., 2020; Medema et al., 2020; Naughton et al., 2021; Navarro et al., 2021; Prado et al., 2020; Randazzo et al., 2020; Rimoldi et al., 2020; Westhaus et al., 2021). For the monitoring of COVID-19 through wastewater surveillance, a set of intricate environmental microbiology methods are employed. These procedures encompassed wastewater sampling techniques, isolation of genetic fragments from complex wastewater matrices leading to the identification and quantification of viral RNA. This primary method employed for this purpose involved utilizing polymerase chain reaction (PCR)-based assays (Ahmed, Angel et al., 2020; Ahmed, Simpson et al., 2022; Pecson et al., 2021). However, wastewater samples often contain inhibitors, such as pharmaceuticals, personal care products, household detergents, industrial effluents, and metals which, may affect PCR amplification (Cao et al., 2012; Schrader et al., 2012). PCR inhibition can be minimized using digital PCR (dPCR) (Ahmed, Smith et al., 2022, Tiwari et al., 2022). However, sample analyzing using dPCR is expensive and often not high throughput. Besides this, it requires trained personnel to perform and interpret results. The availability of microfluidic technologies is a critical barrier, and many reagents and equipment are unavailable in underdeveloped countries where they are more vulnerable to viral infections (Kojabad et al., 2021).

This review paper particularly focused on the rapid and alternative methods which are needed for SARS-CoV-2 RNA detection in wastewater for routine wastewater monitoring and the social implementation of diseases surveillance. The developed methods described in this study are efficient and applied virus detection systems with comparable reliable sensitivity. This paper provides an overview of current available methods used for virus concentration in wastewater and the sensitivity analysis for the specific recovery of SARS-CoV-2 in sewage.

2. Sampling Strategy, Handling and Storage

Wastewater sampling for the pathogen is used to evaluate the trends in infection within the community contributing water to the sewer system. According to the centers for disease control and prevention (CDC, NWSS), there are two primary sample collection methods for wastewater surveillance, grab and composite samples.

Collecting a grab sample is straightforward and does not require expensive auto sampler. The grab sample provides a snapshot of wastewater at the time of sample collection and could be less representative.

Composite samples are collected by putting multiple grab samples at a specified frequency over time, either by

continuous sampling or mixing discrete samples. Collection of composite samples can be possible manually or by using automated samplers. A composite sample represents the average wastewater characteristics during the compositing period. Composite samples are more representative of fecal community contributions than grab samples, and 24-hour composite sample is a more reliable daily average of viral concentration (Sherchan et al., 2020). The suggested sampling depth for surface water samples should be 6 - 12 inches below the water surface.

Wastewater samples containing SARS-CoV-2 must be managed in accordance with guidelines. During transit to the laboratory, water samples should be either iced or refrigerated at a temperature below 10°C. It is crucial to prevent samples from freezing, and the use of insulated containers is recommended to maintain the storage temperature effectively. Additionally, ensure that sample bottles are tightly closed and remain above the water level during transportation. The experiment should be done as soon as possible after the collection of samples. Also, sample storage and pre-treatment steps including temperature, time, and handling may impact the concentration of virus recovered (Ahmed, Smith et al., 2022, Islam et al., 2022). Therefore, many research for WBE of pathogens has focused on developing the best practices for viral concentration, extraction, and quantification (Ciesielski et al., 2021; LaTurner et al., 2021; Perez-Cataluna et al., 2021) however, a better understanding of sample storage and pre-processing steps is necessary to ensure effective detection and recovery regardless of the methods used.

3. Concentration Methods

Throughout the Coronavirus 2 (SARS-CoV-2) pandemic, a range of strategies has been implemented to detect the virus's spread in the population. Wastewater-based epidemiology (WBE) has emerged as an excellent tool for assessing viral circulation in communities. To ensure reliable results, (Salvo et al., 2021) assessed three low-cost virus enrichment methods: polyethylene glycol (PEG) precipitation, skim milk flocculation (SM), and aluminum polychloride flocculation (PAC). They utilized *Pseudomonas aeruginosa* bacteriophage PP7 as a surrogate for non-enveloped viruses and Bovine Coronavirus (BCoV) as a surrogate for enveloped viruses, with a specific focus on SARS-CoV-2.

The research findings indicate that PEG precipitation is a suitable approach for virus concentration, proving effective for both enveloped and non-enveloped viruses in wastewater. It demonstrates greater sensitivity compared to SM flocculation and PAC flocculation. Moreover, a literature review reveals that many other countries have also adopted PEG precipitation methods to concentrate SARS-CoV-2 nucleic acids (Table 1). This methodology can be applied in WBE studies to monitor the dynamics of the SARS-CoV-2 pandemic, especially in developing countries with limited economic resources.

3.1 PEG precipitation method

Polyethylene glycol (PEG) precipitation is one of the most conventional methods for virus concentration (Haramoto et al., 2018; Lewis and Metcalf, 1988; Torii et al., 2022). As PEG is an inert and biocompatible polymer, PEG is

Table 1. Concentration methods used to detect SARS-CoV-2 nucleic acids in different countries wastewater treatment plants

Country	Sampling site	Sample volume (ml)	Concentration method	References
China	Sewage	100	Subjected to polyethylene glycol precipitation	Zhang, Ling et al., 2020
Japan	Sewage	200-5000	Electronegative membrane-vortex (EMV) and membrane adsorption	Haramoto et al., 2020
Australia	Sewage	100-200	Electronegative membrane filter Ultrafiltration	Ahmed, Angel et al., 2020
USA	Sewage	40	Polyethylene glycol (PEG) precipitation	Wu et al., 2020
Brazil	Sewage	40	Ultracentrifugation	Prado et al., 2020
Spain	Sewage	200	Aluminum flocculation (beef extract precipitation)	Randazzo et al., 2020
France	Sewage	11	Ultracentrifugation	Wurtzer et al. 2020
Italy	Sewage	250	Polyethylene glycol (PEG) precipitation/dextran	La Rosa et al., 2020
Germany	Sewage	45	Ultrafiltration	Westhaus et al., 2020
Netherlands	Sewage	250	(PEG) precipitation	Medema et al., 2020
India	Sewage	50	PEG precipitation	Kumar et al., 2020
Turkey	Sewage	250	Ultrafiltration and PEG precipitation	Kocamemi et al., 2020
Israel	Sewage	250 – 1000	PEG/alum precipitation	Bar Or et al., 2020

preferentially applied for trap solvents and acts as an “inert solvent sponge” (Atha and Ingham, 1981). When the concentration exceeds the saturation solubility (Atha and Ingham, 1981; Lewis and Metcalf, 1988), PEG methods are frequently applied for the concentration and precipitation of proteins where sequestrating water molecules from the solvation layer around the proteins of the viral capsid, enhancing the virus-virus interactions and resulting in the precipitation (Torii et al., 2022). The advantages of PEG precipitation are that it can be performed using essential laboratory equipment (Ahmed, Bivins et al., 2020) with relatively low running costs compared to other methods (e.g., ultrafiltration). Other studies have also reported the applicability for the detection of SARS-CoV-2 RNA in wastewater (Hata et al., 2021; Kumar et al., 2020; Torii et al., 2021; Wu et al., 2020) and resulted in high efficiency in the recovery of RNA viruses (Amdiouni et al. 2012). The PEG method is beneficial for concentrating viruses from wastewater samples, given the presence of multiple DNA/RNA viruses in such samples (Adriaenssens et al., 2018; Ng et al., 2012). Also, the procedures of PEG precipitation methods are primarily dependent on executors, like several analytes as supernatant or filtrate of raw wastewater and non-pretreated raw wastewater were added with a different concentration of salt and PEG and the incubation time for the precipitation varied from 0 h to overnight incubation (Ahmed, Angel et al., 2020; Alexander et al., 2020; Barril et al., 2021; Chavarria-Miró et al., 2021; D’Aoust et al., 2021; Gerrity et al., 2021; Graham et al., 2021; LaTurner et al., 2021; Pecson et al., 2021; Pérez-Cataluña et al., 2021; Philo et al., 2021; Sapula et al., 2021; Torii et al., 2021).

In their 2021 report, Pecson et al. highlighted varied process recovery efficiencies (ranging from 0.03% to 78%) for human coronavirus OC43 using PEG precipitation methods. Interestingly, these discrepancies were observed even when employing identical wastewater samples. A drawback of this method is that PEG induces the precipitation of diverse proteins, including enzymes. This precipitation may interfere with or inhibit subsequent viral genome detection through PCR amplification methods, leading to non-selective precipitation (Masclaux et al., 2013; Shieh et al., 1995).

3.2 Skim milk and Aluminum polychloride flocculation

Skim milk flocculation, initially developed (Calgua et al. 2008) as the primary concentration method for adenovirus recovery from seawater, is also employed for retrieving viruses from wastewater samples. The process involves three key physical steps: i) the virus adsorbs to pre-aggregated skim milk proteins, ii) flocs containing the adsorbed virus precipitate, and iii) the precipitate dissolves in a phosphate buffer solution. In a previous study, a successful combination of elution with glycine buffer and skim milk flocculation was employed to recover HAdV, JCPyV, and NoVGII from raw municipal sewage samples (Calgua et al., 2013; Salvo et al., 2021). Their study shows that PEG precipitation and skim milk flocculation have a similar percentage of recovery for enveloped and non-enveloped viruses using PP7 and BCoV as surrogates of each one. Another study shows skim milk flocculation for HAdV and RoV recovery from WWTP wastewater samples (Assis et al., 2018). They also revealed that higher recoveries of HAdV and RoV were obtained by eliminating the initial centrifugation step and doubling the concentration of skim milk. The centrifugation step was eliminated because the treated effluent contained less solids. The advantages of this concentration method are that a large number of samples can be concentrated because no special equipment is required, and the number of processing steps is reduced (Calgua et al., 2008).

The aluminum polychloride (PAC) flocculation concentration technique exhibited high efficiency in the recovery of feline calicivirus (FCV) from wastewater. To mitigate the risk of handling SARS-CoV-2, FCV was utilized as a process control for this concentration technique. Among eleven concentration methods, two protocols, one based on PEG precipitation and the other on PAC flocculation, demonstrated notable effectiveness in FCV recovery from wastewater (62.2% and 45.0%, respectively). Subsequently, both methods were tested for the specific recovery of SARS-CoV-2. The PAC flocculation technique exhibited a lower limit of detection (4.3×10^2 GC/mL) compared to PEG precipitation (4.3×10^3 GC/mL) (Barril et al., 2021). However, the study revealed that while this method recovered PP7 with a low percentage of efficiency, it did not successfully recover BCoV. Consequently, aluminum polychloride flocculation exhibited lower recovery efficiency and success in viral concentration compared to PEG and SM flocculation methods (Salvo et al., 2021).

4. Extraction Methods

All viruses possess genome materials that are either RNA or DNA (Artika et al., 2020). The viral genomic material can be classified as either single-stranded or double-stranded, with nucleic acid strands having positive (+) or negative (-) polarities. The structure of the viral genome may be linear or circular, and viruses can have either segmented or complete genomes (Guttman, 2013; Murphy, 1988; O’Carroll

and Rein, 2016). In most PCR-based amplification processes, the template is DNA; however, in the case of RNA viruses, the RNA is reverse-transcribed into complementary DNA (cDNA). The quality and purity of these bio-macromolecules significantly affect the efficiency of amplification and quantification methods. The isolation and purification of DNA/RNA involve dissolution, purification, and recovery steps. DNA extraction methods encompass boiling, column methods, magnetic beads, and FTA cards (Barbosa et al., 2016).

Studies focusing on virus detection in wastewater samples often rely on commercially available DNA and RNA kits. The most common DNA extraction kits utilize columns with silica-based membranes (Barbosa et al., 2016), categorized as solid phase-DNA extraction methods (Barbosa et al., 2016; Butler, 2010). Examples of silica-based membrane kits frequently used for extracting viral nucleic acids from wastewater samples include those mentioned by Barbosa et al. (2016).

For RNA extraction, researchers commonly employ kits such as the RNeasy Power Microbiome kit and RNeasy Water Kit (Ahmed, Bertsch et al., 2020; Ando et al., 2022). Automated extractors, as utilized by Ibrahim et al. (2017) and Di Bonito et al. (2017), facilitate the extraction of viral nucleic acids from influent and effluent wastewater samples. Most automated extractors use magnetic beads that bind to nucleic acids, leaving impurities in the solution. Elution is then performed to recover DNA bound to the beads (Barbosa et al., 2016). The advantages of using an automated extractor include high throughput and low variability of assay results (Dundas et al., 2008).

This review paper aims to provide guidelines for sensitive and cost-effective virus detection, aiding in the development, optimization, and validation of the SARS-CoV-2 assay to achieve successful virus detection and consistent measurements in wastewater samples. Immunoassays are employed when quantifying an unknown concentration of an analyte within a sample. To ensure accurate determination, an immunoassay must be developed based not only on standard assay development criteria but also on its ability to accurately measure the value of a wastewater sample. Firstly, there is a need to establish the critical success factors of the assay. Subsequently, the assay is developed to establish proof of concept. During the optimization phase, the quantifiable range of the immunoassay method is determined by calculating a precision profile in the matrix in which the

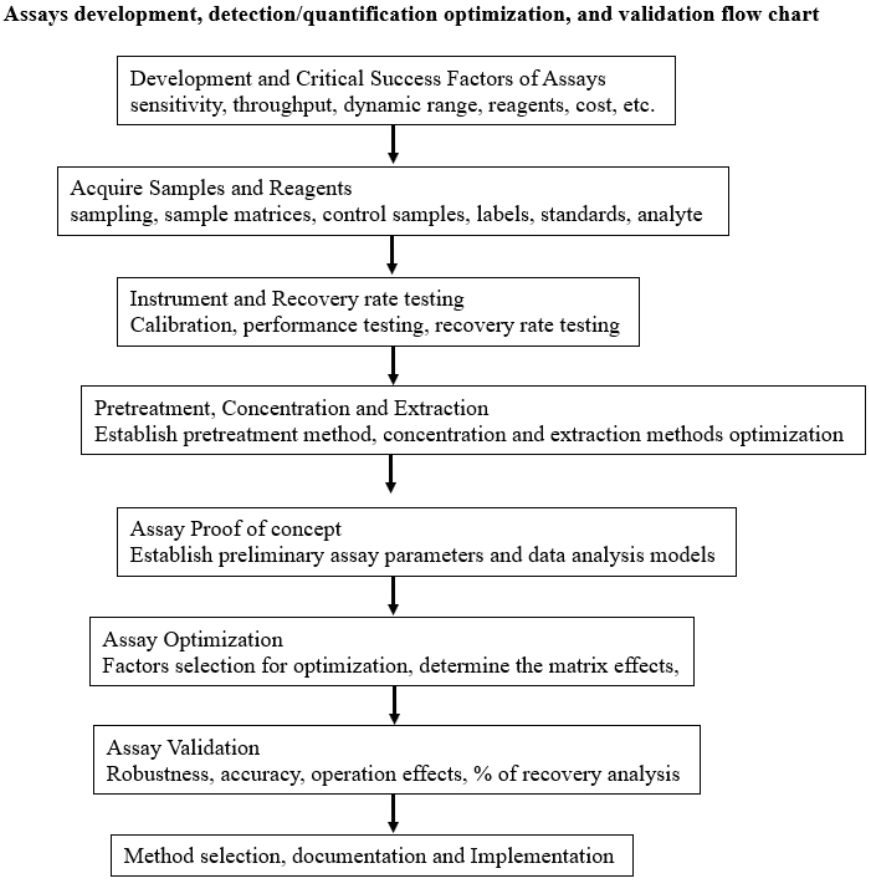

Fig. 1. Detection and quantification development, optimization, and validation flow chart.

experimental wastewater samples will be measured. A spiked recovery is then conducted by adding the analyte to the matrix and determining the percent recovery of the analyte in the matrix. If the precision profile falls within the desired working range, the immunoassay validation is completed by assaying spiked recovery samples over several days. However, if the precision profile limits do not meet the desired working range, further immunoassay optimization is necessary before validation (Cox et al., 2019). Fig. 1 depicts the flowchart illustrating the development, optimization, and validation processes for detection and quantification.

5. Alternative Detection Methods

5.1 RT-LAMP

The standard for COVID-19 testing is RT-PCR to detect the genetic material of SARS-CoV-2 in nasopharyngeal (NP) samples. Although highly reliable, RT-PCR diagnostics are complex, laborious, and expensive. Their global use needed more sample collection steps and reagents for viral RNA extraction early in the pandemic (Amaral et al., 2021). On the other hand, Loop-mediated isothermal amplification (LAMP) is a DNA amplification method that allows rapid and sensitive detection of specific genes (Nagamine et al., 2002; Notomi et al., 2000; Tomita et al., 2008). LAMP combined with reverse transcription (RT-LAMP) has been successfully used for the detection of several respiratory RNA viruses (Ahn et al., 2019; Bhadra et al., 2015; Hong et al., 2004; Jayawardena et al., 2007; Lee et al., 2017) including SARS-CoV-2 (Thompson and Lei, 2020). RT-LAMP stands out as a reliable substitute for RT-PCR, characterized by its exceptional specificity and sensitivity, cost-effectiveness, and rapid turnaround time, typically within 30 minutes. Because RT-LAMP amplifies the genetic material of viruses at a constant temperature and diagnostic tests based on RT-LAMP require only a heat block or a water bath, set to a single temperature and they can be performed anywhere essential resources are available. Reaction products can be analyzed via conventional DNA intercalation dyes, agarose gel electrophoresis, UV illumination, or real-time fluorescence (Quyen et al., 2019). Alternatively, end-point colorimetric readouts are also possible through the detection of reaction by-products, such as pyrophosphate and protons, which are released during DNA polymerization after the incorporation of deoxynucleotide triphosphates. LAMP colorimetric methods detect turbidity, triggered by the accumulation of magnesium pyrophosphate (Nagamine et al., 2002), or color changes, occurring when complexometric indicators (Goto et al., 2009; Tomita et al., 2008), pH-sensitive dyes (Tanner et al., 2015) or even DNA-intercalating dyes (Fischbach et al., 2015; Lamb et al., 2020; Park et al., 2020) are incorporated into the reaction. The simple technical and instrumental requirements of colorimetric RT-LAMP tests make them extremely attractive for point-of-care (POC) use and implementation in low-resource settings (Fig. 2). Colorimetric RT-LAMP has been successfully used for the detection of SARS-CoV-2 in NP fluids from COVID-19 patients (Anahtar et al., 2020; Buck et al., 2020; Butler, 2020; Dao et al., 2020; Huang et al., 2020; Kellner et al., 2020; Park et al., 2020; Rabe and Cepko, 2020; Yu et al., 2020; Zhang, Odiwuor et al., 2020).

Therefore, LAMP offers a practical and swift substitute for traditional PCR or qPCR in the viral context. The amplification in LAMP doesn't necessitate sophisticated equipment, as the reaction is maintained at a constant temperature, typically around 65 °C (Tomita et al., 2000). Many amplification methods are susceptible to contamination, often stemming from products of prior experiments transmitted through the environment, researcher attire, or laboratory apparatus. Contaminant products may serve as templates in new reactions, leading to false positives in certain instances (Dhama et al., 2014; Hsieh et al., 2014). In this regard, the LAMP process is notably vulnerable and responsive compared to alternative detection methods.

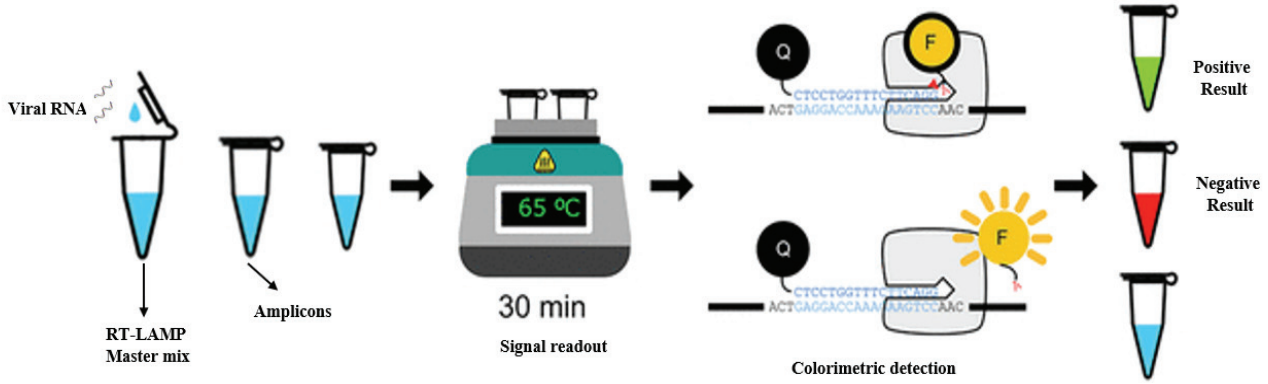

Fig. 2. Colorimetric RT-LAMP method.

Studies demonstrate the potential application of RT-LAMP for detecting SARS-CoV-2 in wastewater, offering a more cost-effective and expeditious alternative to RT-qPCR or RT-ddPCR for the epidemiological monitoring of COVID-19 and other viral infections (Amoah et al., 2021).

LAMP, developed by Notomi et al. in (2000), relies on the utilization of a minimum of four primers to initiate the polymerase-driven extension of the gene sequence.The mechanism of RT-LAMP is based on automated cyclic strand displacement DNA synthesis. In the LAMP reaction, polymerase gene amplification proceeds by repeating two elongation reactions that occur through loop regions. Two pairs of primers are used, inside and outside primer pairs. These primers are specifically designed for the reaction. Each internal primer is complementary to one amplification chain and has the same sequence as the internal region of the same chain. The elongation reaction is sequentially repeated by DNA polymerase-mediated strand-displacement synthesis with the stem mentioned above loop region as a step. This method works on the basic principle of producing large quantities of DNA amplification products with complementary sequences and alternating and repeating structures (Notomi et al., 2015).

However, a primer set to be used for detecting the SARS-CoV-2 virus using RT-LAMP has been developed. This assay can detect the virus even with low sample concentrations. The sample preparation for this can be carried out in just one tube within minutes. Furthermore, only three buffers, a pulse-spin mini-centrifuge, and a 65°C heat block are needed to apply this method at institutions.

RT-LAMP can achieve high specificity due to its targeting sequence. Unlike other techniques, RT-LAMP uses six independent sequences initially and four independent sequences later to recognize the target sequence. Primer recognition of the target genome results in a robust colorimetric response, allowing detection without requiring highly specialized or costly equipment. The primers designed for the target several key areas of coronavirus genomes, including the ORF1ab gene, S gene, and N gene. ORF1ab is involved in the replication of the viral genome, whereas the S gene is important for COVID-19 binding to human ACE2 protein. The N gene is a nucleocapsid protein conserved in most coronaviruses. A key improvement in the COVID-19 LAMP assay is the speed and ease at which it can be carried out.

Furthermore, the color change associated with the presence of viral RNA, at levels as low as 80 copies per mL sample, is visible by the eye, and therefore detection equipment is not needed. This was achieved by using a pH indicator. Amplification of nucleic acids causes the release of pyrophosphate and hydrogen ions, which lead to decreases in pH, therefore making it possible to combine RT-LAMP with a visible pH indicator to infer the presence of COVID-19. A similar method relies on the turbidity of the sample, which increases with the amount of genetic material, to measure viral content. Amplification and detection can also be performed by agarose gel analysis. Therefore, RT-LAMP can be one of preferable technology for using COVID-19 detection due to its accuracy and relatively simple equipment. This technology is possible to applied in non-standard institutions, such as airports or rural hospitals, medical centers, and wastewater treatment plants. Designing robust, field-based platforms that can withstand variations in environmental conditions will broaden the utility of RT-LAMP for on-site testing in both clinical and environmental surveillance scenarios. Addressing the present challenges and embracing future perspectives will contribute to the continued advancement and widespread adoption of the RT-LAMP method including diagnostics, environmental monitoring, and point-of-care applications.

5.2 ELISA

Enzyme-linked immunosorbent assay (ELISA) is a method that detects the presence of microbial antigens in various matrices which uses plates coated with viral proteins, usually the N or S protein, to detect specific antibodies (Boonham et al., 2014; Lino et al., 2022). The principle of this method is antigen binding to its specific antibody and eliciting a change in color or fluorescence due to the resultant enzyme activity. After adding the sample, the binding of any antibodies to the viral proteins occurs. In the case of a positive sample, the presence of the antibody–protein complex will be detected by a color change or fluorescence after adding a marked antibody. The first step of the process is binding an antigen at a specific antibody immobilized on a surface, commonly in a set of 96-well microtiter plates. A second enzyme-linked antibody, specific for the same antigen, forms an antibody-antigen-antibody sandwich. The enzyme-coupled antibody reacts with a substrate that changes color when modified by the enzyme. The change in color or fluorescence is correlated with the concentration of the probed antigens in the sample (Gan and Patel, 2013). This method is faster than RT-qPCR and requires minimal equipment; However, there is a risk of cross-reactivity to antibodies from other coronaviruses (Lv et al., 2020). Additionally, these tests are inconsistent during the first 15 days after infection. Early detection is impossible because the human immune system takes several days to create a detectable antibody response (Udugama et al., 2020).

Moreover, this diagnosis is usually based on detecting just

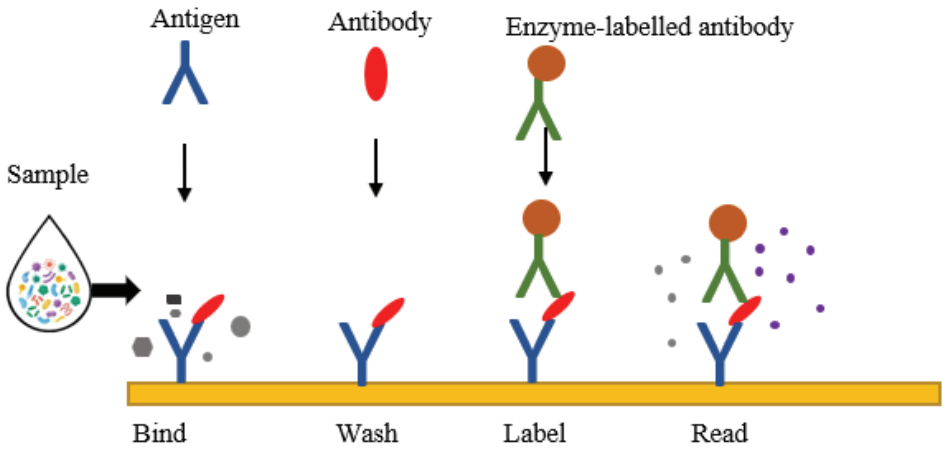

Fig. 3. Schematic representation of the mechanism of ELISA.

one protein. These limitations make these tests prone to inaccurate results, given the high mutation rate of the virus. Although limited in practice for diagnosis, these tests help estimate the number of individuals who have been in contact with SARS-CoV-2 and whether or not they develop symptoms (Katsarou et al., 2019).

An ELISA test requires one or more antibodies with specificity for a particular antigen. Samples containing an unknown antigen are non-specifically or immobilized explicitly on solid support (Fig. 3). After the antigen is immobilized, a detection antibody is added to form a complex with the antigen. The detection antibody may be covalently linked to the enzyme or may itself be detected by a secondary antibody linked to the enzyme via bioconjugation. The antibody incubation part of ELISA is similar to the western blot. The plate is usually washed with a mild detergent solution between each step to remove specifically unbound proteins or antibodies. After a final wash step, the plate is spread with the addition of enzyme-substrate to generate a visual queue indicating the amount of antigen in the sample.

5.3 Bio-Sensors

A biosensor is a device that combines a biological component that detects an analyte and a transducer that detects a physicochemical reaction to produce a measurable signal. A biosensor consists of three components: a bioreceptor, a transducer, and a signal processor. A bioreceptor is a biological element, and the binding of an analyte to a bioreceptor will cause the type of change to be detected by the transducer. This change is converted into a measurable signal, and the signal processor is responsible for displaying it to the electronics (Misra et al., 2021). Biosensors can be largely classified into electrochemical, thermal, optical, and piezoelectric types according to the type of transducer. One of the techniques used to increase the sensitivity of biosensors and lower the detection limit is the addition of nanoparticles. Depending on the type of material, it can exhibit photoluminescence, magnetic ability, low toxicity, high stability, or good biocompatibility and conductivity (Ibrahim et al., 2021). Conversely, an additional benefit is their adaptability for chemical modification to conjugate with nucleic acid probes, viral proteins, antibodies, or other ligands. Various biosensors based on nanoparticles are currently under development for the detection of COVID-19. Nevertheless, the advantages are the same. It is fast, cheap, portable, user-friendly, highly sensitive, and specific. However, the use of nanoparticles usually comes with a need to optimize these systems due to their very untapped potential. Although several biosensors have already been developed or adapted to detect SARS-CoV-2, their use is rare, as most are still in the process of optimization and validation and general commercialization still needs to be improved (Lino et al., 2022).

A biosensor comprises two main components: a biological part, encompassing enzymes, antibodies, etc., that primarily interact with analyte particles and induce a physical change in these particles, and a transducer part that collects information from the biological segment, converting, amplifying, and displaying it. To create a biosensor, biological particles are immobilized on the transducer surface, serving as a point of contact between the transducer and analyte. Biosensors are capable of detecting biological substances, with bioreceptors derived from DNA, enzymes, antibodies, etc. Transducers utilized in biosensors find applications in various fields, including electrochemical, piezoelectric, optical, and thermal (Fig. 4). Biomarkers and biosensors enable the detection and tracing of bacteria and pathogens, while biomarkers and biosensors also facilitate drug delivery to target tissues.

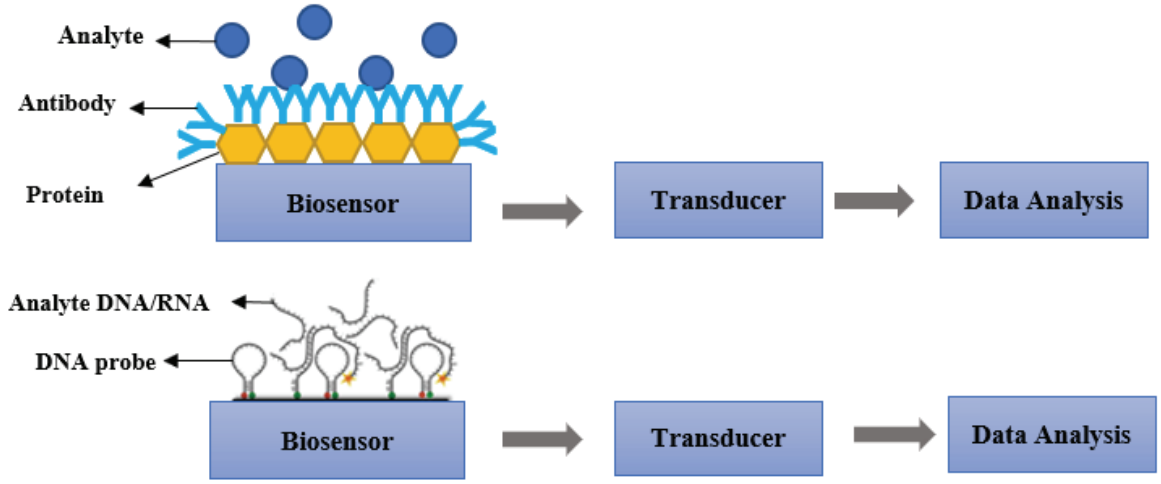

Fig. 4. Function of a biosensor (Kumar et al., 2018).

5.4 EPISENS-S

The Efficient and Practical virus Identification System with ENhanced Sensitivity for Solids (EPISENS-S) method presents a practical approach for detecting SARS-CoV-2 RNA in wastewater, employing direct RNA extraction from wastewater pellets formed through low-speed centrifugation. This technique involves two distinct steps: a first-step RT-preamplifier before total RNA extraction and qPCR from the solid fraction of wastewater, utilizing SARS-CoV-2 and Pepper Mild Mottle Virus (PMMoV)-specific reverse primers for qPCR of targets with different concentrations in wastewater of RT-preamplifier products, allowing for quantification.

To evaluate detection sensitivity, the method was tested using wastewater samples injected with heat-inactivated SARS-CoV-2 at concentrations ranging from 2.11×10^3 to 2.11×10^6 copies/L. Results demonstrated that the EPISENS-S method exhibited a sensitivity 2-fold higher than the conventional method (general RT-qPCR after PEG precipitation; PEG-QVR-qPCR) (Ando et al., 2022).

The limited sensitivity of existing methods for detecting SARS-CoV-2 RNA in wastewater has hindered the widespread adoption of WBE in Japan. The development of a highly sensitive method for detecting low-concentration SARS-CoV-2 RNA in wastewater is urgently needed (Ando et al., 2022). Consequently, it has been suggested that the solid-phase wastewater assay may offer greater sensitivity in SARS-CoV-2 RNA detection compared to the aqueous phase assay.Effective social implementation of WBE demands a method that is simple, time-efficient, and highly sensitive, as timely data collection is crucial for authorities to make informed decisions to mitigate infections or promote socio-economic activity. Table 2 provides a comparative analysis of sensitive SARS-CoV-2 detection methods (Lino et al., 2022).

Based on this research background, Ando et al. (2022) developed an advanced and efficient method for detecting SARS-CoV-2 RNA in wastewater. EPISENS-S, was specifically designed for routine monitoring to facilitate the social implementation of WBE (Fig. 5). The EPISENS-S method involves low-speed centrifugation of wastewater, direct RNA extraction from the resulting pellet, RT pre-amplification, and qPCR using a commercial kit. To enhance accuracy, the method also incorporates the quantification of the endemic PMMoV, an RNA virus prevalent in wastewater (Kitajima et al., 2018), to prevent misinterpretation of SARS-CoV-2 results. The concentrations of RNA in wastewater can be influenced by transient fecal intensity and precipitation-induced dilution (Ando et al., 2022; Graham et al., 2021; Kim et al., 2022).

5.5 GeneXpert

GeneXpert is a molecular diagnostic platform commonly used for the detection of various infectious diseases, including tuberculosis and COVID-19. The GeneXpert system is a cartridge-based rapid molecular clinical test for SARS-CoV-2 on a portable platform that can use wastewater as an input. GeneXpert demonstrated a detection limit of SARS-CoV-2 of 32 copies/mL in wastewater with a sample turnaround time of less than 1 hour (Daigle et al., 2022). An alternative possible option for rapid detection for wastewater sample testing is the Cepheid GeneXpert system, which enables rapid, fully automated, cartridge-based clinical testing. Recently, Cepheid launched the Xpert Xpress-SARS-CoV-2/Flu/RSV combination test for the detection of SARS-CoV-2, Influenza A, and Influenza, a rapid diagnostic multiplex test with a run time of 37 minutes (Johnson et al., 2021) and respiratory syncytial virus (RSV).

Table 2. Summary and comparison of the sensitive detection method of SARS-CoV-2 (Lino et al., 2022)

Methods	Principle	Positive	Negative	Cost
RT-LAMP	Converting COVID-19's RNA to cDNA by transcriptase enzyme is performed and temperature is between 60 and 65°C.	Fast, easy to perform high specificity and sensitivity, no expensive equipment required.	Difficulty in primer design, there are challenges to using LAMP for multiplex assays in a single sample and in quantitation of target DNA.	Cost effective
ELISA	Antibody binding to coated COVID-19 Antigens on ELISA plates to form and detect complexes with a labeled secondary antibody generated color or fluorescence.	Excellent sensitivity and specificity, faster and cheaper than RT-PCR.	Only detects 1 target, risk of cross-reactivity, needs a laboratory setting and technicians.	Moderate
Bio-Sensors	Depends on the type of sensor	Rapid, Fast, portable, continuous, cheap, high specificity and sensitivity.	Needs optimization, can be affected by environmental changes and contamination.	Expensive
EPISENS-S	Extraction of RNA from solid fraction and one step RT-Preamp prior to qPCR.	Highly sensitive and practically usable, effective for untreated and undiluted wastewater samples.	Difficult to apply this method in secondary-treated wastewater or environmental water, which contains only a small number of suspended solids	Cheap
GeneXpert	Cartridge based clinical test on a portable platform	Sensitive and rapid detection possible for SARS-CoV-2. Also, time consuming effective method.	Detection limit is less than 50 copy (cp)/mL in a clinical setting	Moderate

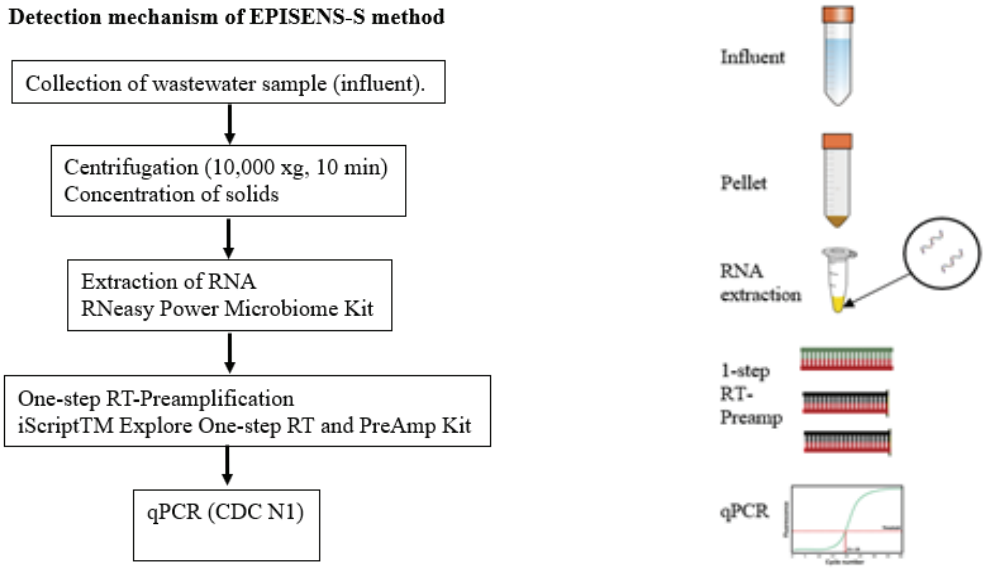

Fig. 5. Detection function of EPISENS-S method (Ando et al., 2022).

This assay performs reverse transcription-quantitative PCR (RT-qPCR) targeting the envelope (E) and nucleocapsid (N2) regions of the SARS-CoV-2 genome. Compared to other rapid diagnostic tests, GeneXpert has several characteristics that make it an ideal candidate for detection of SARS-CoV-2 in wastewater.

The extraction phase of the assay uses a filtration system that separates and concentrates viral particles while removing many of the inhibitors often present in wastewater. Moreover, this assay is one of the most sensitive rapid tests reported with a detection limit of less than 50 copy (cp)/mL in a clinical setting (Becker et al., 2020; Johnson et al., 2021; Wolters et al., 2020; Zhen et al., 2020). GeneXpert's detection limit can be further improved by monitoring the endpoint fluorescence of the assay, a method used to improve sensitivity in clinical settings when performing high multiplex sample pooling. Finally, this test is quantitative and provides cycle threshold (CT) values from which SARS-CoV-2 can be estimated using a standard curve. At this observed level of sensitivity, GeneXpert can act as an early detection system in remote communities in conjunction with a preprocessing method for concentration (Daigle et al.,

2023). Therefore, the summary and comparison of the sensitive detection methods for SARS-CoV-2 have been presented in Table 2.

6. Conclusions

The worldwide pandemic caused by SARS-CoV-2 has emphasized the importance of effective detection methods. Although several technologies are already developed, COVID-19 diagnosis fundamentally relies on PCR techniques. To better track and anticipate COVID-19 disease trends, there is a need for an easy to-use, sensitive, and rapid wastewater test for SARS-CoV-2, particularly in remote communities or in resource-limited settings. Consequently, this study aimed to explore the use several methods as solution for SARS-CoV-2 testing in wastewater, which would allow for the decentralization of testing to sampling sites and the capacity to generate near-real-time data to better guide public health actions. However, the current research and development of sensitive and rapid technologies are RT-LAMP, ELISA, Biosensors, GeneXpert allows a wide range of potential options for SARS-CoV-2 detection and also for other viruses as well. Nonetheless, there are parameters to consider before choosing the best test for each situation. The factors that may limit testing costs are response time, availability of infrastructure, equipment, and specialized personnel.

Additionally, the emergence of new virus strains poses a challenge, potentially impacting the efficacy of currently commercialized detection methods. Hence, there is a crucial need for ongoing genomic surveillance of the SARS-CoV-2 virus worldwide. This continuous monitoring is essential to anticipate potential failures in COVID-19 tests and to facilitate the timely replacement and update of affected testing methods. In conclusion, the foremost challenge posed by the SARS-CoV-2 epidemic to human health necessitates robust research aimed at developing rapid, cost-effective, sensitive, and portable early diagnostic tools. The detection of SARS-CoV-2 in wastewater and sewage from municipal treatment plants holds the potential to expedite mass COVID-19 diagnosis even before clinical tests are universally accessible. Therefore, a persistent focus on monitoring COVID-19 threats in sewage and wastewater, coupled with environmental monitoring of public spaces and the advancement of more effective disinfection methods, promises to mitigate the spread and impact of the global COVID-19 pandemic. It can be confidently asserted that technological advancements in virus detection will empower the scientific community and medical institutions to better prepare for future biological threats and viral pandemics.

Wastewater-based surveillance is a powerful tool to provide an impartial measure of the spread of COVID-19 in a community. This work describes wastewater rapid test for SARS-CoV-2 based on a widely deployed technique. The advantages of easy-to-use wastewater testing for SARS-CoV-2 are important, to deliver faster results that support surveillance in remote communities, improve access to testing, and enable an immediate public health response. The application of wastewater rapid testing in remote communities also demonstrated the usefulness of rapid detection technology by facilitating the detection of COVID-19 clusters and triggering public health actions. Wastewater surveillance will become increasingly important in post-vaccination pandemic settings as individuals with asymptomatic/mild infection continue to transmit SARS-CoV-2 but are unlikely to be tested.

Acknowledgments

This research was supported by National R&D Program through the National Research Foundation of Korea(NRF) funded by Ministry of Science and ICT(NRF-2021K1A4A8A 01079319)

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