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Prospects and challenges of using electrochemical immunosensors as an alternative detection method for SARS-CoV-2 wastewater-based epidemiology

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HIGHLIGHTS

• Discuss the types, mechanisms, and advantages of electrochemical immunosensors.
• Focus on the current efforts on using electrochemical immunosensors in virus detection.
• Elaborate the challenges of using electrochemical immunosensors in COVID-19 WBE.

ABSTRACT

Given its potential applications in confronting the COVID-19 pandemic, wastewater-based epidemiology (WBE) has attracted tremendous attention. Developing a fast, cost-effective, and practical method for SARS-CoV-2 detection in wastewater is of great significance to facilitate future WBE development. By now, the PCR-based approach serves as the reference method and “gold standard” to detect the virus in wastewater. However, we found a trend that the PCR-based method becomes almost an unshakable choice as more and more studies were published regarding SARS-CoV-2 WBE. Of note, the importance of exploring new, alternative approaches for SARS-CoV-2 detection in wastewater should not be underestimated. In this context, the prospect of using electrochemical immunosensors as the alternative detection method was investigated in this survey. Based on the previous efforts towards different virus immunoassay studies and newly published PCR-based COVID-19 WBE works, this survey provides new insights into the electrochemical immunoassay that have been widely adopted in body fluids virus detection, along with an extensive discussion of the detection mechanism, detection performance, past performances, current efforts, and potential challenges with wastewater detection. In the end, this survey concludes that using electrochemical immunosensors to analyze SARS-CoV-2 in wastewater samples quantitatively may have better feasibility and practicability than using the conventional PCR-based approach, especially when considering its fast detection, ease of miniaturization, and potential on-site measurement.
1. Introduction

Since 2019, a global pandemic (i.e., COVID-19), caused by a novel coronavirus SARS-CoV-2, has posed a significant global health threat due to its relatively high mortality rate of 2.20% (WHO, 2021) and the great reproductiveness of 2 to 2.5 (Bukkitgar et al., 2020). As of February 13, 2021, more than 100,000,000 infected patients had been confirmed worldwide (WHO, 2021). Besides the two main transmission routes (i.e., respiratory and contact (WHO, 2020)), substantial evidence points out that the RNA of the SARS-CoV-2 virus could be found in the feces of an infected person (Holshue et al., 2020; Tang et al., 2020; Wang et al., 2020; Wu et al., 2020a, 2020b; Young et al., 2020). Based on this observation, wastewater has attracted significant attention from environmental scientists and engineers because using wastewater-based epidemiology (WBE) may provide a much cheaper and more efficient means of tracking infectious agents in communities (Hamouda et al., 2020; Hart and Halden, 2020).

Recently, using WBE to track the magnitude and distribution of SARS-CoV-2 infection has been proposed and applied in many epidemic areas over the world (Lu et al., 2020), including Spain, India, Netherlands, Australia, Italy, Japan, Israel, France, and Singapore (Ahmed et al., 2020a, 2020b; Balboa et al., 2021; Bar Or et al., 2020; Green et al., 2020; Hamouda et al., 2020; Kocamemi et al., 2020; Kumar et al., 2020; La Rosa et al., 2020; Nemudryi et al., 2020; Peccia et al., 2020; Randazzo et al., 2020; Rimoldi et al., 2020; Sharif et al., 2020; Sherchan et al., 2020; Wu et al., 2020a, 2020b; Wurtzer et al., 2020). As shown in Table S1, approximately 20 different SARS-CoV-2 WBE studies have been conducted over the past several months, and most of these studies detected SARS-CoV-2 viral genetic materials from wastewater treatment plant (WWTP) influent samples. For example, in May 2020, Medema et al. (2020) published the earliest study to investigate if SARS-CoV-2 RNA is present in domestic wastewater of cities and a main airport during the early stages of the COVID-19 pandemic in the Netherlands. They reported that SARS-CoV-2 RNA was detected in WWTP Amersfoort on March 5 earlier than the report of the first two local clinical cases on March 11, suggesting the SARS-CoV-2 virus may preemptively circulate in the population before the local health surveillance system even noticed.

Among all the WBE studies, it is worth noting that various viral concentration methods were adopted to concentrate the SARS-CoV-2 RNA, such as PEG-based separation, membrane filtration, ultrafiltration, and ultracentrifugation (Ahmed et al., 2020a, 2020b). Comparing with different concentration methods, all WBE studies conformably chose the reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) as their detection method. As of now, RT-qPCR is still the “gold standard” for the detection of low concentrations of viral particles in different matrices due to its relatively high sensitivity and excellent selectivity (Hamouda et al., 2020). Nevertheless, it should be noted that using RT-qPCR technology to detect a specific type of virus in wastewater has some inherent limits, which would reduce the practical significance and hinder WBE’s future development. For example, a PCR test typically requires 4–6 h to complete, but the logistical requirement to ship clinical samples means the turnaround time is 24 h at best (Sheridan, 2020). In addition, for testing a low concentration environmental sample (e.g., wastewater), the RT-qPCR is highly susceptible to the presence of PCR inhibitors and sample contamination that can potentially prevent further steps of amplification, resulting in false-negative results (Hamouda et al., 2020).

In this context, research focus has been driven to develop alternative technologies and methods to ensure sensitive, cost-effective, and fast detection of SARS-CoV-2, such as field-effect transistors (FET) biosensor, plasmonic photothermal biosensor, lateral flow immunoassay, clustered regularly interspaced short palindromic repeats (CRISPR) assay, and electrochemical immunosensor (Khan et al., 2020; Qiu et al., 2020; Seo et al., 2020; Zhao et al., 2021). Among many possible alternatives, the electrochemical immunosensor demonstrates the capability of performing relatively fast, sensitive, and cost-effective detection, and most importantly, the possibility of miniaturization for quick, on-site measurement. The adaptation of a conventional, optical ELISA assay to an electrochemical form is the core of an electrochemical immunosensor and endows it with superior specificity and accuracy (Ricci et al., 2012). Based on a comprehensive review, some main advantages and disadvantages of using PCR-based or immunosensor-based approaches are listed in Table 1 (Bukkitgar et al., 2020; Khan et al., 2020; Pilevar et al., 2020; Ricci et al., 2012; Schuurs and Van Weemen, 2006).

This work will collectively examine the mechanisms and advantages of some previously published studies, which successfully detected or diagnosed the virus using electrochemical immunosensors, and then extensively discuss the main challenges and prospects of using electrochemical immunosensors to detect SARS-CoV-2 in wastewater samples. Moreover, several tables summarizing detailed information regarding different detection methods will be presented to elaborate their potential applications in wastewater SARS-CoV-2 detection.

2. Mechanisms and advantages of electrochemical immunosensors

A biosensor typically consists of a bioreceptor, a transducer, and a detector. The bioreceptor, such as antibodies and antigens, is designed to conjugate or react with the target analyte, thereby producing a specific biological change in the detecting environment (Li et al., 2010). Afterward, the transducer can convert and transfer the biological change into a physicochemical signal through a quantifiable reaction and then allows a detector to collect the physicochemical signal (Khan et al., 2020). Based on transducers’ different principles, biosensors can be optical, fluorescent, surface plasmon resonant, piezoelectric, electrochemical, and magnetic, etc. (Khan et al., 2020). Among all biosensing techniques, the electrochemical immunosensor is one of the most feasible options due to its ease of preparation and operation, rapid detection,
low-cost sensor design, in situ detection of pathogens in various matrices, and potentially simultaneous detection of multiple targets (Cesewski and Johnson, 2020). Moreover, unlike the PCR-based detection method, which requires an additional nucleic acid extraction step, an electrochemical immunoassay can directly convert ligand-based biological reaction into a quantifiable electrochemical signal, including voltammetric, impedimetric, amperometric, potentiometric, and conductometric (Silva et al., 2014; Silvestrini et al., 2015). Regardless of the different signal types, the analyzing target of the electrochemical immunoassay is similar to that of other immunoassays, such as enzyme-linked immunosorbent assay (ELISA), which focuses on detecting either a species-specific antigen from a virus itself or an antibody against the corresponding antigen derived from a host’s immune system (Mao et al., 2021). According to several reviews, the conventional approaches for developing electrochemical immunosensors can be categorized under two headings, namely the label-free format and the label-based format (sandwich-type) (see Fig. 1) (Khan et al., 2020; Ricci et al., 2012; Schuurs and Van Weemen, 2006).

2.1. Label-free electrochemical immunosensors

The fundamental mechanism of using a label-free electrochemical immunosensor to detect viral antigen or antibody is that the binding of the immobilized recognition element and the target analyte could modify the electrode surface with some bulky sized antibody-antigen immunocomplexes and therefore alter the electrochemical signal of a standardized redox couple, which is almost always the pair of potassium ferricyanide/ferrocyanide (Fe(CN)$_6^{3-}/^{4-}$) (Bukkitgar et al., 2020; Ricci et al., 2012; Schuurs and Van Weemen, 2006). Of note, when using the label-free format, a relatively high background signal obtained from the preemptively added redox couple is required for testing a sample without any analyte. The detected signal will then decrease in proportion to the increase of an analyte’s concentration due to the electrode surface covered by a specific immunocomplex.

As shown in Fig. 1, two approaches could be used when preparing a label-free electrochemical immunosensor, and the main difference lies
in selecting the immobilized biological recognition element (i.e., antigens or antibodies). Unlike the label-based electrochemical immunosensor (i.e., sandwich-type), the label-free electrochemical immunosensor requires no addition of an enzyme (or inorganic label) conjugated secondary antibody but, in some cases, relies on the competition between the target analyte and immobilized recognition element, thereby causing it to be sometimes named the “competitive approach” method in publications (Bukkitgar et al., 2020; Khan et al., 2020; Pei et al., 2013; Tang and Tang, 2015). For example, when detecting a specific type of virus through antigen assay, instead of immobilizing the corresponding antibody as the recognition element, the competitive approach (label-free) immunosensor could choose to immobilize the target antigen and then spike a fixed concentration of antibodies to initiate a competitive effect between the immobilized antigens and target antigens, thereby changing the preset electrochemical signal in a testing solution.

Because of the elimination of preparing a labeled secondary antibody, the label-free format is a relatively low-cost and straightforward technology developed to diagnose many different biological elements, such as rheumatoid factor, tumor necrosis factor, and fig mosaic virus (Chinnadayyala et al., 2019; Haji-Hashemi et al., 2019; Mazloum-Ardakani et al., 2015). As per the nature of a label-free electrochemical immunosensor, the main difficulty is precisely aligning and orienting the biological recognition element (i.e., immobilized antibodies or antigens) on an electrode surface during the immobilization step (Khan et al., 2020). More specifically, when dealing with immobilization, the recognition element’s alignment not only affects the efficiency of ligand-based conjugation but also significantly alters the working electrode surface, which may result in the overly-passivated electrode surface and consequently degradation in the sensitivity of detection.

In 2019, Layqah and Eissa’s (2019) developed a competitive, label-free electrochemical immunosensor to detect the Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in nasal samples (see Fig. 2). According to the study, the immobilized MERS-CoV recombinant spike protein S1 (the recognition antigen) can compete with the free MERS-CoV S1 antigen in nasal samples for binding a fixed concentration of antibody (10 μg mL−1) added to the samples preemptively. This competitive design allows binding the target MERS-CoV antigen and the fixed amount of antibody to be carried out in the testing solution instead of a working electrode surface, thereby overcoming the small-space limitation of a typical working electrode. The voltammetric signals (i.e., reduction peak currents) derived from 5 mM of ferrocyanide/ferri cyanide were recorded and compared before and after the addition of different antigens concentrations. The developed method demonstrated an excellent linear response over 0.001 to 100 ng mL−1 with a detection limit of 1.0 pg mL−1. The immunosensor has a high specificity to MERS-CoV, allowing it to differentiate MERS-CoV from other respiratory tract illnesses, such as Influenza A or B, which are potentially coexisting in wastewater. Of note, the study used a well-established technology of thiol-gold self-assembled monolayers (SAMs) on top of the electrodeposited gold nanoparticle (AuNPs) to immobilize the recognition element of the spike protein S1. Similar immobilization procedures were previously reported using solid gold electrodes to create the SAMs with a linkage agent (e.g., cysteamine) containing a gold-affinity thiol at one end and a functional group (e.g., amine) for subsequent attachment of recognition element at the other end (Escamilla-Gomez et al., 2008; Feng et al., 2008; Ricci et al., 2012). Compared with traditional solid gold electrodes, Layqah and Eissa’s (2019) immobilization strategy no only allows the formation of SAMs through thiol-gold chemistry in a highly ordered way but also enhance the voltammetric response over the redox couple through the electrodeposited AuNPs.

2.2. Label-based (sandwich-type) electrochemical immunosensor

At the core of the sandwich-type electrochemical immunosensor is the adaptation of the ELISA assay to an electrochemical platform (Ricci et al., 2012). Several crucial factors require more thorough consideration and analysis because of the different transducers mechanisms used in an optical ELISA and a sandwich electrochemical immunosensor. The number one key factor lies in selecting an efficient electroactive label to indicate the antigen-antibody reaction (Pei et al., 2013). According to several recent reviews, two labeling strategies could be conceived, namely the enzyme label and the nanoparticle tag, when designing a sandwich-type electrochemical immunosensor (Pei et al., 2013; Ricci et al., 2012). A first strategy typically in which the electrochemical signal is derived from the enzymatic reaction between the labeled enzyme (e.g., alkaline phosphatase) on the secondary antibody and the corresponding enzymatic substrate (e.g., 1-naphthyl phosphate) in the solution (Centi et al., 2010; Pandey et al., 2012; Zani et al., 2011). A second one, using novel nanoparticle tags, such as copper, silver, and Fe3O4 nanoparticles, to first attach to the secondary antibody and then react with a specifically designed electrochemical substrate.

The nanomaterial tag is a relatively new technology that has been recently reported in some electrochemical immunoassay studies, which provides a whole new perspective to amplify the electrochemical signal of a sandwich-type immunosensor (Ahmadi et al., 2014; Huang et al., 2016; Zhang et al., 2014). Unlike the label-free approach, an obvious advantage of the sandwich-type electrochemical immunosensor is that the derived electrochemical response originates directly from the ligand-based biological reaction and does not require a redox couple to indicate the binding of the recognition element and analyte indirectly. However, it should be noted that the preparation of a specific labeled secondary antibody could significantly increase the total workload of developing a sandwich-type immunoassay. For example, the following study is a representative example of how novel nanocomposite material can significantly improve the voltammetric response of a sandwich-type electrochemical immunosensor while increasing the difficulty in preparation and detection.

Zhang et al. (2014) proposed using dual-functional copper-doped titanium dioxide nanoparticle (CuTiO2) as signal amplification tag (a comparable technology to the conventional enzymatic label) to develop an electrochemical immunoassay using a carboxyl functionalized graphene oxide (CFO) modified glassy carbon electrode to detect human IgG in serum samples (see Fig. 3). The dual-functional characteristic allows the transducer to freely switch between two electrochemical signals, namely, the square wave voltammetric response derived

Fig. 2. Immobilization of MERS-CoV recombinant spike protein S1 using thiol-gold chemistry (image adapted from Layqah and Eissa (2019)).
from the Cu-Cu$^{2+}$ oxidation current and the amperometric response due to the TiO$_2$ catalyzed hydrogen peroxide (H$_2$O$_2$) reaction. For the square wave voltammetric method, the linear response range is $0.1$ pg.mL$^{-1}$ to $100$ ng.mL$^{-1}$, with a limit of detection of $0.052$ pg.mL$^{-1}$. Meanwhile, the amperometric method obtained a linear range over $0.01$ pg.mL$^{-1}$ to $100$ ng.mL$^{-1}$, with a limit of detection of $0.0043$ pg.mL$^{-1}$. In summary, the developed Cu@TiO$_2$ label did demonstrate excellent detection performance and incredible versatility, but the lengthy preparation process, particularly of which designing and fabricating the Ab$_1$-CFG0 modified electrode and the Cu@TiO$_2$ labeled Ab$_2$ secondary antibody, is still a sore point and does not seem currently feasible for mass production.

Despite the bright future ahead of the nanomaterial tag approach, the conventional enzyme label approach still represents the mainstream of developing a sandwich-type immunosensor, particularly when the priority comes down to practical application like clinical diagnosis. For example, the selection of lactate oxidase as the enzyme label in a clinical immunosensor development was reported to be used in detecting carcinoma antigen 125 (CA125) in human serum (Samadi Pakchin et al., 2018). Accordingly, hydrogen peroxide was added into the testing solution as a substrate to demonstrate the enzymatic reaction activity exerted by the lactate oxidase label. Although they used nanocomposites (i.e., gold nanoparticles, multiwall carbon nanotube, and graphene oxide) to enhance the electrochemical platform's sensing performance, the fundamental mechanisms of how the enzymatic reaction was triggered and how the biochemical signal was transduced fell in line with the classic enzyme label design philosophy (see Fig. 4).

In summary, the sandwich-type electrochemical immunosensor is capable of adapting the conventional optical ELISA to an electrochemical platform with a higher detection sensitivity and better signal-to-interference performance (Pei et al., 2013; Ricci et al., 2012). However, it should be noted that the sandwich-type immunosensor relies not only on the labeling strategy but also significantly associated with the recognition element immobilization approach, thereby requiring a more comprehensive fabrication process than the label-free format. Consequently, when conceiving a new electrochemical immunoassay, the choice of either using a label-free or sandwich-type type must be carefully weighed to take into account the need of following mass production.

3. Current efforts on using electrochemical immunosensors in virus detection

Developing fast, reliable, and point-of-care detection technology is one of the most crucial practices to combat global pandemic such as SARS-COV-2. In recent months, there are many research groups worldwide in different fields that aspire to use available resources as effectively as possible to develop novel detection methods for the SARS-COV-2 clinical diagnosis. For example, one of the most recent SARS-COV-2 electrochemical immunosensor, reported by Fabiani et al. (2021), was developed and aimed to detect Spike (S1) and Nucleocapsid (N) protein in saliva using magnetic beads (MBs) integrated sandwich-type immunosensor. In their study, the secondary antibody is labeled with the typically used alkaline phosphates (AP) for the enzymatic reaction of 1-naphthyl phosphate to 1-naphthol. As can be seen from the detection mechanism, the authors chose a well-proven approach when conceiving and performing the immunoassay, which is probably due to the urgent need for an immunological method to conduct practical application.

This section will focus primarily on the recent electrochemical immunosensor built to detect the virus (e.g., SARS-CoV-2, MERS, HCoV, and influenza) in various testing mediums (e.g., serum, saliva, and nasal fluids), especially their design principles and practical aspects. As shown in Table 2, six recent electrochemical immunosensors, including three newly reported SARS-CoV-2 detection studies (Eissa and Zourob, 2021; Fabiani et al., 2021; Rashed et al., 2021), two previously developed influenza detection studies (Tang and Tang, 2015; Zhou et al., 2013), and one MERS-CoV and HCoV codetection study (Layqah and Eissa, 2019).

Despite the different detected viruses, all the listed studies, in fact, can still be categorized under two main principles: label-free and sandwich-type. When comparing the limit of detection (LoD), the enzymatic labeled (i.e., sandwich-type) immunosensors did not show superior performance as expected, which is probably due to the use of MBs.
during the process of immunological chains (Fabiani et al., 2021; Zhou et al., 2013). It is worth mentioning that the use of MBs could overcome many obstacles in the conventional sandwich-type immunosensors, such as the possibility of passivation of the electrode surface due to the lengthy immunological steps and the confinement of the total recognition elements upon the electrode surface because of the limited space of a working electrode (Ricci et al., 2012). However, using MBs as support for the immobilization of recognition elements could bring in an obvious drawback that the secondary antibody is not in direct contact with the electrode surface, thereby limiting the overall detection sensitivity. In contrast, some label-free immunosensors demonstrated surprisingly high sensitivity, with LoDs being thousands of times lower than the two sandwich-type studies (Eissa and Zourob, 2021; Jarocka et al., 2016; Layqah and Eissa, 2019). As a result, due to the relatively simple and straightforward preparation steps, it is reasonable to give priority to the label-free immunosensor when developing a new electrochemical immunosensor for virus detection.

Moreover, it can be found that, in most cases, the actual sampling sizes are below the milliliter level (e.g., 10 and 50 μL), and the tested samples are relatively homogeneous and predictable, such as serum and saliva. Hence, before developing a new electrochemical immunosensor for SARS-COV-2 WBE, it is necessary to come up with

![Fig. 4. Schematic diagram of the sandwich-type electrochemical immunosensor using lactate oxidase conjugated secondary antibody as the enzyme label approach to detect CA 125 in human serum (Image adapted from Samadi Pakchin et al. (2018)).](image)

### Table 2

| Target virus Detection element | Design principle | Electrode | Electrochemical measurement | Immobilization approach | Sample | Sample size | LoD | References |
|-------------------------------|------------------|-----------|-----------------------------|--------------------------|--------|-------------|-----|------------|
| SARS-CoV-2 S1 and N protein   | Enzymatic labeled (AP) sandwich immunosassay | SPE (modified with carbon nanomaterial) | DPV<sup>a</sup> | Magnetic beads | Untreated saliva | 300 μL | 19 ng/mL (S1) 8 ng/mL (N) | (Fabiani et al., 2021) |
| SARS-CoV-2 CR3022 antibody | Label-free immunosassay | Impedimetry | non-specific adsorption | Human serum | 50 μL | Qualitative only | (Rashed et al., 2021) |
| SARS-CoV-2 N protein | Label-free immunosassay | Cotton-tipped SPE | SWV<sup>b</sup> | Carbon nanofiber-diazonium salt | Nasal sample | 100 μL | 0.8 pg/mL | (Eissa and Zourob, 2021) |
| Influenza H5N1 Hemagglutinin | Label-free immunosassay | SPE (gold) | SWV | Thiol-gold SAMs (4'-4-Thiobis-benzenethiol) | Spike in PBS | 10 μL | 0.9 pg/mL | (Jarocka et al., 2016) |
| Influenza H9N2 Hemagglutinin | Bionzymatic labeled (HRP<sup>c</sup> & GOD-A<sup>d</sup>) sandwich immunosassay | Magnetic gold electrode (well configuration) | DPV | Magnetic beads | Chicken serum | n/a<sup>e</sup> | 1.0 ng/mL | (Zhou et al., 2013) |
| MERS-CoV/HCoV<sup>f</sup> Oc43 N protein/725 S1 protein | Label-free immunosassay (competitive) | Gold nanoparticles modified SPE (electrodeposited) | SWV | Thiol-gold SAMs (cysteamine and glutaraldehyde) | Nasal fluid (artificial) | 10 μL | 1.0 pg/mL | 0.4 pg/mL | (Layqah and Eissa, 2019) |

<sup>a</sup> Alkaline phosphatase (AP).
<sup>b</sup> Differential pulse voltammetry (DPV).
<sup>c</sup> Square wave voltammetry (SWV).
<sup>d</sup> Horseradish peroxidase (HRP).
<sup>e</sup> Glucose oxidase-conjugated avidin (GOD-A).
<sup>f</sup> Not available (n/a).
<sup>g</sup> Human coronavirus (HCoV).
some countermeasures to be taken when handling wastewater samples with the characters of large sample volume, low virus concentration, and overly-complex testing matrix. The main challenges of adapting electrochemical immunoassay for WBE will be surveyed in more detail in the following section.

4. Challenges of using electrochemical immuno sensors in WBE

As mentioned above, using electrochemical immunosensors to detect the presence of a specific type of virus in wastewater samples is more challenging than in body fluid samples. It can be primarily ascribed to the complex matrix of a typical wastewater sample and its relatively low virus concentration (Pilevar et al., 2020). Consequently, it is necessary to pretreat, concentrate, or extract the target virion from a wastewater sample before conducting the subsequent immunoassay in a comparatively small-volume test medium. As mentioned, in the recent SARS-COV-2 WBE cases, all of the studies selected RT-qPCR as their virus detection approach. While the PCR-based methods are still the “gold standard” as of now and can offer high sensitivity due to the target genes amplification step, its lengthy processing time, varieties of required internal/external references and kits, and susceptibility to environmental inhibitors together are likely to create bottlenecks in the future development of WBE. In contrast, the electrochemical immuno sensor can provide a result within the shortest possible time of 15 min and only require a buffer solution for electrochemical reactions to take place (Pilevar et al., 2020).

Based on the recent review by Lu et al. (2020), almost all published PCR-based SARS-COV-2 WBE studies, to a certain extent, had adopted pretreatment and concentration processes before conducting the PCR test. A relatively low sensitive method could be compensated in part by having a large-volume initial sample and then condensing it into a much smaller final sample through one or multiple concentration steps. Therefore, we believe that if employing pretreatment and concentration steps is a must-have in both PCR-based and immuno sensor-based virus detection in wastewater samples, their performance’s differences in terms of the detection sensitivity might not be as significant as we commonly understood. Consequently, choosing appropriate pretreatment and concentration methods during the sample preparation is of vital importance to overcome the challenges regarding using electrochemical immuno sensors in SARS-COV-2 WBE.

A municipal wastewater sample matrix usually consists of human excreta (i.e., feces and urine), shower/bath water, food waste, household maintenance products, along with a wide variety of trace amounts of organic and inorganic compounds. Some important wastewater constituents, which can potentially affect SARS-COV-2 detection in wastewater samples, are listed in Table 3. For instance, suspended solids, captured by a filter with a nominal pore-size varying from 0.45 μm to about 2.0 μm (Tchobanoglous, 2014), can attract small virus particles suspended in the wastewater sample (WHO, 2003). Therefore, employing a relatively large pore-size filter (e.g., 1.0 μm) or relatively slow-speed centrifugation (1000 × g, 5 min) step to pretreat a wastewater sample may eventually cause a considerable loss of the target virus particles. To avoid such losing, Wu et al. (2020a, 2020b) retained the pre-centrifuged pellet and simultaneously eluted it with the subsequent PEG precipitated layer. The presence of biodegradable organics is another factor that can potentially affect the virus concentration efficiency. For example, using electrostatically charged membranes to adsorb viruses from the wastewater can be subject to a significant reduction of virus recovery efficiency due to the presence of organic matter, which can result in a preferential attachment to the charged filters and raise the risk of the membrane-clog situation (Lu et al., 2020). For electrochemical immunoassay, crossing-reacting species that may exist in a test sample are the most intractable problem, resulting in both over-or-under-estimation of the detected virus concentration (Schuurs and Van Weemen, 2006; Tate and Ward, 2004).

| Table 3 Constituents of concern for virus detection in wastewater. |
|---------------------------------------------------------------|
| Constituent | Reason for concern |
|----------------|-------------------|
| Suspended solids | A significant cause of the development of sludge deposits in the sewage system and may lead to the electrostatic attachment of viruses on suspended solids (Tchobanoglous, 2014). |
| Biodegradable organics (proteins, carbohydrates, and fats) | Measured in BOD/COD, the presence can significantly reduce virus concentration efficiency when using the electrostatically charged membrane method due to a preferential attachment of dissolved organic molecules over virus particles (Fout et al., 2015). |
| Other pathogens (virus, bacteria, protozoa) | Other seasonal, infectious diseases with similar symptoms may be transmitted with SARS-CoV-2 simultaneously; the cross-react species may result in the over-or under-estimation of the target virus (Tate and Ward, 2004). |
| Nutrients (N, P, and trace nutrients) | The essential nutrients for biofilm growth in the sewage system, the resulted biofilm can be associated with the confinement, retardation, and decay of virus particles during transport in sewage pipes (Lu et al., 2020; Tchobanoglous, 2014). |
| Heavy metals (Cr, As, Pb, Hg, etc.) | Highly toxic to biological activities when having a high concentration; can lead to antigen denaturation (Tchobanoglous, 2014). |
| Dissolved inorganics (Ca, Na, Cl, etc.) | Dramatic changes of cations present in water samples can cause antigen conformation change and result in under-estimation (Tate and Ward, 2004; Tchobanoglous, 2014). |
| Refractory organics (surfactants, etc.) | Present typically in trace amount; would elicit various unpredictable biochemical reactions with virus particles (Tchobanoglous, 2014). |

It should be noted that clean up all potential crossing-reacting species in a given wastewater sample, no matter how a comprehensive pre-treatment is adopted, is neither feasible nor practical. Before preparing an electrochemical immunosensor, selecting a pair of antibody-antigen that contains high specificity is a simple, affordable, and standard approach to solve the problem of crossing-reacting species (Tate and Ward, 2004). Moreover, carrying out a preemptive blocking test in the antigen-spiked wastewater matrix to validate the specificity before performing the actual test can directly demonstrate the proposed immunoassay’s feasibility (Guttman-Bass et al., 1987; Morinet et al., 1984). For example, Guttman-Bass et al. (1987) evaluated a commercial immunoassay’s specificity by incubating the spiked SA-11 rotavirus and rotavirus antisera for 1 h at 37 °C before the actual test in the wastewater.

To the best of our knowledge, there is no electrochemical immunosensor currently developed to detect the SARS-CoV-2 antigen in the wastewater sample specifically. However, after a comprehensive review, we found several previously reported studies that focused on detecting viral antigens in wastewater samples using immunological-based approaches. As shown in Table 4, all these studies adopted preconcentration methods before the subsequent immunoassays, and all of them successfully detected the target viral antigens from raw sewage samples. These three studies chose three different technologies in terms of the preconcentration methods, including the adsorption-elution method (Guttman-Bass et al., 1987), the filtration method (Palmer et al., 1993), and the ultra centrifugation method (Calgua et al., 2011). It is worth noting that two of these studies were published relatively early, and the sensitivities of both optical immunoassays were considerably lower than today’s nanomaterials-integrated immunosensors. Collectively, based on all the information that had been found, it is our confident expectation that the development of a feasible electrochemical immunosensor as the alternative detection approach with an effective concentration method could achieve a fast, sensitive, and cost-effective...
**Table 4**

Comparison of different pretreatment/concentration methods used for virus/bacteria detection in wastewater matrix.

| Detection type | Target | Matrix | Pretreatment/concentration | Note | References |
|----------------|--------|--------|-----------------------------|------|------------|
| Immunological-based (antigen detection) | Legionella spp. | Raw sewage/effluent | Filtration-Resuspension-Coagulation | 2% Formalin for antigen coagulation (Palmer et al., 1993) | (Palmer et al., 1993) |
| Immunological-based (antigen detection) | Adenoviruses/Polymaviruses | Raw sewage | Ultracentrifugation-Elution | 229,600 × g | (Calgua et al., 2020) |
| PCR-based SARS-COV-2 (RNA detection) | SARS-CoV-2 (or surrogate) | Primary influent | Centrifugation-Precipitation | Standing overnight after PEG-based precipitation | (La Rosa et al., 2020) |
| Conventional culture (live virus detection) | Poliovirus | Untreated sewage | Centrifugation-Precipitation | Addition of 39.5 mL of 22% dextran, 287 mL 29% (WHO, 2003) | (WHO, 2003) |
| WBE analysis | | | | | | (Ahmed et al., 2020a, 2020b) |

Although numerous SARS-COV-2 wastewater surveillance studies have been published recently by different research groups worldwide, their analytical tool and practical application are still in an early stage in reflecting and monitoring the prevalence of SARS-COV-2 through testing the viruses in sewage. The RT-qPCR detection method seems as though it is the sole and exclusive approach for the quantitative measure of SARS-COV-2 in wastewater due to its high sensitivity. Nevertheless, we should not ignore and downplay the possibility of using other advanced analytical technologies. Besides, RT-qPCR is not flawless in terms of the time-consuming process, the complexity of testing reagents and references, and its susceptibility to environmental contamination and inhibition. As a result, it is of great importance to develop and try other alternative wastewater virus detection methods. Due to its excellent portability and quantifiability, an electrochemical immunosensor may be one of the most promising technologies when considering the possibility of miniaturization and on-site detection for WBE.

Two available approaches can be referenced regarding the electrochemical immunosensor development: the label-free format and the label-based (sandwich-type) format. Comparing both approaches, the sandwich-type format, in general, is believed to be capable of delivering a better sensitivity (Pei et al., 2013). However, we found that label-free immunosensors sometimes can offer better sensitivity when detecting viruses through the antigens-antibodies reaction (Jarocka et al., 2016; Layqah and Eissa, 2019). This phenomenon is in part associated with the rapidly developed nanomaterials technology, which endows the label-free electrode surface with significantly greater sensing capability, while the sensitivity of the sandwich-type format is subject to the activity of the labeled enzymatic reaction. Besides, the relatively sizeable virus particle, compared with other conventional immunological analytes such as human thyroglobulin (Costia et al., 2020), can result in a more remarkable electrode surface change, thereby favoring the detection performance of the label-free format. Moreover, the preparation of a specific labeled secondary antibody could considerably increase the total workload of developing a sandwich-type immunosensor.

As of now, two electrochemical immunosensors have been developed to detect SARS-CoV-2 in human body fluids (i.e., saliva and serum), and both showed promising performance (Fabiani et al., 2021; Rashed et al., 2021). It should be noted that similar to PCR-based studies, the adaptation of clinical diagnosis to wastewater detection requires additional pretreatment and concentration steps to reduce the wastewater matrix’s complexity and elevate the diluted virus concentration. Thanks to the numerous reported PCR-based SARS-COV-2 studies and a few early published other virus immunoassays in wastewater, it is not difficult to adopt or refine an existing pretreatment and concentration steps prior to an electrochemical immunoassay based on substantial evidence and reliable results, see Fig. 5.

WBE has a proven track record of being implemented as a large-scale surveillance tool to combat infectious diseases, such as poliovirus (Asghar et al., 2014; Ikner et al., 2012). It now holds an excellent promise for surveillance of the SARS-COV-2 prevalence at the community level. Yet, the SARS-COV-2 WBE is still in its infancy and remains only a preliminary tested tool in many scientific research articles. In part, this situation is associated with the RT-qPCR assay, which relies on the amplification cycle to achieve the goal of virus quantification. Due to the high variability of wastewater matrix and different concentration methods adopted, the amplification step could be subject to inhibition and render unreproducible assay performance. For the electrochemical immunosensor, although there are constituents in wastewater that may trigger the non-specific immunological reaction, the mechanism in terms of quantitative measurement is more straightforward.

**5. Prospects**

Detection of SARS-CoV-2 in the wastewater, thereby providing critical information for analyzing the prevalence of SARS-CoV-2 in a community.
6. Conclusions

Collectively, we believe the electrochemical immunosensor method has a great potential to cut an outstanding figure regarding the SARS-CoV-2 WBE. In the end, based on the previous efforts towards different virus immunosensor studies and new PCR-based COVID-19 WBE works, we present the following concise conclusions for those audiences who aspire to develop new detection or diagnosis methods to protect human society from further damage brought on by the SARS-CoV-2 pandemic:

1. Due to the nature of the electrochemical immunosensor, it is possible to demonstrate better feasibility and practicability than the PCR-based approach, especially when considering its fast detection, ease of miniaturization, and potential on-site measurement.

2. Developing electrochemical immunosensors to detect viral antigens’ presence has been done primarily in the body fluids, such as nasal fluid and saliva. To the best of our knowledge, only three early works tried immunological-based methods to detect viruses in raw wastewater samples.

3. Two commonly used strategies can be referenced when developing a new electrochemical immunosensor: the label-free and the sandwich-type format. Based on past performance, the label-free format is relatively straightforward to be prepared. It shows comparable performance as the sandwich-type format in virus detection, sometimes even more sensitive than the sandwich-type immunosensor.

4. No matter what method is chosen for virus detection, it is necessary to pretreat and concentrate the wastewater samples due to the matrix’s complexity and the diluted virus concentration. Various pretreatment and concentration methods, such as PEG precipitation, electrostatic membrane adsorption, and ultracentrifugation, are available to be selected, refined, and adapted to electrochemical immunosassay.

5. It is crucial to give equal attention to the detection method’s sensitivity and practicability to facilitate future development of SARS-CoV-2 detection in wastewater.

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Author contributions

DL and DZ contributed conception and design; DL, HG, and ZY contributed collection and assembly of relevant information; DL contributed drafting of the article; DZ, QF, and XZ contributed reviewing. All authors had final approval of the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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