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PII: S0304-3894(20)32646-7

DOI: https://doi.org/10.1016/j.jhazmat.2020.124656

Reference: HAZMAT124656

To appear in: *Journal of Hazardous Materials*

Received date: 5 September 2020
Revised date: 12 November 2020
Accepted date: 20 November 2020

Please cite this article as: Mohsen Pilevar, Keug Tae Kim and Woo Hyoung Lee, Recent advances in biosensors for detecting viruses in water and wastewater, *Journal of Hazardous Materials,* (2020) doi:https://doi.org/10.1016/j.jhazmat.2020.124656

Recent advances in biosensors for detecting viruses in water and wastewater

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Abstract

As there is a considerable number of virus particles in wastewater which cause numerous infectious diseases, it is necessary to eliminate viruses from domestic wastewater before it is released in the environment. In addition, on-site detection of viruses in wastewater can provide information on possible virus exposures in the community of a given wastewater catchment. For this purpose, the pre-detection of different strains of viruses in wastewaters is an essential environmental step. Epidemiological studies illustrate that viruses are the most challenging pathogens to be detected in water samples because of their nano sizes, discrete distribution, and low infective doses. Over the past decades, several methods have been applied for the detection of waterborne viruses which include polymerase chain reaction-based methods (PCR), enzyme-linked immunosorbent assay (ELISA), and nucleic acid sequence-based amplification (NASBA). Although they have shown acceptable performance in virus measurements, their drawbacks such

as complicated and time-consuming procedures, low sensitivity, and high analytical cost call for alternatives. Although biosensors are still in an early stage for practical applications, they have shown great potential to become an alternative means for virus detection in water and wastewater. This comprehensive review addresses the different types of viruses found in water and the recent development of biosensors for detecting waterborne viruses.

Keywords: antibody; biorecognition element; biosensor; waterborne virus

1. Introduction

Pathogens are classified as bacteria, protozoan parasites, fungi, worms, viruses, and any other microorganism that could cause a disease. One of the great threats to public health is the water and foodborne pathogens. Based on previous reports published by the United States (U.S.) Centers for Disease Control and Prevention (CDC), 1,000 cases of disease outbreaks, and approximately 48 million illnesses are originated by foodborne pathogens annually in the U.S. [1, 2]. The Economic Research Service (ERS) of the U.S. Department of Agriculture (USDA) has announced that the annual cost of illness by Salmonella disease which is a bacterial infection is approximately $2.7 billion [3]. Various virus particles may infiltrate from animal and human excreta to aquatic environments via leachate from septic systems, runoff from agricultural lands, and effluent discharge. Studies report that more than 150 types of enteric viruses have been identified in domestic wastewater [4]. The majority of the identified enteric viruses are rotaviruses (RVs), noroviruses (NVs), enteroviruses (EVs), hepatitis A virus (HAV), adenoviruses (AdVs), and astroviruses (AVs) [4]. Epidemiological studies illustrate that viruses are the most challenging pathogens to be detected in aquatic environments. It is because of their

nano sizes, discrete distribution, and low infective doses which makes them the major source of infectious diseases [5-7].

Common methods for wastewater disinfection are chlorination, ozonation, and UV irradiation before effluent discharge. However, some of the methods have low efficiency in eliminating viruses from wastewater for certain viruses. For instance, inactivation of poliovirus in municipal wastewater requires the residual chlorine with a concentration of 9.0 mg Cl_2/L (with a contact time of 30 min), while the lower concentration of chlorine (1.0–2.0 mg Cl_2/L) is sufficient for sewage disinfection of bacteria Salmonella [8]. It is a crucial necessity to eliminate or track viruses from domestic wastewater before it is released in the environment. For this purpose, the pre-detection of virus particles in wastewaters is an essential step and it is imperative to develop novel techniques to detect different strains of viruses in wastewater environments during the processes.

Over the past decades, several methods have been applied for virus detection in medical diagnosis, and some of them are modified to be used for virus detection in environmental samples as well. The conventional methods of virus detection in water and wastewater include polymerase chain reaction (PCR)-based methods such as quantitative PCR (q-PCR) and real-time (RT) q-PCR, enzyme-linked immunosorbent assay (ELISA), and nucleic acid sequence-based amplification (NASBA). Other analytical methods for the detection of virus species include transmission electronic microscopy (TEM), epifluorescence microscopy, pulsed-field gel electrophoresis (PFGE), flow cytometry, immunofluorescence assay (IFA), traditional cell-culture, and molecular methods. Some of them can only provide qualitative data while others can also present quantitative information regarding the virus particles in environmental samples. For

instance, a TEM method, an imaging technique with nanometer-scale resolution, allows identification and quantification of viruses based on the morphology of viral species in samples [9]. Epifluorescence microscopy can provide quantitative data by counting fluorescent dots generated by staining the nucleic acids in the virus particles by a fluorescent dye [10]. The IFA method offers a great advantage over other quantitative analytical methods due to its ability to analyze the infectivity of detected viruses [11]. In the PFGE method, the separation of high molecular weight DNA fragments (based on their molecular sizes) is enabled by a pulsating electric field [12].

Nevertheless, it should be mentioned that these methods have some disadvantages such as low sensitivity (ELISA method) and time-consuming (PCR based techniques) [13]. These flaws hinder them to become a practical method for the real-time detection of viruses in environmental samples. Virus detection in aquatic environments such as wastewater, sewage, and drinking water requires techniques that fulfill all needed factors such as high sensitivity, rapid detection, reliability, reproducibility, representativeness, and being cost-effective. Recently, biosensors have become a popular technique for the pre-screening of viruses in environmental samples. Although biosensors are still in their infancy, they have great potential to become the main method for the pre-detection of viruses in aquatic samples.

This review article aims to address different types of viruses found in water and wastewater and their characteristics in the water environment, summarize conventional methods for virus detection, and present recent advance on biosensors and their application in the detection of waterborne viruses in wastewater.

2. Viruses and their Characteristics in Water Environment

Viruses generally consist of a virus particle (virion 10–100 nm) containing nucleic acid (DNA or RNA genome) surrounded in a protein capsid [14]. Viruses tend to reproduce and require host cell structures for viral replication. The viral nucleic acid and the capsid are the fundamental structure for viral replication. The capsid protects the nucleic acid from nucleases and aids in the attachment of the virus to the host cell, while the nucleic acid carries the required genetic information to stimulate the synthetic machinery of the host cell [15]. Viruses are intercellular pathogenic microorganisms unable to reproduce outside of the host cells. After infection of the host organism, viruses initiate their reproduction which leads to the excretion of virus progeny by an infected organism. This would cause a threat to other healthy organisms and would even result in an outbreak [16].

Enteric viruses that are associated with water and foodborne diseases could infiltrate the general water supply systems through agricultural activities, sewage waste, wastewater discharge, and landfill run-off. The enteric viruses are capable of contaminating recreational waters, irrigation waters, and drinking water sources [17, 18]. The most known waterborne viruses that could cause human diseases are hepatitis A, hepatitis E, adenovirus, norovirus, and rotavirus. Enteric viruses are extremely persistent in aquatic environments and could survive in wastewaters for long periods. Moreover, they are highly resistant to acidic conditions, disinfectants, high temperatures, and pressure. They are also infectious at low doses, indicating that only a few virion particles can infect the host cells. The characteristics of most common waterborne viruses are summarized in Table 1 [4, 19-26]. Studies indicate that approximately

13% of the global population lack access to safe, sanity drinking water which evidently would result in millions of deaths caused by waterborne diseases annually [24, 27, 28].

The detection and removal of threatening microbiological agents from the water supply system are critically important to increase global access to safe drinking water. There have been numerous studies on the detection and inactivation of different types of harmful bacteria in wastewaters which resulted in the developing bacterial standards for water disinfection [29-32]. On the other hand, viruses could not be easily investigated because of numerous waterborne virus strains that could not be cultured *in vitro*. The inability to culture waterborne viruses hinders the researchers to identify the viral behavior of them to be inactivated [33]. It should also be mentioned that the detection of waterborne viruses through molecular diagnostic techniques is challenging compared to other types of viruses because waterborne viral nucleic acids contain RNA rather than DNA genomes which makes them more dynamic, complex, and genetically variable [34-36]. Consequently, guidelines and regulations for microbiological quality of water mainly use bacteriological indicators such as fecal coliforms, total coliforms, fecal streptococci, and *E.coli*. Hence, the correlation between indicator bacteria and waterborne viruses in municipal surface water and secondary effluents may not be substantially reliable [37]. Currently, there is no diagnostic method that could assure a sample is completely free of viruses [38].

Aside from waterborne viruses, there is another crucial reason to pursue the detection of viruses in wastewaters and more specifically in municipal sewage water. In particular, the emergence of the COVID-19 pandemic in early 2020 has put unprecedented stress on the public health infrastructure across the world. While the current human biomonitoring (HBM) of clinical

testing is expensive and time-consuming and may be limited to those who have symptoms, tracking the disease-causing coronavirus in sewage samples offers the opportunity to monitor outbreaks at a community level. For example, the genetic material (RNA) behind the coronavirus which causes COVID-19, known as SARS-CoV-2, can be tracked in sewage water to reflect the severity of the outbreak in the area it came from [39] since an infected individual could excrete 10^5 to 10^{11} virus particles per gram of feces [40, 41]. Although the viral infections are hard to diagnose before the outbreak, a prescreening method for virus presence qualitatively or quantitatively in the sewage water could potentially estimate the severity of the outbreaks in the population of the community which could facilitate the decision of lockdown or release measures.

3. Conventional Methods for Virus Detection in Water Environment

Several methods have been used for virus detection in medical diagnosis and are modified to be used for virus detection in environmental samples. The current traditional methods for virus detection include q-PCR [42], RT-PCR [43], ELISA [44], enzyme-linked immunospotting (ELISPOT) [45], NASBA [46], fluorescent microscopy [47], microarray technique [48], and flow cytometry [49]. Some of these methods have been combined to improve their response in detecting viruses. For instance, PCR combines with plaque-forming units (PFU) tests [50]. Table 2 shows a summary of conventional methods for virus detection in the water environment [14, 51-64]. ELISA and ELISPOT methods are relatively popular for virus detection in water samples as they could be applied for high-throughput analysis of multiple samples. However, they are becoming obsolete due to their time-consuming procedures (6 hrs to run) and lack of sensitivity which would result in false-negative results under low virus concentration.

PCR-based methods have recently become the most popular choice for virus detection in water samples. This is because PCR-based techniques can collect epidemiological information about the viruses and genotype data which can be applied in vaccination programs [65, 66]. Another benefit of using PCR-based methods is that viral nucleic acids can be amplified and detected in a single step by nucleic acid amplification, reducing the required time for virus detection (e.g., 3 hrs) compared to enzyme-based methods (e.g., ELISA and ELISPOT). However, they are relatively expensive and only usable for detecting organisms that are already known by designing a specific primer. Moreover, they are not always able to amplify and detect the viral nucleic acids particularly in low concentrations of viruses [67]. To improve sensitivity, sample preparation techniques such as preconcentration, immunomagnetic separation (IMS), and nucleic acid purification of pathogens have been used to extract the required amount of viral genome to improve the sensitivity of PCR and immunoassay. [68-70] Recently, microfluidic platforms were combined with the PCR reaction to reduce the required time and costs of the assay. For this purpose, a specific target amplification (STA) reaction was used to increase the number of target genes prior to detection. STA reaction is especially necessary when a small amount of target molecules is to be quantified by the microfluidic quantitative PCR (MFQPCR) platform.

4. Virus Detection in Water Environment using Biosensors

Biosensors could become an emerging alternative to the traditional virus measurements as they can offer the fast detection of viruses (e.g., 7–16 min using a lab-on-a-chip), reduced detection cost, reduction of sample volume and volume of reagents, and high sensitivity [71, 72]. There are several factors to be considered for the characterization and evaluation of virus detection

performance, including LOD, specificity/selectivity, sensitivity, detection rage, sample volume, assay time, and sample matrix (Table 3) [73-75]. For decades, biosensors have shown great potential for practical applications of continuous monitoring and/or prescreening [76-78]. However, their evaluations are mostly limited to the well-controlled laboratory setups and still need more validation for the use in real systems. The associated protocols of sample collections and pretreatment of the samples are also required to be developed for target viruses.

Biosensor technology has been initiated with the concept of using a biorecognition element (e.g., enzyme) for detecting various biological components (e.g., glucose) [50] and expanded to the applications in the field of food, water [79], bioprocess [80], and environmental monitoring [81], agricultural process [82], and clinical diagnostics [83]. One of the most significant advances in biosensors is pathogen detection. A biosensor consists of two fundamental parts, a biorecognition element (bioreceptor) and a physical or physicochemical transducer. The biorecognition element interacts with the analyte biologically and the physicochemical transducer detects the interaction between the analyte and the bioreceptor which causes changes in the transducer which could be optical, electrical, thermal, and thermodynamic properties of the transducer. Then, the transducer converts this change to electrical signals (e.g., pA or mV). The transduced signal can be further amplified and converted from analogue into digital form for display [84]. Biosensors can be categorized based on their biorecognition elements or their transduction mechanisms (Fig. 1) [85, 86]. The biorecognition element could be an antibody, nucleic acid (DNA, RNA, Peptide nucleic acid [PNA], and aptamer), enzyme, phage, and whole cells/microorganisms based on the genome of the analyte [87-89]. Transduction mechanisms of the biosensors used for pathogen detection are predominantly optical, piezoelectric, and

electrochemical. Accuracy, sensitivity, robustness, and LOD are main features that are highly dependent on the type of transducers used in biosensors [90].

Most studies have been applied biosensors for virus detection in field water samples (e.g., tap water, reclaimed water, or virus-spiked wastewater) [91-93] and the direct use of the biosensors in the fields are still rare. Chung et al. (2019) developed a microfluidic paper analytic device (μPAD) for effective detection of norovirus in field water samples (tap water and reclaimed wastewater) without any sample concentration or nucleic acid amplification steps [91]. For this purpose, the antibody-conjugated fluorescent submicron particles were added to the μPAD structure. The correlation between the concentration of virus particles and the number of aggregated particles (formed by antibody-antigen binding) played the key role in the functionality of the detection device. The biosensing platform was able to achieve detection limits of 1 genome copy/μL and 10 genome copy/μL in DI water and reclaimed wastewater samples, respectively.

Paper-based biosensors have shown great potential for rapid and *in situ* detection of SARS-CoV-2 in wastewater samples [94], however, it is extremely challenging due to the complexity of water matrix. An extensive clean up step to remove the matrix interferences from wastewater samples (prior to biosensor detection) could potentially bolster the accuracy and practicality of a paper-based biosensor method [94, 95]. For the current efforts of tracking COVID-19 using wastewater [96, 97] although many researchers have extensively conducted studies on this, the technique and its application to responses and preventions to viral threats are very preliminary and the research is still in an early stage to connect levels of virus in sewage mapping to levels of

infection in humans. There are still several questions to be addressed with regard to sample collection and test methods of the wastewater samples.

One of the biggest challenges in virus detection in water matrix is the relatively low concentrations of virus particles in water. In general, samples are treated to concentrate and extract RNA from the pre-concentrated samples [98, 99]. The current virus concentration methods include electronegative membrane, ultrafiltration (UF), and molecular imprinting polymers (MIP) method combined with magnetic nano particles [100, 101]. A recent study on tracking COVID-19 in wastewater shows that although the SARS-CoV-2 virus is very dilute in wastewater, it was still detectable and that the viral signal can be measured using RNA markers [102]. However, it is critical to develop quicker and cheaper virus concentration methods and to develop standardized protocols for uniform benchmarking and adaptation.

The type of sample matrix is another factor that can significantly influence the detection and quantification of viruses in wastewater samples. For instance, the influents are generally composed of higher humic acids and heavy metals compared to other environmental samples [103]. Raw wastewater samples, on the other hand, have shown higher turbidity [104, 105] with higher concentrations of suspended solids [106], and organic matters [107] than other wastewater samples. Moreover, sludge samples, another wastewater sample that could be used for virus detection, are extremely heterogeneous sample matrices, in which viruses are adsorbed on the surface of flocs [108]. These characteristics could potentially interfere with the molecular methods of assaying for viruses [106, 109]. It has been reported that applying an ultra-high affinity probe for specific targets can minimize the challenging matrix effects of the wastewater samples [110]. Based on the fact that environmental samples pose a great number of challenges

to assay development, appropriate sample pre-treatments and concentration steps can amplify the accuracy of virus detection methods especially through molecular methods.

The biosensor platform and other molecular methods, used for the detection and quantification of viruses in water media, are only able to assess the presence of segments of the genetic materials (DNA or RNA) of the viruses in the wastewater sample. Therefore, the viral species will be identified even when the viral capsid is compromised, or when the genetic material of the target virus is fragmented. The viability of a virus is generally determined by immunological and cell-culture based methods, in which the cytopathogenic effect of the infected sample in suitable cell lineages is investigated [9].

4.1. Piezoelectric biosensors

Piezoelectric sensors are mass sensitive detectors consisting of a crystal surface layered with the biorecognition element. After being exposed to the analyte, a change happens in the resonant frequency (MHz) of the crystal which relates to the mass change at the crystal surface. Piezoelectric transducers are vastly used in acoustic wave biosensors since they generate acoustic waves in a frequency-dependent manner. Quartz (SiO_2) and lithium niobate ($LiNbO_3$) are the most popular materials used in piezoelectric transducers [111]. Quartz crystal microbalance (QCM) is the most recognizable piezoelectric sensor which could be considered as the simplest acoustic wave device in operation. The structure of QCM sensors is comprised of parallel electrodes located on a thin cut piece of the crystal (e.g., 1-inch diameter) which would be deformed when a periodic electric field (V/m) is applied. As expected, the physical properties of the crystal, such as density (g/cm^3) and size (mm), highly affect the vibrational frequency (Hz) [112]. A schematic view of a typical QCM biosensor is illustrated in Figure 2 [113].

Piezoelectric biosensors have been broadly used for clinical virus detections such as hepatitis B virus (HBV) [114-116], African swine fever virus [117], Herpes simplex [118-120], Ebola virus [121], and Dengue virus [122-125]. However, there are only a few publications regarding the detection of waterborne viruses via piezoelectric sensors [126, 127].

König and Grätzel (1993) studied the detection of rotavirus and adenovirus through piezoelectric immunosensors with the application of three different antibody immobilization methods to a gold (Au) electrode [126]. For this purpose, the protein A, γ-amino propyltriethoxy silane, and polyethyleneimine were coated to the Au electrode for the immobilization. The goat anti-Rotavirus antibody was coated to the crystal surface as the biorecognition element for the detection of rotavirus in a purified rotavirus sample. Goat anti-Rotavirus antibody reacts with rotavirus and is specific for infected cell proteins (ICPs) and late structural (virion) antigens. It is a known antibody for rotavirus that demonstrates 90% cross-reactivity with human rotavirus and does not react with human epithelial type 2 (HEp-2) cells in immunofluorescence [128]. Results indicated that protein A generated the most satisfactory frequency changes ($\Delta F = 775 \pm 10$ Hz) for the detection of rotavirus [126]. With regards to immobilization methods, similar results were obtained for the detection of adenovirus with the application of antibody-coated crystal surface ($\Delta F = 782 \pm 10$ Hz). Moreover, the optimal value of incubation time and the antibody solution concentration was 45 min and 5 mg/L respectively [126]. They also worked on the detection of hepatitis viruses type A and type B with the application of a piezoelectric immunosensor [126]. A 16-MHz (as a basic resonance frequency) AT-cut crystal was coated with the specific virus antibodies immobilized via protein A. A linear frequency change was achieved for the virion concentrations of 10^5 to 10^{10} viruses in the solution with the detection limit of 10^5 virions [127].

The protein A was used to immobilize the anti-virus antibodies onto the gold surface of the crystal. The coated crystal was stable for four weeks (n=10) when stored dry over silica gel blue at room temperature, without detectable loss of activity [127].

Kim et al (2015) have worked on detecting canine parvovirus (CPV) by measuring frequency changes associated with antigen-antibody interaction using the QCM biosensor and ProLinker^{TM} B which facilitates the attachment of antibodies to a gold-coated quartz surface in a regular pattern and the correct orientation for antigen binding [93]. 261 field fecal samples were tested and results were compared to PCR. The QCM biosensor showed 95.4% sensitivity (104/109 as positive) and 98.0% specificity (149/152 as negative) with a cut-off value of -205 Hz [93].

The piezoelectric biosensor has also been applied for the detection of norovirus based on the shift in the resonance frequency that is proportional to the deposited mass [129]. Neumann et al (2018) conducted experiments to detect GII.4 Sydney norovirus virus-like particles (NorVLPs) using a localized QCM (L-QCM) biosensor of a 10 MHz TC-cut quartz crystal with a 100 nm thick gold layer that was deposited on a 60 nm adhesive titanium layer [129]. To achieve extremely low LOD of down to one molecule, rolling circle amplification (RCA)-based assay methods were combined with a QCM. In particular, proximity ligation assay (PLA) which is an RCA-based method for protein-based method was evaluated on an L-QCM platform for NorVLPs detection. It is known that RCA, as an isothermal amplification method, offers excellent specificity due to its padlock probes (PLPs), linear oligonucleotides that are ligated in a strictly sequence-dependent manner to form a circle that can be amplified subsequently [129]. When PLA probes (specific secondary antibodies) which are bound to a primary antibody

specific to the protein of interest, are in close proximity, connector oligonucleotides hybridize to the probes, get circularized, then can be amplified via RCA process [129]. That is why the PLA method offers a high degree of specificity [130, 131]. The applied PLA consisted of an aptamer (100 µg/mL) and a primary antibody for norovirus (1 µg/mL). This study showed that the QCM biosensor utilized with an adequate PLA could exceptionally increase the efficiency of virus detection [129].

Rydell et al (2009) used a QCM with dissipation (QCM-D) for detecting NorVLPs (0–20 µg/mL, Norwalk (GI.1) and Dijon (GII.4) strains) [132]. For this purpose, an H type 1 and Lewis a pentaglycosylceramides incorporated in laterally fluid supported lipid bilayers were used as biorecognition elements. The objective of the study of norovirus detection through QCM-D [132] was to evaluate and compare the efficiency of bioreceptors in binding with the target analytes and it was found that only H type 1 was able to display binding with both target analytes (GI.1 and GII.4). That is because the structure of H type is more suitable for binding with the distinct binding sites of GI.1 and GII.4 strains. This study also demonstrated that the GI.1 and GII.4 were not able to bind with the H type 1 at low concentration levels (1 wt% and 2-4 wt%, respectively).

In a study by Imai et al (2011), a gene of an enteric virus-binding protein (EVBP) derived from the GroEL (bacterial chaperon protein) was applied for binding with the NorVLPs (GI.7 and GII.4) strains, group A rotavirus, and poliovirus type 1 [133]. Based on the QCM method, the equilibrium dissociation constants of the EVBP candidate with GI.7 and GII.4 strains were found out to be 240 nM (standard deviation [SD]=60, n=4) and 210 nM (SD=50, n=4) respectively. The study showed that viral particles with rugged structure, viruses with rugged

topography of capsid protein, could more effectively bind with the small hydrophobic protein molecule of EVBP [133].

Aptamers are relatively novel biorecognition elements synthesized specifically to identify and bind with the analyte target in nucleic acid-based biosensors. Aptamers have been used in piezoelectric biosensors as well. Wang and Li (2013) studied the detection of avian influenza virus (AIV H5N1) by developing a novel hydrogel-based QCM coated with a synthesized aptamer with high affinity [134]. The binding between the aptamer and the ssDNA of AIV H5N1 formed a crosslinker in the polymer hydrogel which caused the dissolution of the linkage between the aptamer and ssDNA, resulting in the abrupt swelling of the hydrogel which activated the QCM sensor. Three different ratios of polymeric hydrogels (100:1 hydrogel I, 10:1 hydrogel II, 1:1 hydrogel III) were examined for AIV H5N1 detection and the study indicated that hydrogel III obtained the highest sensitivity with the detection limit of 0.0128 hemagglutinin unit (HA unit). The QCM aptasensor used by Wang and Li (2013) shortened the detection time (30 min) previously obtained by an anti-H5 antibody-coated QCM immunosensor (1 h) [134]. That is due to the fact that aptamers are single-chain, redox-insensitive, and self-refolding that do not aggregate since they lack the large hydrophobic cores of proteins. Moreover, they are more resistant to harsh physical and chemical conditions compared to proteins. In a similar coating amount, antibodies were found to partially lost their activities during the chemical covalent bonding procedure which resulted in a decrease of sensitivity.

Although piezoelectric biosensors have many advantages (e.g., being label-free, cost-effective, and easy to use), relatively long incubation times, difficulties in regenerating crystal surfaces, and multiple washing and drying steps are drawbacks of the piezoelectric biosensors.

Moreover, immobilization of antibodies on the crystal surface is challenging as each receptor pair requires a unique optimized immobilization technique, indicating more compact and simple immobilization steps need to be developed for sensor preparation [135].

4.2. Optical biosensors

Optical changes in the properties of the surface caused by the interaction between the biorecognition element and the target analyte can be used to detect various viruses (Fig. 3) [136]. These optical changes are mostly physico-chemical properties such as refractive index, fluorescence (expressed as relative fluorescence unit [RFU])/phosphorescence, bio/chemiluminescence (expressed as a relative light unit [RLU]), simple light absorption (expressed as absorbance unit [Au]), and reflectance. Optical biosensors are generally categorized into surface plasmon resonance (SPR) biosensors, evanescent field-based fiber-optic biosensors, fluorescence, and chemiluminescence biosensors, and colorimetric biosensors. SPR and fiber-optic biosensors are popular optical biosensors in detecting pathogens [137]. They are particularly popular in food safety since they are capable of detecting analytes in complex matrices with minimal sample treatment. Moreover, using fiber optic cables for the detection of optical signals offers advantages such as low loss of signal, low noise, and less interference [138].

Ashiba et al (2017) developed an SPR-assisted fluoroimmunosensor and optimized the sensor using CdSe-ZnS-based quantum dots (QDs) for the norovirus detection [139]. A V-shaped trench sensor chip was fabricated by injection molding of polystyrene. An Al film, formed by sputtering (CFS-4EP-LL) with an RF power of 200 W, was applied as the SPR excitation layer. The vertex length, angle, and opening width of the V-trench were set to be 10

mm, 52.9°, and 0.3 mm, respectively. An anti-norovirus monoclonal antibody was applied as the biorecognition element for binding with the analyte while the SPR was stimulated by an excitation wavelength (390 nm). The results demonstrated that the detection region of the V-tech corresponds to 100 norovirus virus-like particles (VLPs) with a LOD of 0.01 ng/mL [139]. In Fahmida et al (2018), a localized surface plasmon resonance (LSPR) was tested with gold nanoparticles (AuNPs) and fluorescent CdSeTeS QDs were used to detect norovirus [63]. Exposing the norovirus particles to the CdSeTeS QDs/AuNPs nanocomposites caused the stimulation of the fluorescence enhancement of QDs in proportion to the concentration of the target norovirus particles with 12.1×10^{-15} g/mL of LOD [63].

Weerathunge et al (2019) applied a colorimetric biosensor technique for the detection of murine norovirus particles [140]. The biorecognition element used for targeting the analyte was an enzyme-mimic catalytic activity of gold nanoparticles with high target specificity of a murine norovirus aptamer (NanoZyme aptasensor) which generates a blue color (with different intensity depending on concentrations) in the presence of the norovirus particles. This method has obtained the LOD of 30 viruses/mL in 10 min which is the most sensitive biosensor method applied for the detection of norovirus to date. Moreover, the NanoZyme aptasensor is capable of detecting the norovirus particles in complex matrixes in the presence of non-target microorganisms and others (e.g., human serum and shellfish homogenate) [140].

Gu et al. (2013) tested the detection of adenovirus in adenovirus-spiked wastewater samples (200 and 2000 pfu/mL) by using an evanescent wave optical immunosensor [92]. They found that there is no significant interference from different wastewater matrix on antibody-

based fiber optic biosensor analysis even without any sample pre-treatments. The overall sensing process was completed in less than an hour with a detection limit of 100 pfu/mL. Optical biosensors have also been used for clinical diagnosis of human adenovirus DNA by Jin et al (2018) [141]. They investigated the detection of human adenovirus DNA samples by optical biosensors with the application of adenovirus primer DNA as the biorecognition element. This study showed that the optical biosensor could detect ten copies of adenovirus particles in clinical samples in only 30 min which could potentially provide a fast and sensitive diagnostic platform for future applications [141].

Kim et al. (2020) have studied the detection of bovine viral diarrhea virus (BVDV) by a peptide-based optical biosensor utilized with BVDV-specific bioreceptors anchored on the AuNPs to produce seeding sites for effective signaling [142]. The copper polyhedral nanoshell (CuP) growth on the surface of AuNPs was used to amplify the optical signal reflecting the amount of BVDV in the sample with a detection limit of 4.4 copies/mL. A detection of Dengue virus (DENV) was visualized using a reverse transcription loop-mediated isothermal amplification (RT-LAMP) method under UV light at 365 nm with high selectivity toward DENV RNA with a detection limit of 3.5 copies/mL [143].

Prabowo et al (2017) studied the label-free detection of enterovirus 71 (EV71), another waterborne virus, using a portable SPR biosensor [144]. The main objective of their study was to shorten the required detection time to several minutes from 6 days using the conventional viral plaque assay. For this purpose, the crystal structure of EV71 was coated by capsid proteins (VP1, VP2, VP, and VP4) which contained amino acid chains [144]. Based on previous studies, the amino acid can be attached to gold particles due to strong binding between them [145, 146]. This

direct detection method was utilized a rich variety of amino acid structures in EV71 to immobilize the viral particle to the gold metal sensing component. They were able to successfully detect the virus particles in approximately 20 min with the application of major capsid protein of enterovirus particles (VP1) as the biorecognition element. The portable SPR biosensor was able to obtain an approximate detection limit of 4.8 pg/mL in the PBS buffer [144]. More recently, Liu et al (2017) developed a novel thermosensitive surface-imprinted polymer coating SiO_2 for a resonance light scattering (RLS) thermosensitive virus-affinity sensor to detect the hepatitis A virus (HAV) through the application of thermal control process at 40 °C [147]. The temperature-sensitive element applied in the study was *N*-isopropylacrylamide. It was reported that the biosensor successfully monitored the HAV with a LOD of 1.1 pM. The RLS intensity of the thermosensitive HAV-MIPs at 555nm had excellent linearity with the increase of HAV concentration in the range of 5-25 pM [147].

4.3. Electrochemical biosensors

Electrochemical biosensors operate by measuring the biological changes (at biosensor surface) that occur because of the interaction between the biorecognition element and the analyte. The biological changes could be in conductance (S/m), current (A), resistance (Ω), or capacitance (F) at the sensing surface. In electrochemical biosensors, an electrode is immobilized with a biorecognition element that would interact with the analyte that would generate signals that could further be amplified for the detection of virus particles (Fig. 4) [148]. Enzyme-catalyzed reactions are the main mechanism of generating current/potential difference which is detected in electrochemical biosensors [149]. This type of sensor is generally categorized into voltammetric, amperometric, and potentiometric.

The electrochemical biosensor was first developed in 1962 for the determination of glucose levels [50]. At the early stages, amperometric electrochemical biosensors were applied to detect small analytes metabolites such as urea and lactate [150]. The applications of electrochemical biosensors have been developed to detect larger macromolecules such as pathogens and proteins in the past decades [151, 152]. Cyclic voltammetry (CV) and differential pulse voltammetry (DPV) have been used to detect Epstein Barr virus, cytomegalovirus, Herpes simplex virus (HSV), and hepatitis B virus (HBV) with an application of DNA-based biorecognition elements [153]. Ionescu et al (2007) developed an amperometric immunosensor through the electropolymerization of T7 bacteriophage for the detection of the West Nile virus (WNV) [154]. The T7 phages were modified by an additional peptide sequence taken from the virus and used as an antigen [154]. The amperometric biosensor successfully detected the WNV antibody. A calibration curve was created based on different WNV-antibody dilutions in the range between 1:10 and $1:10^6$ with an extremely sensitive detection titer ($1:10^7$) which indicated the great potential of amperometric sensors for pathogen detection.

Manzano et al (2018) developed an electrochemical biosensor based on DNA hybridization to effectively detect the hepatitis A virus (HAV) in contaminated food and water samples [155]. The sensor was utilized with a disposable gold electrode which was functionalized with a specific capture probe for the detection of HAV single-strand DNA (5'-GGTAACAGCGGCGGATATTGGTGAGTTGTTAAGACAAAAACCATTCAACG-3') and complementary DNA (5'-ThiC6-AAAAATCTTAACAACTCACCAATATCCGC-3') [155]. The cyclic voltammetry (CV) technique was applied to monitor the oxidative hybridization of the indicator tripropylamine. The biosensor successfully detected the HAV ssDNA and cDNA

with the detection limit of 0.65 pM and 6.94 fg/µL, respectively [155]. In another attempt to simultaneously detect the hepatitis viruses (types A-E), Tang et al (2010) designed a novel integrated automatic electrochemical immunosensor platform [156]. A self-made electrochemical sensor array was utilized with nanogold particles and protein A as matrices. Then, 5-type hepatitis virus antibodies were immobilized onto the sensor for binding with their corresponding antigens from the sample solution. The antigens/antibodies were dissolved in a PBS at pH 7.4 containing Tween 20 (0.1 mL/L) at 4 ˚C. The developed electrochemical biosensor was able to simultaneously detect all 5-type hepatitis viruses using a two-electrode system based on the antigen-antibody reaction. Detection time was only 5 min, while LOD was ≤1.0 ng/mL for most analytes [156]. For further validation, the results were compared with the data obtained from the ELISA method and the results of the two methods were relatively similar [156].

Several studies showed a possible electrochemical detection of human norovirus using peptides as biorecognition elements [157, 158]. A NoroBP-nonFoul-$(FlexL)_2$-coated Au-SPE biosensor showed highly selective detection of human norovirus with a detection limit of 1.7 copies/mL for a potential use in food samples [157]. Another human norovirus detection was tested using a peptide functionalized AuNPs decorated tungsten disulfide nanoflower (WS_2NF/AuNP) by measuring impedances which are elevated by hindering charge transfer between the working electrode and redox species ($[Fe(CN)_6]^{3-/4-}$) without significant interference with other viruses (e.g., rotavirus and dengue virus), but with a low detection limit of 2.37 copies/mL [158].

The electrochemical biosensor has also been used to detect rotavirus. Liu et al (2011) designed a highly sensitive graphene-based electrochemical biosensor [159]. They synthesized a graphene oxide (GO) film from GO colloidal suspensions and generated a reduced GO film as a working electrode in a three-electrode system. An Ag/AgCl electrode and a platinum (Pt) wire were used as the reference electrode, and the counter electrode, respectively. The synthesized GO with a great electron transfer property was then modified with pyrene derivatives and was covalently linked with rotavirus antibodies. The reduced GO film significantly improved the capture of virus cells caused by antibody-antigen interactions. A 10^5 pfu/mL of input cells was measured with the anodic current peaks of ca. 30.7% sensitivity while 10^3 pfu/mL of input cells was detected with ca. 1.3% sensitivity.

Liu et al (2013) designed a micropatterned reduced GO field-effect transistor (MIRGO-FET) which displays a typical p-type semiconducting behavior [160]. The rotavirus samples were injected into the biosensor with a concentration ranged from 10 to 10^5 pfu/mL. LOD of 10^2 pfu/mL was reported for the detection of rotavirus cells which is superior to the conventional ELISA method [160, 161].

Caygill et al (2012) developed a novel impedimetric based assay platform to detect and quantify human adenoviruses (Ads) [162]. The polyclonal antibodies were synthesized against the human Ad (Ad5) capsid protein as biorecognition elements. Furthermore, the antibody fragments were immobilized onto the polyaniline and 2-aminobencylamine copolymer matrix. The fabricated sensors were incubated with different serotypes (Ad, Ad5, and Ad3) in the virial concentration of 10 to 10^{12} virus particles/mL. The results from the electrochemical impedance spectroscopy indicated that the impedimetric based assay platform was able to detect each

human Ad serotype specifically with the detection limit of 10^3 virus particles/mL. Several researchers have investigated the detection of waterborne viruses through biosensor platforms and a summary of these studies including LOD is presented in Table 4 [63, 126, 127, 139-141, 144, 147, 155, 156, 159, 160, 162, 163].

5. Future Direction and Challenges of Biosensors for Virus Detection in Wastewaters

5.1. Biosensors modified with nanomaterials

The immobilization matrix has a crucial role in bolstering the performance of different biosensors (piezoelectric, optical, and electrochemical) for the detection of pathogenic viruses. Recently, nanomaterials have become extremely popular among researchers as they have exceptional optical and electrical properties due to their electron and phonon confinement, modified surface work function, high surface reactivity, high surface-to-volume ratio, strong adsorption ability, and high catalytic efficiency [164, 165]. Major advantages of nanomaterials in fabricating sensors include their size, morphology, functionality, adsorption ability, effective surface area, and great electron-transfer properties [86, 166]. Different nanomaterials such as nanoparticles [167], nanorods [168], nanowires [169], carbon nanotubes [170], hybrid materials [171], and nanocomposite materials [172, 173] have been applied to improve the selectivity, sensitivity, and generally the performance of biosensors for detection of pathogens. Lee et al (2018) utilized nanoparticle (NP)-carbon nanomaterial hybrid structures to detect norovirus DNA [163]. Gold (au)/iron-oxide magnetic NP-decorated CNTs (Au/MNP-CNT) were synthesized for norovirus DNA sensing channels. Then a thiol-group-functionalized probe DNA was attached to the gold nanoparticle surface that was obtained by aligning a platinum (Pt)-interdigitated electrode with the CNTs. The synthesized hybrid structure was applied for the detection of the

target DNA with a concentration ranged from 1 pM to 10 nM. The sensor was able to specifically detect the norovirus DNA with a LOD of 8.8 pM [163]. Perez et al (2003) developed novel magnetic nanoparticles conjugated with virus-surface-specific antibodies for the detection of adenovirus particles in complex biological media. The fabricated magnetic nanosensors were able to specifically detect the adenovirus particles, with a LOD of 10^2 pfu/mL, without the need for extensive sample preparation. Moreover, it was found that magnetic nanosensors are independent of optical properties of the suspension which is a significant advantage of this technique [174].

5.2. The use of biosensors for virus detection in sewage water

Although there are numerous infectious diseases caused by different viruses that are present in wastewaters, to the best of our knowledge, there are no clear guidelines or standard methods for monitoring of viruses present in raw wastewater [175]. The RT-qPCR method is the currently popular practice for detecting viruses with high sensitivity from wastewater samples [99]. However, the volume of the environmental sample is usually too large for PCR reaction, thus requiring sample concentration (e.g., direct RNA extraction from electronegative membranes and ultrafiltration) [99]. In addition, the method may not distinguish between infectious viruses and noninfectious ones in the sample. Moreover, the PCR-based methods are only able to detect a specific type of virus in wastewater samples based on the specifically designed primers while there could be several types of infectious viruses in the sample. Besides, the PCR-based methods are known to be time-consuming and complex with high-cost procedures [51, 59]. Therefore, it is vital to develop an alternative method to overcome the previously mentioned drawbacks of the PCR method for the detection of viruses in wastewater samples. One possible solution would be

a combination of different methods such as a combination of molecular biology techniques (e.g., PCR) with cell cultures (e.g., plaque-forming tests) and a combination of atomic force microscopy (AFM) with protein microarray technology [175]. Along with appropriate biorecognition elements selection for target analytes and optimized immobilization techniques, the platform of the biosensor can be used for pre-detection or pre-screening purposes due to their time-saving and cost-effectiveness [51, 161].

Infectious viruses are present in human feces in large quantities and could survive in human feces for up to several days after exciting the human bodies [176]. While the current human biomonitoring (HBM) of clinical testing is expensive, time-consuming, and may be limited to those who have symptoms, the sewage tracking can keep monitoring all cases. With this context, biosensors could be further modified to be applied for the prescreening of viruses in the sewer systems and can be used as an early warning sign of disease outbreak in the community of a given wastewater catchment (Fig. 5). Recently, paper-based analytical devices have been developed to filter the genetic material of pathogens from wastewater samples through a biochemical reaction with preloaded reagents that can detect whether the genetic material of SARS-CoV-2 is present [177]. The cost is relatively cheap less than $1.25 and the detection time is short about 30 minutes. The results are visible to the naked eye as a green circle indicating positive and a blue circle negative [177]. Although the developed paper-based sensors are rapid and easy to use, such devices tend to have lower sensitivity, accuracy, and specificity compared to PCR methods.

Rapid detection, ease of operation, and portability are major advantages of biosensors which make them suitable for further application in virus detection in sewage water samples.

Further modifications of biosensors combined with automated and portable methods could potentially make them excellent candidates for virus detection in wastewater samples. For the future development of biosensors, it is recommended that the research focus on signal enhancement by novel nanomaterials, improved selectivity and sensitivity through modified recognition elements such as synthesized aptamers, and miniaturized detection platforms for simultaneous detection of various viruses.

6. Conclusion

A considerable number of infectious diseases are caused by waterborne viruses that could easily enter wastewater treatment systems or water supply systems. Hence, cautionary measures and regulations should be developed for the detection and removal of virus particles in water and wastewater. Currently, the ELISA, PCR-based methods, and biosensors are the most known methods for virus detection. Although each method has its limitations, biosensors seem to be a more attractive method as an early monitoring tool for unknown and asymptomatic carriers in communities as they are cost-effective, easy to use, and less-time required compared to conventional methods. Among many biosensing techniques, electrochemical biosensors can provide the most feasible options among other types of biosensors including sample preparation-free detection of pathogens in various matrices, rapid detection, low-cost sensor design, *in situ* detection of viruses on the surfaces of objects, multiplexed detection of pathogens in practical sample matrices, and detection of pathogens via wireless actuation and data acquisition formats [178]. Moreover, in the case of any viral pandemic, not only are electrochemical biosensors able to detect the virus but also they are capable of estimating the concentration of the target analyte which would facilitate the estimation of the infected population from wastewater. However, the

biosensor technology for the application in wastewater is still in its infancy and there is still more research required to bring the technology to the field.

It is crucial to identify the highly sensitive biorecognition elements of the biosensor (e.g., antibodies, aptamers, and imprinted polymers) to monitor and detect the target analyte in a complicated water matrix. Nucleic acid and antibodies are the most reported choices for the detection of waterborne viruses. With regards to nucleic acid-based biosensors, aptamers are promising bioreceptors since they could be designed specifically for each target virus, particularly, novel viruses with no known antibodies. Nevertheless, other types of bioreceptors should not be neglected because with a given time and by conducting enough research, there may be an enzyme, cell, or other microorganisms that could react with a specific virus and facilitate the detection of it. Moreover, novel developments in nanotechnology and materials science can be advantageously used to obtain engineered nanomaterials that could improve the immobilization of biorecognition elements which could lead to the development of useful and reliable biosensor devices.

Acknowledgments

This research was supported by a grant (20UGCP-B157945-01) from Plant R&D Program funded by Ministry of Land, Infrastructure and Transport of the Korean government.

References

[1] E. Scallan, R.M. Hoekstra, F.J. Angulo, R.V. Tauxe, M.-A. Widdowson, S.L. Roy, J.L. Jones, P.M. Griffin, Foodborne illness acquired in the United States—major pathogens, Emerging infectious diseases, 17 (2011) 7. https://doi.org/10.3201/eid1701.p11101.

[2] E. Scallan, P.M. Griffin, F.J. Angulo, R.V. Tauxe, R.M. Hoekstra, Foodborne illness acquired in the United States—unspecified agents, Emerging infectious diseases, 17 (2011) 16. https://doi.org/10.3201/eid1701.p21101.

[3] U.F.I.C.C.A.f. http://, www.ers.usda.gov/Data/FoodborneIllness/. [Accessed 12 April 2012].

[4] K. Wong, T.-T. Fong, K. Bibby, M. Molina, Application of enteric viruses for fecal pollution source tracking in environmental waters, Environment International, 45 (2012) 151-164. https://doi.org/10.1016/j.envint.2012.02.009.

[5] H. Xiao, D. Guan, R. Chen, P. Chen, C. Monagin, W. Li, J. Su, C. Ma, W. Zhang, C. Ke, Molecular characterization of echovirus 30-associated outbreak of aseptic meningitis in Guangdong in 2012, Virology journal, 10 (2013) 263. https://doi.org/10.1186/1743-422x-10-263.

[6] G.F. Craun, J.M. Brunkard, J.S. Yoder, V.A. Roberts, J. Carpenter, T. Wade, R.L. Calderon, J.M. Roberts, M.J. Beach, S.L. Roy, Causes of outbreaks associated with drinking water in the United States from 1971 to 2006, Clinical Microbiology Reviews, 23 (2010) 507-528. https://doi.org/10.1128/cmr.00077-09.

[7] E.J. Dziuban, J.L. Liang, G.F. Craun, V. Hill, P.A. Yu, J. Painter, M.R. Moore, R.L. Calderon, S.L. Roy, M.J. Beach, Surveillance for waterborne disease and outbreaks associated with recreational water—United States, 2003–2004, Morbidity and Mortality Weekly Report: Surveillance Summaries, 55 (2006) 1-30.

[8] Z. Dymaczewski, J.A. Oleszkiewicz, M.M. Sozański, J.F. Lemański, Poradnik eksploatatora oczyszczalni ścieków, Polskie Zrzeszenie Inżynierów i Techników Sanitarnych, 1997.

[9] P. Roingeard, P.I. Raynal, S. Eymieux, E. Blanchard, Virus detection by transmission electron microscopy: Still useful for diagnosis and a plus for biosafety, Rev Med Virol, 29 (2019) e2019. https://doi.org/10.1002/rmv.2019.

[10] A.C. Ortmann, C.A. Suttle, Determination of virus abundance by epifluorescence microscopy, in: Bacteriophages, Springer, 2009, pp. 87-95.

[11] B. Calgua, C.R.M. Barardi, S. Bofill-Mas, J. Rodriguez-Manzano, R. Girones, Detection and quantitation of infectious human adenoviruses and JC polyomaviruses in water by immunofluorescence assay, Journal of virological methods, 171 (2011) 1-7. https://doi.org/10.1016/j.jviromet.2010.09.013.

[12] E. Nassonova, Pulsed field gel electrophoresis: theory, instruments and application, Cell and Tissue Biology, 2 (2008) 557. https://doi.org/10.1134/S1990519X08060011.

[13] S. Xu, Electromechanical biosensors for pathogen detection, Microchimica Acta, 178 (2012) 245-260. https://doi.org/10.1007/s00604-012-0831-4.

[14] R. Yadav, S. Dwivedi, S. Kumar, A. Chaudhury, Trends and perspectives of biosensors for food and environmental virology, Food and Environmental Virology, 2 (2010) 53-63. https://doi.org/10.1007/s12560-010-9034-5.

[15] Z. Altintas, M. Gittens, J. Pocock, I.E. Tothill, Biosensors for waterborne viruses: Detection and removal, Biochimie, 115 (2015) 144-154. https://doi.org/10.1016/j.biochi.2015.05.010.

[16] C.A. Belon, D.N. Frick, Hepatitis C virus NS3 helicase inhibitors, Hepatitis C: Antiviral Drug Discovery and Development, (2011) 237-256.

[17] D. Radin, New trends in food-and waterborne viral outbreaks, Archives of Biological Sciences, 66 (2014) 1-9. https://doi.org/10.2298/ABS1401001R.

[18] K.R. Bradbury, M.A. Borchardt, M. Gotkowitz, S.K. Spencer, J. Zhu, R.J. Hunt, Source and transport of human enteric viruses in deep municipal water supply wells, Environmental science & technology, 47 (2013) 4096-4103. https://doi.org/10.1021/es400509b.

[19] USEPA (2012) Guidelines for water reuse. United States Environmental Protection Agency.

[20] K. Wong, B. Mukherjee, A.M. Kahler, R. Zepp, M. Molina, Influence of inorganic ions on aggregation and adsorption behaviors of human adenovirus, Environmental science & technology, 46 (2012) 11145-11153. https://doi.org/10.1021/es3028764.

[21] C.-C. Liang, Fate of bacterial and viral indicators in an advanced wastewater treatment plant, in, 2013.

[22] J.C. Crittenden, R.R. Trussell, D.W. Hand, K.J. Howe, G. Tchobanoglous, MWH's water treatment: principles and design, John Wiley & Sons, 2012.

[23] R. Sinclair, E. Jones, C.P. Gerba, Viruses in recreational water-borne disease outbreaks: A review, Journal of applied microbiology, 107 (2009) 1769-1780. https://doi.org/10.1111/j.1365-2672.2009.04367.x.

[24] C. Woodall, Waterborne diseases–What are the primary killers?, Desalination, 248 (2009) 616-621.

[25] J. Angel, M.A. Franco, H.B. Greenberg, Rotavirus vaccines: recent developments and future considerations, Nature reviews microbiology, 5 (2007) 529-539. https://doi.org/10.1038/nrmicro1692.

[26] S.J. Flint, Adenoviruses, e LS, (2001). https://doi.org/10.1038/npg.els.0000409.

[27] P.o.s.a.d.-w. WHO & UNICEF, Available from:, h.a.w.i.i.b.e.p.u. (2013).

[28] J.W. Woods, Waterborne Diseases of the Ocean, Enteric Viruses, in: Infectious Diseases, Springer, 2013, pp. 381-398.

[29] H. Fan, Q. Wu, X. Kou, Co-detection of five species of water-borne bacteria by multiplex PCR, Life Sci. J, 5 (2008) 47-54.

[30] H. Tsen, C. Lin, W. Chi, Development and use of 16S rRNA gene targeted PCR primers for the identification of Escherichia coli cells in water, Journal of Applied Microbiology, 85 (1998) 554-560. https://doi.org/10.1046/j.1365-2672.1998.853535.x.

[31] D.-Y. Lee, H. Lauder, H. Cruwys, P. Falletta, L.A. Beaudette, Development and application of an oligonucleotide microarray and real-time quantitative PCR for detection of wastewater bacterial pathogens, Science of the total environment, 398 (2008) 203-211. https://doi.org/10.1016/j.scitotenv.2008.03.004.

[32] P.K. Pandey, P.H. Kass, M.L. Soupir, S. Biswas, V.P. Singh, Contamination of water resources by pathogenic bacteria, Amb Express, 4 (2014) 51. https://doi.org/10.1186/s13568-014-0051-x.

[33] K.R. Wigginton, T. Kohn, Virus disinfection mechanisms: the role of virus composition, structure, and function, Current opinion in virology, 2 (2012) 84-89. https://doi.org/10.1016/j.coviro.2011.11.003.

[34] Z. Pang, A. Li, J. Li, J. Qu, C. He, S. Zhang, C. Li, Q. Zhang, M. Liang, D. Li, Comprehensive multiplex one-step real-time TaqMan qRT-PCR assays for detection and quantification of hemorrhagic fever viruses, PloS one, 9 (2014) e95635. https://doi.org/10.1371/journal.pone.0095635.

[35] M.S. Cheng, C.-S. Toh, Novel biosensing methodologies for ultrasensitive detection of viruses, Analyst, 138 (2013) 6219-6229. https://doi.org/10.1039/c3an01394d.

[36] K. Kleo, A. Kapp, L. Ascher, F. Lisdat, Detection of vaccinia virus DNA by quartz crystal microbalance, Analytical biochemistry, 418 (2011) 260-266. https://doi.org/10.1016/j.ab.2011.07.016.

[37] C. Zhang, X. Wang, J. Zhou, Z. Ji, Comparative study on enteric pathogens and their relations with fecal indicator bacteria in urban surface waters, Acta Sci Circum, 32 (2012) 2789-2794.

[38] J.T. Connelly, A.J. Baeumner, Biosensors for the detection of waterborne pathogens, Analytical and bioanalytical chemistry, 402 (2012) 117-127. https://doi.org/10.1007/s00216-011-5407-3.

[39] K. Gander. *Future coronavirus outbreatks could be predicted using sewage water*. 2020 [Accessed May 7 2020]; Available from: https://www.newsweek.com/future-coronavirus-outbreaks-could-predicted-using-sewage-water-scientists-say-1502463?fbclid=IwAR0XSrw4jv9zgb4uzD-SLnxOxGQJyiqWfEM4O087_F1Ugu7mCYUCVti8azw.

[40] A. Bosch, Human enteric viruses in the water environment: a minireview, Int Microbiol, 1 (1998) 191-196.

[41] A. Bosch, S. Guix, D. Sano, R.M. Pinto, New tools for the study and direct surveillance of viral pathogens in water, Current Opinion in Biotechnology, 19 (2008) 295-301. https://doi.org/10.1016/j.copbio.2008.04.006.

[42] J. Cubero, J. Graham, T. Gottwald, Quantitative PCR method for diagnosis of citrus bacterial canker, Applied and environmental microbiology, 67 (2001) 2849-2852. https://doi.org/10.1128/AEM.67.6.2849-2852.2001.

[43] P. Carrasco, J. Daros, P. Agudelo-Romero, S. Elena, A real-time RT-PCR assay for quantifying the fitness of Tobacco etch virus in competition experiments, Journal of virological methods, 139 (2007) 181-188. https://doi.org/10.1016/j.jviromet.2006.09.020.

[44] N. Boonham, J. Kreuze, S. Winter, R. van der Vlugt, J. Bergervoet, J. Tomlinson, R. Mumford, Methods in virus diagnostics: from ELISA to next generation sequencing, Virus research, 186 (2014) 20-31. https://doi.org/10.1016/j.virusres.2013.12.007.

[45] F.K. Lee, A.J. Nahmias, S. Lowery, S. Nesheim, S. Reef, S. Thompson, J. Oleske, A. Vahlne, C. Czerkinsky, ELISPOT: a new approach to studying the dynamics of virus-immune system interaction for diagnosis and monitoring of HIV infection, AIDS research and human retroviruses, 5 (1989) 517-523. https://doi.org/10.1089/aid.1989.5.517.

[46] J.W. Romano, R.N. Shurtliff, E. Dobratz, A. Gibson, K. Hickman, P.D. Markham, R. Pal, Quantitative evaluation of simian immunodeficiency virus infection using NASBA technology, Journal of virological methods, 86 (2000) 61-70. https://doi.org/10.1016/S0166-0934(99)00184-6.

[47] N. Hamilton, Quantification and its applications in fluorescent microscopy imaging, Traffic, 10 (2009) 951-961. https://doi.org/10.1111/j.1600-0854.2009.00938.x.

[48] A.J. Jääskeläinen, L. Maunula, Applicability of microarray technique for the detection of noro-and astroviruses, Journal of virological methods, 136 (2006) 210-216. https://doi.org/10.1016/j.jviromet.2006.05.015.

[49] F.X. Abad, R.M. Pintó, A. Bosch, Flow cytometry detection of infectious rotaviruses in environmental and clinical samples, Applied and Environmental Microbiology, 64 (1998) 2392-2396. https://doi.org/10.1128/AEM.64.7.2392-2396.1998.

[50] L. Clark, L. BARGERON, Lyons c, Ann. NY Acad. Sci, 102 (1962) 29-45.

[51] J.-W. Li, X.-W. Wang, C.-Q. Yuan, J.-L. Zheng, M. Jin, N. Song, X.-Q. Shi, F.-H. Chao, Detection of enteroviruses and hepatitis A virus in water by consensus primer multiplex RT-PCR, World journal of gastroenterology, 8 (2002) 699. https://doi.org/10.3748/wjg.v8.i4.699.

[52] T.M. Straub, I.L. Pepper, C.P. Gerba, Comparison of PCR and cell culture for detection of enteroviruses in sludge-amended field soils and determination of their transport, Appl. Environ. Microbiol., 61 (1995) 2066-2068. https://doi.org/10.1128/aem.61.5.2066-2068.1995.

[53] Y.-L. Tsai, B. Tran, L.R. Sangermano, C.J. Palmer, Detection of poliovirus, hepatitis A virus, and rotavirus from sewage and ocean water by triplex reverse transcriptase PCR, Appl. Environ. Microbiol., 60 (1994) 2400-2407. https://doi.org/10.1128/AEM.60.7.2400-2407.1994.

[54] G. Orrù, G. Masia, G. Orrù, L. Romanò, V. Piras, R.C. Coppola, Detection and quantitation of hepatitis E virus in human faeces by real-time quantitative PCR, Journal of virological methods, 118 (2004) 77-82. https://doi.org/10.1016/j.jviromet.2004.01.025.

[55] L. Kittigul, B. Raengsakulrach, S. Siritantikorn, R. Kanyok, F. Utrarachkij, P. Diraphat, V. Thirawuth, K. Siripanichgon, S. Pungchitton, K. Chitpirom, Detection of poliovirus, hepatitis A virus and rotavirus from sewage and water samples, Southeast Asian journal of tropical medicine and public health, 31 (2000) 41-46.

[56] J. Brown, D. Coates, R. Phillpotts, Evaluation of monoclonal antibodies for generic detection of flaviviruses by ELISA, Journal of virological methods, 62 (1996) 143-151. https://doi.org/10.1016/s0166-0934(96)02095-2.

[57] R.D. Saville, N.T. Constantine, F.R. Cleghorn, N. Jack, C. Bartholomew, J. Edwards, P. Gomez, W.A. Blattner, Fourth-generation enzyme-linked immunosorbent assay for the simultaneous detection of human immunodeficiency virus antigen and antibody, Journal of clinical microbiology, 39 (2001) 2518-2524. https://doi.org/10.1128/JCM.39.7.2518-2524.2001.

[58] J.M. Łoś, P. Golec, G. Węgrzyn, A. Węgrzyn, M. Łoś, Simple method for plating Escherichia coli bacteriophages forming very small plaques or no plaques under standard conditions, Appl. Environ. Microbiol., 74 (2008) 5113-5120. https://doi.org/10.1128/AEM.00306-08.

[59] K. Everett, J. Rees-George, I. Pushparajah, B. Janssen, Z. Luo, Advantages and disadvantages of microarrays to study microbial population dynamics a minireview, New Zealand Plant Protection, 63 (2010) 1-6. https://doi.org/10.30843/nzpp.2010.63.6606.

[60] K.H. Abd El Galil, M. El Sokkary, S. Kheira, A.M. Salazar, M.V. Yates, W. Chen, A. Mulchandani, Real-time nucleic acid sequence-based amplification assay for detection of

hepatitis A virus, Applied and environmental microbiology, 71 (2005) 7113-7116. https://doi.org/10.1128/AEM.71.11.7113-7116.2005.

[61] S.J. Martin, The biochemistry of viruses, CUP Archive, 1978.

[62] N. Guttman-Bass, Y. Tchorsh, E. Marva, Comparison of methods for rotavirus detection in water and results of a survey of Jerusalem wastewater, Applied and environmental microbiology, 53 (1987) 761-767. https://doi.org/10.1128/AEM.53.4.761-767.1987.

[63] F. Nasrin, A.D. Chowdhury, K. Takemura, J. Lee, O. Adegoke, V.K. Deo, F. Abe, T. Suzuki, E.Y. Park, Single-step detection of norovirus tuning localized surface plasmon resonance-induced optical signal between gold nanoparticles and quantum dots, Biosensors and Bioelectronics, 122 (2018) 16-24. https://doi.org/10.1016/j.bios.2018.09.024.

[64] J. Jean, B. Blais, A. Darveau, I.l. Fliss, Detection of hepatitis A virus by the nucleic acid sequence-based amplification technique and comparison with reverse transcription-PCR, Applied and Environmental Microbiology, 67 (2001) 5593-5600. https://doi.org/10.1128/AEM.67.12.5593-5600.2001.

[65] K. Farkas, F. Mannion, L.S. Hillary, S.K. Malham, D.I. Walker, Emerging technologies for the rapid detection of enteric viruses in the aquatic environment, Current Opinion in Environmental Science & Health, (2020). https://doi.org/10.1016/j.coesh.2020.01.007.

[66] C. Larsson, Y. Andersson, G. Allestam, A. Lindqvist, N. Nenonen, O. Bergstedt, Epidemiology and estimated costs of a large waterborne outbreak of norovirus infection in

Sweden, Epidemiology & Infection, 142 (2014) 592-600. https://doi.org/10.1017/S0950268813001209.

[67] T.-L. Chuang, S.-C. Wei, S.-Y. Lee, C.-W. Lin, A polycarbonate based surface plasmon resonance sensing cartridge for high sensitivity HBV loop-mediated isothermal amplification, Biosensors and Bioelectronics, 32 (2012) 89-95. https://doi.org/10.1016/j.bios.2011.11.037.

[68] Y. Kim, A.T. Abafogi, B.M. Tran, J. Kim, J. Lee, Z. Chen, P.K. Bae, K. Park, Y.-B. Shin, D. van Noort, Integrated Microfluidic Preconcentration and Nucleic Amplification System for Detection of Influenza A Virus H1N1 in Saliva, Micromachines, 11 (2020) 203. https://doi.org/10.3390/mi11020203.

[69] M. Bu, T.B. Christensen, K. Smistrup, A. Wolff, M.F. Hansen, Characterization of a microfluidic magnetic bead separator for high-throughput applications, Sensors and Actuators A: Physical, 145 (2008) 430-436. https://doi.org/10.1016/j.sna.2007.12.014.

[70] N. Xia, T.P. Hunt, B.T. Mayers, E. Alsberg, G.M. Whitesides, R.M. Westervelt, D.E. Ingber, Combined microfluidic-micromagnetic separation of living cells in continuous flow, Biomedical microdevices, 8 (2006) 299. https://doi.org/10.1007/s10544-006-0033-0.

[71] L. Su, W. Jia, C. Hou, Y. Lei, Microbial biosensors: a review, Biosensors and bioelectronics, 26 (2011) 1788-1799. https://doi.org/10.1016/j.bios.2010.09.005.

[72] P. Mehrotra, Biosensors and their applications–A review, Journal of oral biology and craniofacial research, 6 (2016) 153-159. https://doi.org/10.1016/j.jobcr.2015.12.002.

[73] H. Bridle, M. Desmulliez, Biosensors for the Detection of Waterborne Pathogens, in: Waterborne Pathogens, Elsevier, 2014, pp. 189-229.

[74] P. Kralik, M. Ricchi, A basic guide to real time PCR in microbial diagnostics: definitions, parameters, and everything, Frontiers in microbiology, 8 (2017) 108. https://doi.org/10.3389/fmicb.2017.00108.

[75] B. Pejcic, R. De Marco, G. Parkinson, The role of biosensors in the detection of emerging infectious diseases, Analyst, 131 (2006) 1079-1090. https://doi.org/10.1039/b603402k.

[76] F. Salam, Y. Uludag, I.E. Tothill, Real-time and sensitive detection of Salmonella Typhimurium using an automated quartz crystal microbalance (QCM) instrument with nanoparticles amplification, Talanta, 115 (2013) 761-767. https://doi.org/10.1016/j.talanta.2013.06.034.

[77] D.E. Baskeyfield, F. Davis, N. Magan, I.E. Tothill, A membrane-based immunosensor for the analysis of the herbicide isoproturon, Analytica chimica acta, 699 (2011) 223-231. https://doi.org/10.1016/j.aca.2011.05.036.

[78] Z. Altintas, Y. Uludag, Y. Gurbuz, I.E. Tothill, Surface plasmon resonance based immunosensor for the detection of the cancer biomarker carcinoembryonic antigen, Talanta, 86 (2011) 377-383. https://doi.org/10.1016/j.talanta.2011.09.031.

[79] P. Leonard, S. Hearty, J. Brennan, L. Dunne, J. Quinn, T. Chakraborty, R. O'Kennedy, Advances in biosensors for detection of pathogens in food and water, Enzyme and Microbial Technology, 32 (2003) 3-13. https://doi.org/10.1016/S0141-0229(02)00232-6.

[80] A. Mulchandani, A.S. Bassi, Principles and applications of biosensors for bioprocess monitoring and control, Critical reviews in biotechnology, 15 (1995) 105-124. https://doi.org/10.3109/07388559509147402.

[81] S. Rodriguez-Mozaz, M.J.L. de Alda, M.-P. Marco, D. Barceló, Biosensors for environmental monitoring: A global perspective, Talanta, 65 (2005) 291-297. https://doi.org/10.1016/j.talanta.2004.07.006.

[82] S. Li, A. Simonian, B.A. Chin, Sensors for agriculture and the food industry, The Electrochemical Society Interface, 19 (2010) 41-46. https://doi.org/10.1149/2.F05104if.

[83] M.S. Belluzo, M.É. Ribone, C.M. Lagier, Assembling amperometric biosensors for clinical diagnostics, Sensors, 8 (2008) 1366-1399. https://doi.org/10.3390/s8031366.

[84] C. Karunakaran, R. Rajkumar, K. Bhargava, Introduction to biosensors, in: Biosensors and bioelectronics, Elsevier, 2015, pp. 1-68.

[85] H. Bridle, Waterborne pathogens: detection methods and applications, Newnes, 2013.

[86] R. Singh, M.D. Mukherjee, G. Sumana, R.K. Gupta, S. Sood, B. Malhotra, Biosensors for pathogen detection: A smart approach towards clinical diagnosis, Sensors and Actuators B: Chemical, 197 (2014) 385-404. https://doi.org/10.1016/j.snb.2014.03.005.

[87] J. Wang, PNA biosensors for nucleic acid detection, Curr Issues Mol Biol, 1 (1999) 117-122. https://doi.org/10.21775/cimb.001.117.

[88] J. Wang, DNA biosensors based on peptide nucleic acid (PNA) recognition layers. A review, Biosensors and Bioelectronics, 13 (1998) 757-762. https://doi.org/10.1016/s0956-5663(98)00039-6.

[89] K. Kerman, M. Kobayashi, E. Tamiya, Recent trends in electrochemical DNA biosensor technology, Measurement Science and Technology, 15 (2003) R1. https://doi.org/10.1088/0957-0233/15/2/R01.

[90] X. Guo, C.-S. Lin, S.-H. Chen, R. Ye, V.C. Wu, A piezoelectric immunosensor for specific capture and enrichment of viable pathogens by quartz crystal microbalance sensor, followed by detection with antibody-functionalized gold nanoparticles, Biosensors and Bioelectronics, 38 (2012) 177-183. https://doi.org/10.1016/j.bios.2012.05.024.

[91] S. Chung, L.E. Breshears, S. Perea, C.M. Morrison, W.Q. Betancourt, K.A. Reynolds, J.-Y. Yoon, Smartphone-based paper microfluidic particulometry of norovirus from environmental water samples at the single copy level, ACS omega, 4 (2019) 11180-11188. https://doi.org/10.1021/acsomega.9b00772.

[92] N. Yildirim, D. Li, F. Long, A.Z. Gu, Direct detection of adenovirus in environmental waste waters by portable optical fiber sensor platform, in: SENSORS, 2013 IEEE, IEEE, 2013, pp. 1-5.

[93] Y.K. Kim, S.-I. Lim, S. Choi, I.-S. Cho, E.-H. Park, D.-J. An, A novel assay for detecting canine parvovirus using a quartz crystal microbalance biosensor, Journal of virological methods, 219 (2015) 23-27. https://doi.org/10.1016/j.jviromet.2015.03.015.

[94] K. Mao, H. Zhang, Z. Yang, Can a paper-based device trace COVID-19 sources with wastewater-based epidemiology?, in, ACS Publications, 2020.

[95] D. Barceló, Wastewater-Based Epidemiology to monitor COVID-19 outbreak: Present and future diagnostic methods to be in your radar, Case Studies in Chemical and Environmental Engineering, 2 (2020) 100042. https://doi.org/10.1016/j.cscee.2020.100042.

[96] K. Mao, H. Zhang, Z. Yang, The potential of an integrated biosensor system with mobile health and wastewater-based epidemiology (iBMW) for the prevention, surveillance, monitoring and intervention of the COVID-19 pandemic, Biosensors & Bioelectronics, (2020). https://dx.doi.org/10.1016%2Fj.bios.2020.112617.

[97] B. Taha, N. Arsad, Y. Al Mashhadany, An Analysis Review, Detection Coronavirus Disease 2019 (COVID-19) based on Biosensor Application, (2020). https://doi.org/10.20944/preprints202008.0597.v1.

[98] W. Lodder, A.M. de Roda Husman, SARS-CoV-2 in wastewater: potential health risk, but also data source, The Lancet Gastroenterology & Hepatology, 5 (2020) 533-534. https://doi.org/10.1016/S2468-1253(20)30087-X.

[99] W. Ahmed, N. Angel, J. Edson, K. Bibby, A. Bivins, J.W. O'Brien, P.M. Choi, M. Kitajima, S.L. Simpson, J. Li, First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater surveillance of COVID-19 in the community, Science of The Total Environment, (2020) 138764. https://doi.org/10.1016/j.scitotenv.2020.138764.

[100] A.A. Lahcen, A. Amine, Recent advances in electrochemical sensors based on molecularly imprinted polymers and nanomaterials, Electroanalysis, 31 (2019) 188-201. https://doi.org/10.1002/elan.201800623.

[101] M.V.A. Corpuz, A. Buonerba, G. Vigliotta, T. Zarra, F. Ballesteros Jr, P. Campiglia, V. Belgiorno, G. Korshin, V. Naddeo, Viruses in wastewater: occurrence, abundance and detection methods, Science of the Total Environment, 745 (2020) 140910. https://doi.org/10.1016/j.scitotenv.2020.140910.

[102] CDMSmith. *A Look in the Sewer: Using Wastewater-Based Epidemiology to Track COVID-19* 2020 [Accessed 7/10 2020]; Available from: https://cdmsmith.com/en/Client-Solutions/Insights/Using-Wastewater-Based-Epidemiology-to-track-COVID-19.

[103] A.C. Mumford, J.L. Barringer, W.M. Benzel, P.A. Reilly, L. Young, Microbial transformations of arsenic: mobilization from glauconitic sediments to water, Water research, 46 (2012) 2859-2868. https://doi.org/10.1016/j.watres.

[104] Y. Qiu, B.E. Lee, N.J. Ruecker, N. Neumann, N. Ashbolt, X. Pang, A one-step centrifugal ultrafiltration method to concentrate enteric viruses from wastewater, Journal of virological methods, 237 (2016) 150-153. https://doi.org/10.1016/j.jviromet.2016.09.010.

[105] J.P. Sidhu, W. Ahmed, S. Toze, Sensitive detection of human adenovirus from small volume of primary wastewater samples by quantitative PCR, Journal of virological methods, 187 (2013) 395-400. https://doi.org/10.1016/j.jviromet.2012.11.002.

[106] T. Prado, A. de Castro Bruni, M.R.F. Barbosa, S.C. Garcia, A.M. de Jesus Melo, M.I.Z. Sato, Performance of wastewater reclamation systems in enteric virus removal, Science of The Total Environment, 678 (2019) 33-42. https://doi.org/10.1016/j.scitotenv.2019.04.435.

[107] J.C. Falman, C.S. Fagnant-Sperati, A.L. Kossik, D.S. Boyle, J.S. Meschke, Evaluation of secondary concentration methods for poliovirus detection in wastewater, Food and environmental virology, 11 (2019) 20-31. https://doi.org/10.1007/s12560-018-09364-y.

[108] E. Symonds, M. Verbyla, J. Lukasik, R. Kafle, M. Breitbart, J. Mihelcic, A case study of enteric virus removal and insights into the associated risk of water reuse for two wastewater treatment pond systems in Bolivia, Water research, 65 (2014) 257-270. https://doi.org/10.1016/j.watres.2014.07.032.

[109] Y. Yuan, Y. Wu, X. Ge, D. Nie, M. Wang, H. Zhou, M. Chen, In vitro toxicity evaluation of heavy metals in urban air particulate matter on human lung epithelial cells, Science of The Total Environment, 678 (2019) 301-308. https://doi.org/10.1016/j.scitotenv.2019.04.431.

[110] Z. Yang, B. Kasprzyk-Hordern, C.G. Frost, P. Estrela, K.V. Thomas, Community sewage sensors for monitoring public health, in, ACS Publications, 2015.

[111] F. He, L. Zhang, J. Zhao, B. Hu, J. Lei, A TSM immunosensor for detection of M. tuberculosis with a new membrane material, Sensors and Actuators B: Chemical, 85 (2002) 284-290. https://doi.org/10.1016/S0925-4005(02)00144-2.

[112] V.K. Rao, M.K. Sharma, A.K. Goel, L. Singh, K. Sekhar, Amperometric immunosensor for the detection of Vibrio cholerae O1 using disposable screen-printed electrodes, Analytical sciences, 22 (2006) 1207-1211. https://doi.org/10.2116/analsci.22.1207.

[113] U. Schaible, M. Liss, E. Prohaska, J. Decker, K. Stadtherr, H. Wolf, Affinity measurements of biological molecules by a quartz crystal microbalance (QCM) biosensor, in: Molecular Diagnosis of Infectious Diseases, Springer, 2004, pp. 321-330.

[114] C. Yao, T. Zhu, J. Tang, R. Wu, Q. Chen, M. Chen, B. Zhang, J. Huang, W. Fu, Hybridization assay of hepatitis B virus by QCM peptide nucleic acid biosensor, Biosensors and Bioelectronics, 23 (2008) 879-885. https://doi.org/10.1016/j.bios.2007.09.003.

[115] C.-Y. Yao, W.-L. Fu, Biosensors for hepatitis B virus detection, World Journal of Gastroenterology: WJG, 20 (2014) 12485. https://doi.org/10.3748/wjg.v20.i35.12485.

[116] X. Zhou, L. Liu, M. Hu, L. Wang, J. Hu, Detection of hepatitis B virus by piezoelectric biosensor, Journal of pharmaceutical and biomedical analysis, 27 (2002) 341-345. https://doi.org/10.1016/s0731-7085(01)00538-6.

[117] E. Uttenthaler, C. Kößlinger, S. Drost, Characterization of immobilization methods for African swine fever virus protein and antibodies with a piezoelectric immunosensor, Biosensors and Bioelectronics, 13 (1998) 1279-1286. https://doi.org/10.1016/s0956-5663(98)00089-x.

[118] M.A. Cooper, F.N. Dultsev, T. Minson, V.P. Ostanin, C. Abell, D. Klenerman, Direct and sensitive detection of a human virus by rupture event scanning, Nature biotechnology, 19 (2001) 833-837. https://doi.org/10.1038/nbt0901-833.

[119] S.B. Shinde, C.B. Fernandes, V.B. Patravale, Recent trends in in-vitro nanodiagnostics for detection of pathogens, Journal of controlled release, 159 (2012) 164-180. https://doi.org/10.1016/j.jconrel.2011.11.033.

[120] Y. Uludağ, R. Hammond, M.A. Cooper, A signal amplification assay for HSV type 1 viral DNA detection using nanoparticles and direct acoustic profiling, Journal of nanobiotechnology, 8 (2010) 3. https://doi.org/10.1186/1477-3155-8-3.

[121] J.-S. Yu, H.-X. Liao, A.E. Gerdon, B. Huffman, R.M. Scearce, M. McAdams, S.M. Alam, P.M. Popernack, N.J. Sullivan, D. Wright, Detection of Ebola virus envelope using monoclonal and polyclonal antibodies in ELISA, surface plasmon resonance and a quartz crystal microbalance immunosensor, Journal of virological methods, 137 (2006) 219-228. https://doi.org/10.1016/j.jviromet.2006.06.014.

[122] C.-C. Su, T.-Z. Wu, L.-K. Chen, H.-H. Yang, D.-F. Tai, Development of immunochips for the detection of dengue viral antigens, Analytica chimica acta, 479 (2003) 117-123. https://doi.org/10.1016/S0003-2670(02)01529-5.

[123] A. Peh, Y.-S. Leo, C.S. Toh, Current and nano-diagnostic tools for dengue infection, Front Biosci (Schol Ed), 3 (2011) 806-821. https://doi.org/10.2741/189.

[124] F.S.R.R. Teles, Biosensors and rapid diagnostic tests on the frontier between analytical and clinical chemistry for biomolecular diagnosis of dengue disease: a review, Analytica chimica acta, 687 (2011) 28-42. https://doi.org/10.1016/j.aca.2010.12.011.

[125] S.-H. Chen, Y.-C. Chuang, Y.-C. Lu, H.-C. Lin, Y.-L. Yang, C.-S. Lin, A method of layer-by-layer gold nanoparticle hybridization in a quartz crystal microbalance DNA sensing system used to detect dengue virus, Nanotechnology, 20 (2009) 215501. https://doi.org/10.1088/0957-4484/20/21/215501.

[126] B. König, M. Grätzel, Detection of viruses and bacteria with piezoelectric immunosensors, Analytical letters, 26 (1993) 1567-1585. https://doi.org/10.1080/00032719308021481.

[127] B. König, M. Grätzel, A piezoelectric immunosensor for hepatitis viruses, Analytica chimica acta, 309 (1995) 19-25. https://doi.org/10.1016/0003-2670(94)00640-8.

[128] https://www.bio-rad-antibodies.com/polyclonal/viral-rotavirus-antibody-8129-9804.html?f=purified. [Accessed 10 August 2020].

[129] F. Neumann, N. Madaboosi, I. Hernández-Neuta, J. Salas, A. Ahlford, V. Mecea, M. Nilsson, QCM mass underestimation in molecular biotechnology: Proximity ligation assay for norovirus detection as a case study, Sensors and Actuators B: Chemical, 273 (2018) 742-750. https://doi.org/10.1016/j.snb.2018.06.025.

[130] C. Greenwood, D. Ruff, S. Kirvell, G. Johnson, H.S. Dhillon, S.A. Bustin, Proximity assays for sensitive quantification of proteins, Biomolecular detection and quantification, 4 (2015) 10-16. https://doi.org/10.1016/j.bdq.2015.04.002.

[131] M.C. Hansen, L. Nederby, M.O.-B. Henriksen, M. Hansen, C.G. Nyvold, Sensitive ligand-based protein quantification using immuno-PCR: a critical review of single-probe and proximity ligation assays, Biotechniques, 56 (2014) 217-228. https://doi.org/10.2144/000114164.

[132] G.E. Rydell, A.B. Dahlin, F. Höök, G. Larson, QCM-D studies of human norovirus VLPs binding to glycosphingolipids in supported lipid bilayers reveal strain-specific characteristics, Glycobiology, 19 (2009) 1176-1184. https://doi.org/10.1093/glycob/cwp103.

[133] T. Imai, D. Sano, T. Miura, S. Okabe, K. Wada, Y. Masago, T. Omura, Adsorption characteristics of an enteric virus-binding protein to norovirus, rotavirus and poliovirus, BMC biotechnology, 11 (2011) 123. https://doi.org/10.1186/1472-6750-11-123.

[134] R. Wang, Y. Li, Hydrogel based QCM aptasensor for detection of avian influenzavirus, Biosensors and Bioelectronics, 42 (2013) 148-155. https://doi.org/10.1016/j.bios.2012.10.038.

[135] D. Ivnitski, I. Abdel-Hamid, P. Atanasov, E. Wilkins, Biosensors for detection of pathogenic bacteria, Biosensors and Bioelectronics, 14 (1999) 599-624. https://doi.org/10.1016/S0956-5663(99)00039-1.

[136] D. Dey, T. Goswami, Optical biosensors: a revolution towards quantum nanoscale electronics device fabrication, Journal of Biomedicine and Biotechnology, 2011 (2011). https://doi.org/10.1155/2011/348218.

[137] A. Collings, F. Caruso, Biosensors: recent advances, Reports on Progress in Physics, 60 (1997) 1397. https://doi.org/10.1088/0034-4885/60/11/005.

[138] K. Narsaiah, S.N. Jha, R. Bhardwaj, R. Sharma, R. Kumar, Optical biosensors for food quality and safety assurance—a review, Journal of food science and technology, 49 (2012) 383-406. https://doi.org/10.1007/s13197-011-0437-6.

[139] H. Ashiba, Y. Sugiyama, X. Wang, H. Shirato, K. Higo-Moriguchi, K. Taniguchi, Y. Ohki, M. Fujimaki, Detection of norovirus virus-like particles using a surface plasmon resonance-assisted fluoroimmunosensor optimized for quantum dot fluorescent labels, Biosensors and Bioelectronics, 93 (2017) 260-266. https://doi.org/10.1016/j.bios.2016.08.099.

[140] P. Weerathunge, R. Ramanathan, V.A. Torok, K. Hodgson, Y. Xu, R. Goodacre, B.K. Behera, V. Bansal, Ultrasensitive colorimetric detection of murine norovirus using NanoZyme aptasensor, Analytical chemistry, 91 (2019) 3270-3276. https://doi.org/10.1021/acs.analchem.8b03300.

[141] C.E. Jin, T.Y. Lee, B. Koo, H. Sung, S.-H. Kim, Y. Shin, Rapid virus diagnostic system using bio-optical sensor and microfluidic sample processing, Sensors and Actuators B: Chemical, 255 (2018) 2399-2406. https://doi.org/10.1016/j.snb.2017.08.197.

[142] M.W. Kim, H.-J. Park, C.Y. Park, J.H. Kim, C.H. Cho, J.P. Park, S.K. Kailasa, C.-H. Lee, T.J. Park, Fabrication of a paper strip for facile and rapid detection of bovine viral diarrhea virus via signal enhancement by copper polyhedral nanoshells, RSC Advances, 10 (2020) 29759-29764. https://doi.org/10.1039/D0RA03677C.

[143] J.-G. Kim, S.H. Baek, S. Kim, H.I. Kim, S.W. Lee, S.K. Kailasa, T.J. Park, Rapid discriminative detection of dengue viruses via loop mediated isothermal amplification, Talanta, 190 (2018) 391-396. https://doi.org/10.1016/j.talanta.2018.08.019.

[144] B.A. Prabowo, R.Y. Wang, M.K. Secario, P.-T. Ou, A. Alom, J.-J. Liu, K.-C. Liu, Rapid detection and quantification of Enterovirus 71 by a portable surface plasmon resonance biosensor, Biosensors and Bioelectronics, 92 (2017) 186-191. https://doi.org/10.1016/j.bios.2017.01.043.

[145] M. Hoefling, F. Iori, S. Corni, K.-E. Gottschalk, Interaction of amino acids with the Au (111) surface: adsorption free energies from molecular dynamics simulations, Langmuir, 26 (2010) 8347-8351. https://doi.org/10.1021/la904765u.

[146] A. Pakiari, Z. Jamshidi, Interaction of amino acids with gold and silver clusters, The Journal of Physical Chemistry A, 111 (2007) 4391-4396. https://doi.org/10.1021/jp070306t.

[147] Y. Liu, T. Shen, L. Hu, H. Gong, C. Chen, X. Chen, C. Cai, Development of a thermosensitive molecularly imprinted polymer resonance light scattering sensor for rapid and highly selective detection of hepatitis A virus in vitro, Sensors and Actuators B: Chemical, 253 (2017) 1188-1193. https://doi.org/10.1016/j.snb.2017.07.166.

[148] M.-S. Yoo, M. Shin, Y. Kim, M. Jang, Y.-E. Choi, S.J. Park, J. Choi, J. Lee, C. Park, Development of electrochemical biosensor for detection of pathogenic microorganism in Asian dust events, Chemosphere, 175 (2017) 269-274. https://doi.org/10.1016/j.chemosphere.2017.02.060.

[149] P. D'Orazio, Biosensors in clinical chemistry, Clinica Chimica Acta, 334 (2003) 41-69. https://doi.org/10.1016/s0009-8981(03)00241-9.

[150] S.B. Adeloju, S.J. Shaw, G.G. Wallace, Polypyrrole-based amperometric flow injection biosensor for urea, Analytica Chimica Acta, 323 (1996) 107-113. https://doi.org/10.1016/0003-2670(95)00562-5.

[151] A.C. Barton, S.D. Collyer, F. Davis, G.-Z. Garifallou, G. Tsekenis, E. Tully, R. O'Kennedy, T. Gibson, P.A. Millner, S.P. Higson, Labeless AC impedimetric antibody-based sensors with pg ml− 1 sensitivities for point-of-care biomedical applications, Biosensors and Bioelectronics, 24 (2009) 1090-1095. https://doi.org/10.1016/j.bios.2008.06.001.

[152] M. Billah, H.C. Hays, P.A. Millner, Development of a myoglobin impedimetric immunosensor based on mixed self-assembled monolayer onto gold, Microchimica Acta, 160 (2008) 447-454. https://doi.org/10.1007/s00604-007-0793-0.

[153] C. Ding, F. Zhao, M. Zhang, S. Zhang, Hybridization biosensor using 2, 9-dimethyl-1, 10-phenantroline cobalt as electrochemical indicator for detection of hepatitis B virus DNA, Bioelectrochemistry, 72 (2008) 28-33. https://doi.org/10.1016/j.bioelechem.2007.11.001.

[154] R.E. Ionescu, S. Cosnier, G. Herzog, K. Gorgy, B. Leshem, S. Herrmann, R.S. Marks, Amperometric immunosensor for the detection of anti-West Nile virus IgG using a photoactive copolymer, Enzyme and microbial technology, 40 (2007) 403-408. https://doi.org/10.1016/j.enzmictec.2006.07.010.

[155] M. Manzano, S. Viezzi, S. Mazerat, R.S. Marks, J. Vidic, Rapid and label-free electrochemical DNA biosensor for detecting hepatitis A virus, Biosensors and Bioelectronics, 100 (2018) 89-95. https://doi.org/10.1016/j.bios.2017.08.043.

[156] D. Tang, J. Tang, B. Su, J. Ren, G. Chen, Simultaneous determination of five-type hepatitis virus antigens in 5 min using an integrated automatic electrochemical immunosensor array, Biosensors and Bioelectronics, 25 (2010) 1658-1662. https://doi.org/10.1016/j.bios.2009.12.004.

[157] S.H. Baek, M.W. Kim, C.Y. Park, C.-S. Choi, S.K. Kailasa, J.P. Park, T.J. Park, Development of a rapid and sensitive electrochemical biosensor for detection of human norovirus via novel specific binding peptides, Biosensors and Bioelectronics, 123 (2019) 223-229. https://doi.org/10.1016/j.bios.2018.08.064.

[158] S.H. Baek, C.Y. Park, T.P. Nguyen, M.W. Kim, J.P. Park, C. Choi, S.Y. Kim, S.K. Kailasa, T.J. Park, Novel peptides functionalized gold nanoparticles decorated tungsten disulfide nanoflowers as the electrochemical sensing platforms for the norovirus in an oyster, Food Control, (2020) 107225. https://doi.org/10.1016/j.foodcont.2020.107225.

[159] F. Liu, K.S. Choi, T.J. Park, S.Y. Lee, T.S. Seo, Graphene-based electrochemical biosensor for pathogenic virus detection, BioChip Journal, 5 (2011) 123-128. https://doi.org/10.1007/s13206-011-5204-2.

[160] F. Liu, Y.H. Kim, D.S. Cheon, T.S. Seo, Micropatterned reduced graphene oxide based field-effect transistor for real-time virus detection, Sensors and Actuators B: Chemical, 186 (2013) 252-257. https://doi.org/10.1016/j.snb.2013.05.097.

[161] O. Lazcka, F.J. Del Campo, F.X. Munoz, Pathogen detection: a perspective of traditional methods and biosensors, Biosensors and bioelectronics, 22 (2007) 1205-1217. https://doi.org/10.1016/j.bios.2006.06.036.

[162] R.L. Caygill, C.S. Hodges, J.L. Holmes, S.P. Higson, G.E. Blair, P.A. Millner, Novel impedimetric immunosensor for the detection and quantitation of Adenovirus using reduced antibody fragments immobilized onto a conducting copolymer surface, Biosensors and Bioelectronics, 32 (2012) 104-110. https://doi.org/10.1016/j.bios.2011.11.041.

[163] J. Lee, M. Morita, K. Takemura, E.Y. Park, A multi-functional gold/iron-oxide nanoparticle-CNT hybrid nanomaterial as virus DNA sensing platform, Biosensors and Bioelectronics, 102 (2018) 425-431. https://doi.org/10.1016/j.bios.2017.11.052.

[164] V. Van Thu, P.T. Dung, P.D. Tam, Biosensor based on nanocomposite material for pathogenic virus detection, Colloids and Surfaces B: Biointerfaces, 115 (2014) 176-181. https://doi.org/10.1016/j.colsurfb.2013.11.016.

[165] N. Reta, C.P. Saint, A. Michelmore, B. Prieto-Simon, N.H. Voelcker, Nanostructured electrochemical biosensors for label-free detection of water-and food-borne pathogens, ACS applied materials & interfaces, 10 (2018) 6055-6072. https://doi.org/10.1021/acsami.7b13943.

[166] P.R. Solanki, A. Kaushik, V.V. Agrawal, B.D. Malhotra, Nanostructured metal oxide-based biosensors, NPG Asia Materials, 3 (2011) 17-24. https://doi.org/10.1038/asiamat.2010.137.

[167] K. Jayakumar, R. Rajesh, V. Dharuman, R. Venkatasan, J. Hahn, S.K. Pandian, Gold nano particle decorated graphene core first generation PAMAM dendrimer for label free electrochemical DNA hybridization sensing, Biosensors and Bioelectronics, 31 (2012) 406-412. https://doi.org/10.1016/j.bios.2011.11.001.

[168] K.M. Byun, N.-H. Kim, Y.H. Ko, J.S. Yu, Enhanced surface plasmon resonance detection of DNA hybridization based on ZnO nanorod arrays, Sensors and Actuators B: Chemical, 155 (2011) 375-379. https://doi.org/10.1016/j.snb.2010.12.039.

[169] A. Kulkarni, Y. Xu, C. Ahn, R. Amin, S.H. Park, T. Kim, M. Lee, The label free DNA sensor using a silicon nanowire array, Journal of biotechnology, 160 (2012) 91-96. https://doi.org/10.1016/j.jbiotec.2012.04.013.

[170] P.D. Tam, N. Van Hieu, N.D. Chien, A.-T. Le, M.A. Tuan, DNA sensor development based on multi-wall carbon nanotubes for label-free influenza virus (type A) detection, Journal of Immunological Methods, 350 (2009) 118-124. https://doi.org/10.1016/j.jim.2009.08.002.

[171] W. Lv, F.-M. Jin, Q. Guo, Q.-H. Yang, F. Kang, DNA-dispersed graphene/NiO hybrid materials for highly sensitive non-enzymatic glucose sensor, Electrochimica Acta, 73 (2012) 129-135. https://doi.org/10.1016/j.electacta.2011.11.089.

[172] Y.-L. Wu, J.-J. Lin, P.-Y. Hsu, C.-P. Hsu, Highly sensitive polysilicon wire sensor for DNA detection using silica nanoparticles/γ-APTES nanocomposite for surface modification, Sensors and Actuators B: Chemical, 155 (2011) 709-715. https://doi.org/10.1016/j.snb.2011.01.035.

[173] Y. Zhu, P. Chandra, K.-M. Song, C. Ban, Y.-B. Shim, Label-free detection of kanamycin based on the aptamer-functionalized conducting polymer/gold nanocomposite, Biosensors and Bioelectronics, 36 (2012) 29-34. https://doi.org/10.1016/j.bios.2012.03.034.

[174] J.M. Perez, F.J. Simeone, Y. Saeki, L. Josephson, R. Weissleder, Viral-induced self-assembly of magnetic nanoparticles allows the detection of viral particles in biological media, Journal of the American Chemical Society, 125 (2003) 10192-10193. https://doi.org/10.1021/ja036409g.

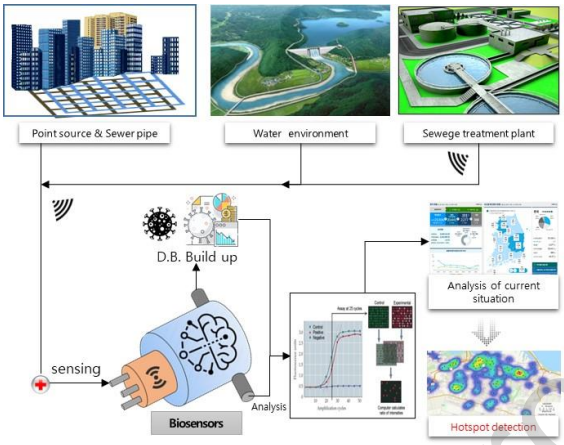
[175] A. Hryniszyn, M. Skonieczna, Methods for detection of viruses in water and wastewater, Advances in Microbiology, 3 (2013) 442. http://dx.doi.org/10.4236/aim.2013.35060.

[176] G. Orive, U. Lertxundi, D. Barcelo, Early SARS-CoV-2 outbreak detection by sewage-based epidemiology, Science of The Total Environment, 732 (2020) 139298. https://doi.org/10.1016/j.scitotenv.2020.139298.

[177] https://india.mongabay.com/2020/04/low-cost-paper-based-device-being-developed-for-rapid-coronavirus-detection/. [Accessed 14 June 2020].

[178] E. Cesewski, B.N. Johnson, Electrochemical biosensors for pathogen detection, Biosensors and Bioelectronics, (2020) 112214.

Graphical Abstract



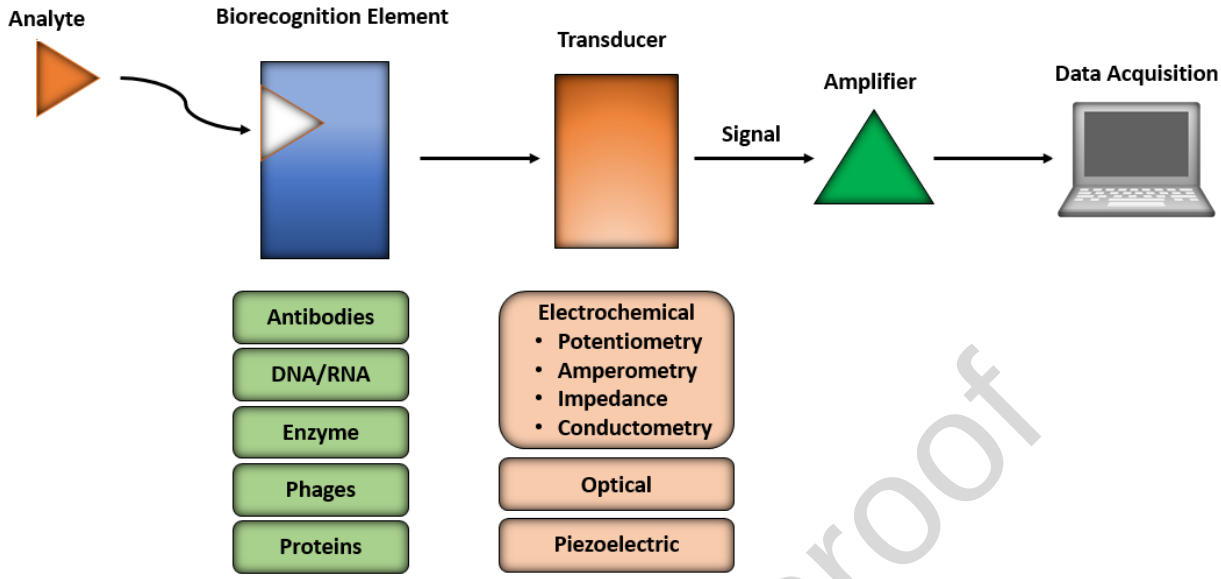


Figure 1. A schematic diagram of a typical biosensor system configuration and the classification of biosensors with various bioreceptors or transducers [85, 86].

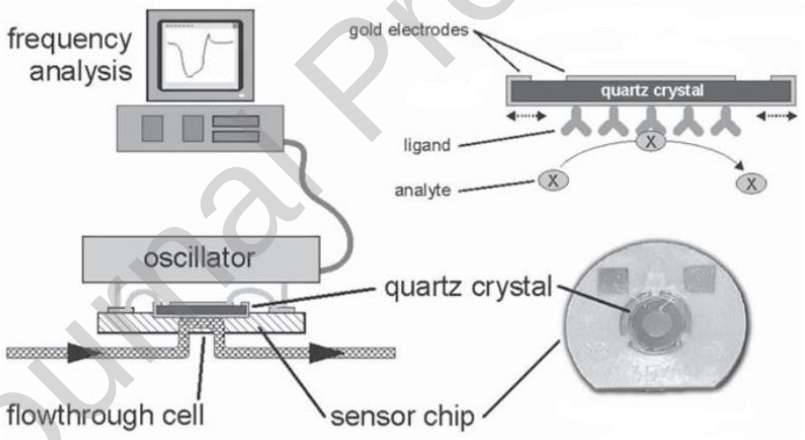


Figure 2. A quartz crystal microbalance (QCM) biosensor (left), the quartz crystal (top right), and the sensor chip with the quartz crystal (bottom right) [113].

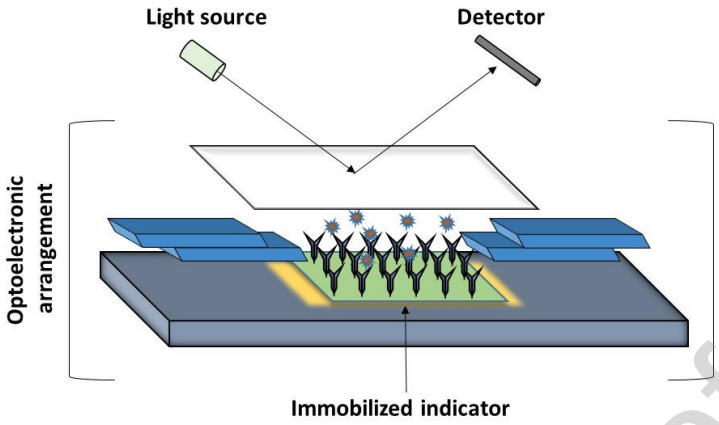


Figure 3. Architecture of an optical biosensor [136].

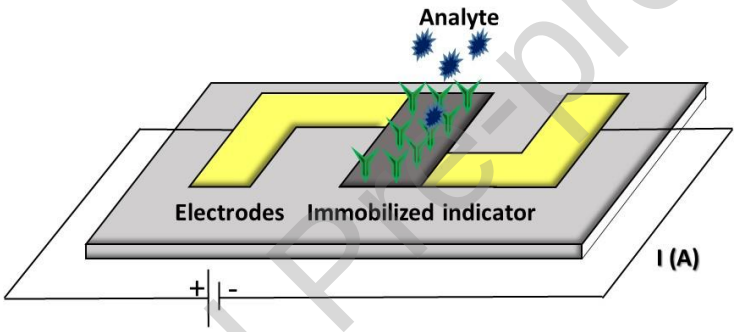


Figure 4. A single-walled carbon nanotubes (SWCNTs)-based electrochemical biosensor for detection of pathogenic microorganisms [148].

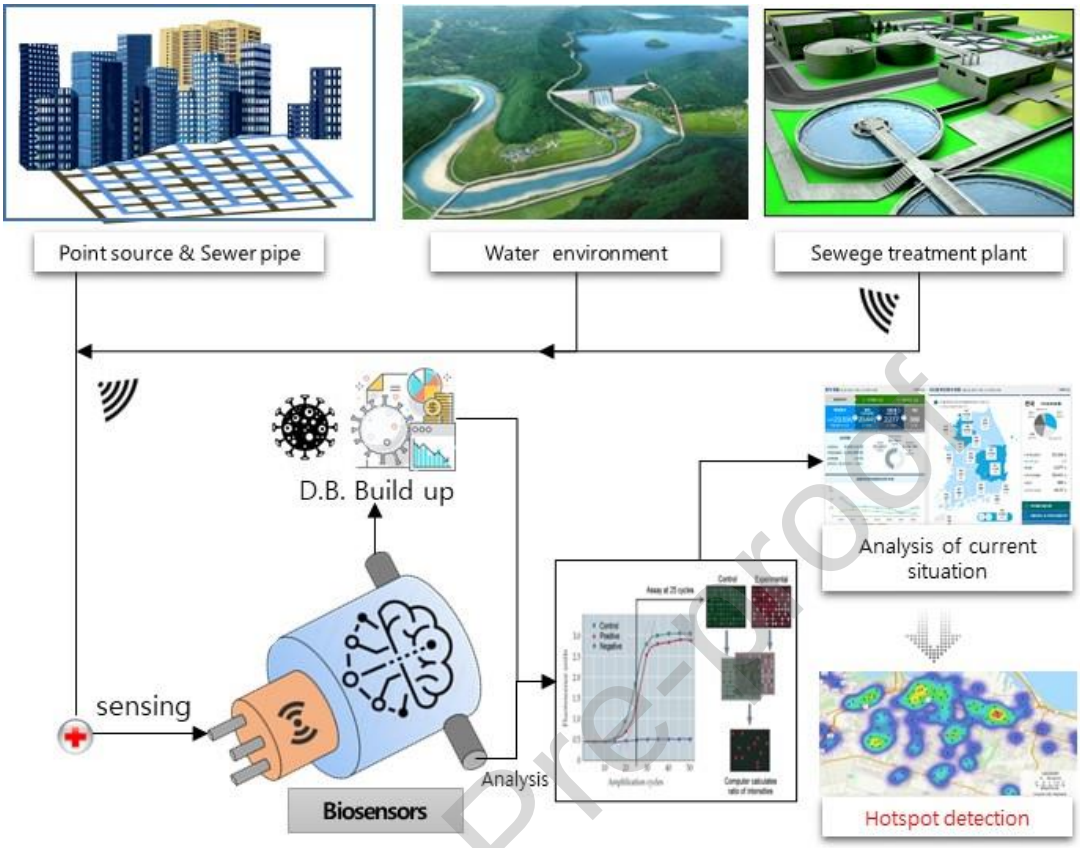


Figure 5. A schematic view of a biosensor-based WBE approach for prescreening of viruses in the wastewater treatment plants and sewer systems.

Table 1. Characteristics of most common waterborne viruses [4, 19-26]

Virus	Genome	Size (nm)	Shape	Symptoms and Diseases	Infectious dose (viral particles)
Norovirus	ssRNA*	35-40	Spherical	Diarrhea, vomiting, nausea, dehydration, fever, abdominal pain, gastroenteritis	10-100
Adenovirus	dsDNA**	60-90	Icosahedral	Gastroenteritis, conjunctivitis, respiratory disease	1-100
hepatitis A virus (HAV)	ssRNA	27-32	Icosahedral	Abdominal cramps and anorexia, jaundice, fever, nausea, fatigue	1-100
hepatitis E virus (HEV)	ssRNA	27-32	Icosahedral	Inflammation of the liver which causes severe pain, fever, vomiting, incapacitation of the patient	N/A****
Rotavirus	dsRNA***	50-65	Icosahedral	Diarrhea, gastroenteritis	1-10
Astrovirus	ssRNA	28	Spherical	Gastroenteritis	1-100
Polyomavirus	dsDNA	35-40	Icosahedral	Sarcoma, cancer	N/A
Coxsackievirus	ssRNA	20-30	Icosahedral	Meningitis, respiratory disease	10-100
Poliovirus	ssRNA	20-30	Icosahedral	Fever, paralysis, meningitis, poliomyelitis	100-500

*ssRNA: single stranded RNA, **dsRNA: double stranded RNA, ***dsDNA: double stranded DNA, ****N/A: not available

Table 2. Summary of conventional methods for virus detection in water environment

Virus detection methods	Advantages	Disadvantages	Ref.
RT-qPCR	• Nucleic acid-based method • Molecular amplification approach • Great specificity, in particular toward enteric viruses	• Two amplification steps which are highly susceptible to contamination by inhibitors • Possible interference with humic acids • Sample pretreatment concentration are required • Not satisfactory in terms of limit of detection (LOD) (10^4 PFU/mL for hepatitis A virus [HAV]) • Relatively time-consuming detection time (e.g., 3 hrs for detection of hepatitis E virus [HEV])	[51-54, 64]
ELISA	• Protein detection method • Cost effective • Relatively simple procedure	• Lack of specificity • Sample pretreatment concentration are required • Inefficient detection performance when bacteria is present in the sample and if the epitopes are hidden in protein structure • Time-consuming (6 hrs for detection of rotavirus in wastewater sample) • Not satisfactory in terms of LOD (10^4–10^6 virus particles/mL for norovirus)	[55-57, 62, 63]
Plaque forming assay	• Capable of recognizing pathogenic from non-pathogenic viruses	• Expensive (~$400 per analysis) • Difficulties associated with plaque observation (arisen plaques can be hooded by bacterial colony) • Extremely time-consuming (3 to 14 days)	[14, 52, 58, 61]

NASBA	• Sensitivity • Simplicity compared to RT-qPCR • Resistant to sample contamination	• Sample pretreatment concentration are required • Relatively low LOD (4×10^2 PFU/mL for hepatitis A virus [HAV]) • Relatively time-consuming (e.g., 3.5 hrs for detection of hepatitis A virus [HAV])	[59, 60]

Table 3. Important factors for characterizing and evaluating biosensor performance [73-75]

Factor/parameters	**Description**
Limit of detection (LOD)	Lowest quantity of pathogen that can be detected by a biosensor
Specificity/Selectivity	The ability of the biosensors to distinguish a specific strain from another; low interference with other chemical and biological compounds; low impact by other environmental parameters (e.g., temperature, pH, etc.)
Sensitivity	The ability to detect very few organisms in a sample (influenced by affinity of the biorecognition element as well as the transduction method)
Detection range	The concentration range which the sensor operates
Sample volume	Total volume required for one analysis
Assay time	Total time necessary to prepare the sample, analyze it and read out the results; sample pretreatment requirement (e.g., concentration and RNA extraction)
Sample matrix	Type of the solution in which detection takes place

Table 4. Summary of waterborne virus detection using biosensors

Type	Target virus	Bioreceptor	Buffer / pH	Temp. (˚C)	LOD	Detection time	Repeatability (n)	Ref.
Piezoelectric	Rotavirus	Goat anti - rotavirus antibody	PBS* (pH 7)	Room temp.	N/A	45 min	12	[126]
Piezoelectric	hepatitis A virus	Anti-hepatitis A virus antibody & protein A	PBS (pH 7)	Room temp.	10^5 viruses/mL	NA	10	[127]
Optical	hepatitis A virus	Thermosensitive surface-imprinted polymer	Britton - Robinson (pH 3.5)	40 ˚C	1.1 pM	30 min	N/A	[147]
Optical	Norovirus	Anti-norovirus monoclonal antibody	PBS/ N/A	Room temp.	0.01 ng/mL	20 min	N/A	[139]
Optical	Norovirus	NanoZyme aptasensor	N/A	N/A	30 viruses/mL	10 min	N/A	[140]
Optical	Adenovirus DNA	Primer DNA	N/A	56 ˚C	10 copies/reaction	30 min	N/A	[141]
Optical	Enterovirus 71	capsid protein of	Acetate (pH 5),	Room	67 viruses/m	N/A	N/A	[144]

		enterovirus particles	and PBS	temp.	L			
Optical	Norovirus	Norovirus antibody	PBS containing 0.1 % Tween 20	N/A	12.1×10^{-15} g/mL	N/A	N/A	[63]
Electrochemical	Adenovirus	Anti-adenovirus polyclonal antibody	PBS (pH 7.4)	N/A	10^3 viruses/mL	20 min	N/A	[162]
Electrochemical	hepatitis A virus ssDNA	Specific capture probes	PBS (pH 7.4)	Room temp.	0.65 pM	N/A	N/A	[155]
Electrochemical	hepatitis A virus cDNA	Specific capture probes	PBS (pH 7.4)	Room temp.	6.94 fg/μL	N/A	N/A	[155]
Electrochemical	hepatitis virus (types A-E)	Anti-hepatitis virus antibody	PBS (pH 7.4) containing 0.1 mL/L Tween 20	37 ˚C	≤1.0 ng/mL	5 min	N/A	[156]
Electrochemical	Rotavirus	Anti-rotavirus antibody	PBS	37 ˚C	N/A	N/A	N/A	[159]
Electroche	Rotavir	Anti-rotavirus	PBS (PH	Room	10^2	N/A	N/A	[16

mical	us	antibody	8.4)	temp.	pfu/mL			0]
Electrochemical	Norovirus DNA	DNA	N/A	N/A	8.8 pM	N/A	N/A	[163]

Declaration of interests

☒ 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.

☐The authors declare the following financial interests/personal relationships which may be considered

as potential competing interests:

Highlights

- It is urgent to develop an effective early monitoring tool for virus detection in water.
- Electrochemical biosensors can provide rapid-detection, low-cost sensor design, and in situ detection.
- Biosensors have shown great potential as an alternative tool for rapid virus detection.
- The challenges and future recommendations of virus detection sensor are also discussed.