Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Biosensors for the detection of disease outbreaks through wastewater-based epidemiology

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ABSTRACT

Wastewater-Based Epidemiology (WBE) is a novel community-wide monitoring tool that provides comprehensive real-time data of the public and environmental health status and can contribute to public health interventions, including those related to infectious disease outbreaks (e.g., the ongoing COVID-19 pandemic). Nonetheless, municipalities without centralized laboratories are likely still not able to process WBE samples. Biosensors are a potentially cost-effective solution to monitor the development of diseases through WBE to prevent local outbreaks. This review discusses the economic and technical feasibility of eighteen recently developed biosensors for the detection and monitoring of infectious disease agents in wastewater, prospecting the prevention of future pandemics.

1. Introduction

Despite the rise in medical advances during the last decades, infectious disease outbreaks continue to represent a great burden to the global population. They constantly challenge global healthcare systems, causing continuous concern worldwide surrounding the increasing occurrence of epidemics (Fig. 1). For instance, only in 2016, infectious diseases represented one-fifth of the total number of registered deaths worldwide [30,98]. Furthermore, socioeconomic and environmental factors, (e.g., climate change, migration, population growth) will likely aggravate this situation within the upcoming years, especially in overpopulated areas [98]. The growing risk of more frequent epidemics and disease outbreaks calls for the development of measures that allow their early detection.

The ongoing COVID-19 pandemic caused by the rapid spread of the novel severe acute respiratory syndrome coronavirus (SARS-CoV-2) is an example of the need for early detection methods to monitor disease outbreaks [32,105]. The pandemic led to a great public health crisis, with 302 million confirmed cases and more than 5 million registered deaths worldwide as of January 7th, 2022 [21]. Health authorities around the world have rushed to contain the virus, ordering nationwide isolation, individual screening and contact tracing measures. However, identifying infected individuals has remained a challenge, mainly due to shortages of effective point-of-contact assays. Efforts to mitigate the spread of the virus have been challenged by difficulties diagnosing asymptomatic, pre-asymptomatic, and mildly symptomatic patients, all of which represent important sources of infection [12,36,52]. Detection methods that can also estimate the number and distribution of

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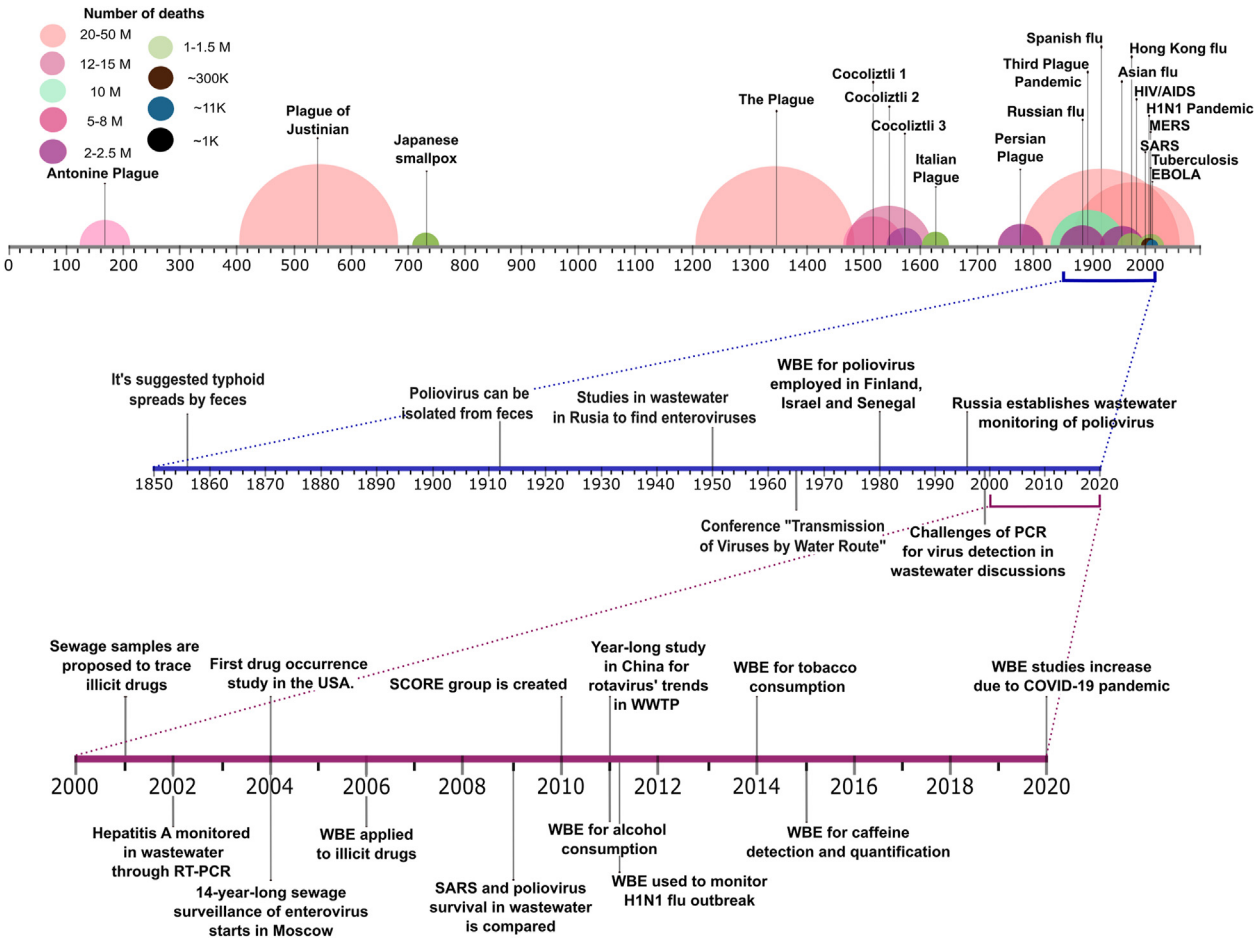

Fig. 1. The timeline of epidemics throughout history coupled with the evolution of wastewater-based epidemiology (WBE). Abbreviations: Human Immunodeficiency Virus (HIV); Acquired Immunodeficiency Syndrome (AIDS); Middle East Respiratory Syndrome (MERS); Severe Acute Respiratory Syndrome (SARS); Polymerase Chain Reaction (PCR); Sewage Analysis CORe group Europe (SCORE); wastewater treatment plant (WWTP).

asymptomatic and pre-symptomatic patients are needed in order to stop the spread of the disease in a timely manner.

The early diagnosis of infectious disease outbreaks is essential to stop their transmission. This is especially important in countries or regions where there is a lack of infrastructure or healthcare coverage, and other conditions, such as informal employment, hinder the efforts to stop disease propagation [53]. Unfortunately, most classic epidemiology strategies depend on the availability of resources and testing technology that many low-middle income countries (LMICs) and resource-constrained municipalities lack [47,83]. Thus, this resource inequality leads to an underrepresentation in reports of disease occurrence, likely biased towards regions that allocate more resources to carry out individual screenings [7]. Furthermore, well-established analytical methods to detect infections, such as reverse transcription-polymerase chain reaction (RT-PCR), generally require skilled personnel and specialized equipment in centralized laboratories [54,77]. Therefore, detection methods need to be cost-effective, and allow for on-site detection.

Wastewater-based epidemiology (WBE) is a promising population-wide disease surveillance tool capable of monitoring the presence of different types of infectious agents in wastewater, as well as to prevent disease outbreaks [6,34,98]. WBE can be used to assess the spatial distribution and temporality of virus occurrence within the catchment area of sewage treatment plants [6]. As a matter of fact, a wide range of xenobiotics [58,63,73] and pathogens [62,82] have been successfully detected and quantified in sewage samples since the development of this monitoring tool [8,17,69,98]. Current WBE protocols for viral monitoring (e.g., SARS-CoV-2) generally involve taking samples from WWTPs to detect viral presence and quantify their concentration (gene copies/L) using PCR techniques. Samples are generally obtained manually, although occasionally autosampler devices are employed [2,94]. Plastic or glass containers are usually filled with wastewater samples, which are then transported to centralized laboratories where the analytical procedures take place [94].

An overview of WBE history is summarized in this work to showcase epidemic events and leaps in WBE technology (as shown in Fig. 1). The WBE field started around the 1950's with the first studies to detect poliovirus and enteroviruses in sewage samples. Years later, it was suggested that typhoid could be spread by feces [41,60,102]. There began to be a need to develop a technology that could detect low levels of viral concentrations in water in the late 60's [60]. These discussions later evolved into the assessment of the challenges of using PCR techniques to detect viruses in wastewater [87]. Finally, in the 2000s, the development of the field accelerated as WBE started to be used to track different types of pathogens [27,35,42,45,50,51,61,95], as well as licit and illicit drug consumption [14,22,27,43] including alcohol and tobacco [71,72], as well as other types of pharmaceuticals [18,44,106].

Since the beginning of the COVID-19 pandemic, multiple research groups reported the detection of SARS-CoV-2 in patient

urine and stool samples, even when their nasopharyngeal tests gave negative results [6,38,98]. Soon after, SARS-CoV-2 RNA was recovered from residential wastewaters in different municipalities around the world [2,29,94,96]. Recently, this technique has been included as part of the reopening strategies of different higher education institutions [5,31].

The longitudinal analysis of WWTPs is a promising approach for the early detection of infectious agents that can be used to estimate the number of infected individuals within a specific population. This can ultimately aid public health authorities to implement effective containment measures on time [6]. WBE is especially useful, as it does not only provide information regarding confirmed active cases but also measures the prevalence of the disease since it considers pre-symptomatic and asymptomatic patients. Nonetheless, even though WBE represents a viable option to monitor and combat diseases, access to the required specialized equipment and skilled personnel can still be challenging for many under-resourced regions. The use of portable biosensors is a possible solution to this problem, allowing to assess the prevalence of pathogens in specific municipalities.

Biosensors enable the detection of different types of biomarkers in healthcare or environmental monitoring. A biosensor is a device that possesses a biological receptor capable of emitting a signal when in contact with the target analyte. In comparison to other conventional analytical tools, biosensors represent suitable instruments for on-site detection in resource-scarce regions, since they are portable and require minimal sample processing [103]. Due to their characteristics, as well as the need to develop portable, sensitive and cost-effective devices to monitor the spread and status of the ongoing COVID-19 pandemic as well as other pathogens, the integrated use of biosensors with WBE is a promising detection tool. This technique would allow a close to real-time community-wide detection of specific targets [4]. The following review analyses several biosensors that have been developed for the detection of infectious disease agents in complex matrices, with the objective of finding those that would suit the needs of scarce-resource regions.

2. Implementation of biosensors in WBE as an affordable community-wide detection system for viral pathogens

The detection of human pathogenic viruses is of special interest when it comes to disease outbreak prediction. A wide variety of pathogenic and waterborne viral families have been successfully detected and quantified in wastewater samples (e.g., *Adenoviridae, Astroviridae, Coronaviridae, Flaviviridae, Herpesviridae, Papillomaviridae, Parvoviridae, Retroviridae, Togaviridae)* [20,57]. Driven by the recent pandemic, large efforts have been carried out focusing on the coronaviruses [10]. Viruses usually infiltrate sewage from body fluids like blood, saliva, feces, and urine. Supposing that an individual average daily release of fecal matter is 100 g, an infected person would excrete around 10^7 - 10^{13} viral particles per day [9]. In the USA, the estimated mean concentration of enteric viruses in wastewater is ~7 K pathogenic organisms per liter [59]. Moreover, some pathogenic viruses may be defecated before infected individuals even present symptoms of the disease (e.g., norovirus) [37]. Norovirus, rotavirus, and enterovirus D38 disease outbreak peaks have been linked to increases in their wastewater detection [37,86].

Urban areas play an important role in infectious disease control because their population density facilitates disease outbreaks when public health authorities are not able to act on time [57]. Wastewater-based epidemiology has been successfully used to detect both waterborne and non-waterborne viruses in wastewater, making it an ideal tool for preventing and detecting viral disease outbreaks [37,56,67,90]. Viruses are one of the main sources of disease transmission through large volumes of contaminated water, as they are capable of remaining stable in aquatic environments for long periods of time [57]. In addition, viral infectious diseases can also be spread through agricultural and food process water, lakes, and reservoirs [11]. Table 1 below shows a list of different types of viral agents that are commonly found in aqueous systems.

Sewage contains valuable information regarding a community's lifestyle, health trends, and consumption or exposure to xenobiotics. Such information is obtained by studying specific biomarkers that enter sewage through the excretion of urine and feces [103]. Wastewater-based epidemiology (WBE) has been successfully employed to trace a wide range of substances such as illegal drugs, pharmaceuticals, and pesticides, as well as to predict and monitor the trends in the circulation of infectious agents within specific communities [3,17,24,69]. Infectious diseases, like COVID-19, require monitoring as close as possible to real-time in order to tackle their spread and mitigate their effects [103]. Even though WBE has proven to be a feasible tool to monitor the onset of the current pandemic, there is still an imperative need to develop affordable and effective on-site analytical tools capable of detecting SARS-CoV-2 with minimal sample processing, and that can preferably be used by unskilled personnel.

Biosensors possess multiple advantages; they are cost-effective, sensitive, specific, portable, and provide real-time information. These devices have been used to detect a wide range of analytes for multiple applications, such as food security, clinical diagnostics and environmental monitoring [6]. Biosensing devices are composed of (1) a selective and sensitive recognition element that can detect the target metabolite; (2) a transduction element that converts the recognition signal into one that can be interpreted; and (3) an amplifier to read such signals [88].

According to the World Health Organization, rapid diagnostic approaches should follow the ASSURED criteria, meaning that they should be affordable, sensitive, specific, user-friendly, rapid, robust, equipment-free, and deliverable to end-users [64,66]. Therefore, in order to deliver affordable and effective biosensing solutions for the cost-effective implementation of WBE in LMICs, special attention should be paid to the analytical considerations of such devices. Wastewater is a complex matrix with many sources of interference which makes it hard to analyze with accuracy. This could potentially be assessed by using ultra-high affinity probes for specific analytes (e.g., an aptamer), as well as nanostructured materials [6].

Nucleic acid amplification-based tests (NAATs) are commonly used methods for the diagnosis of infections. NAATs are an advantageous approach as they detect viruses in the early stages of infection. They are sensitive, specific, and have a very low rate of false positives. In general, PCR-based assays have remained the gold standard of NAATs. As mentioned earlier, PCR requires trained staff and specialized equipment, which poses a significant challenge for many resource-scarce regions [53]. Other nucleic-acid amplification methods exist, such as Loop-mediated Isothermal Amplification (LAMP), Recombinase Protein Amplification (RPA), and Rolling Circle Amplification (RCA). Paper microfluidic devices have also emerged as low-cost alternative tools for NAATs [53,70].

Nano-based diagnostic tools can significantly help increase the sensitivity and selectivity of biosensors due to their large surface area and high surface-to-volume ratio. Nanomaterials (e.g., metal and magnetic nanoparticles, carbon-based structures, nanozymes, polymeric nanovesicles) improve detection as they support functionalization with other biological materials, allowing for more recognition events [64]. Furthermore, they have advantageous physicochemical properties, such as conductivity or catalytic activity, that are useful for the improvement of signal readout [64,93].

Table 1
Different viral agents and their occurrence in aquatic environments.

Viral agent	Family	Epidemiology	Occurrence	Reference
Astroviruses	Astroviridae	Low-level persistent infection	Untreated wastewater	[57]
Cryptosporidium	Cryptosporidiidae	Alters with variation in host susceptibility to infection	Reservoirs Wastewater	[11] [108]
Giardia	Hexamitidae	High susceptibility infection on contaminated water	Reservoirs Lakes Wastewater	[109] [110]
SARS-CoV	Coronaviridae	Easily transmits via respiratory droplets	Drinking water Wastewater	[107]
Poliovirus type 1	Picornaviridae	Transmitted mainly by fecal-oral route, also possible through respiratory secretions	Estuarine water	[11]
Coxsackievirus B3	Picornaviridae	Highly infectious from contaminated food and water	River water Groundwater	[11]
Hepatitis A	Picornaviridae	Epidemic form through contaminated food and water	Wastewater Freshwater	[107]

Affordable biosensors with minimum sample handling, have been developed for the detection of different disease biomarkers in resource-constrained regions as an alternative to costly tests. For example, a plasmonic enzyme-linked immunosorbent (ELISA) assay was designed for the detection of a prostate specific antigen (PSA) and HIV-1 capsid antigen p24. The enzyme label of the ELISA assay controlled the growth of gold nanoparticles, leading to a change in the color of the solution when the analyte was present. Both molecules were successfully detected in whole serum at extremely low concentrations ($1x10^{-18}\ g/mL$) [23].

Paper microfluidic devices are promising alternatives in Nucleic Acid (NA) detection [70]. Channels are designed through patterns made through photolithography, cutting, etching, plasma treatment, or printing. Among these, wax printing is the simplest and most popular technique [53]. These analytical tools have various functions including extraction, purification, elution, amplification, valving, visual detection, and multiplexing. In general, all the steps required for NA testing are integrated through the folding of the paper device [39,54,55]. For instance, a paper-based microfluidic device with >98% sensitivity used paper folding and vertical flow to integrate all LAMP process steps in Uganda to detect malaria [70]. Blood samples of 67 children from different rural primary schools in Uganda were tested, as well as 20 other healthy patient samples. Malaria was successfully detected in the infected patients using the paper-based sensor and was compared to results using PCR.

Similarly, paper microfluidic devices have been combined with electrochemical transduction or amplification elements. One example is a nanoparticle-enhanced cellulose paper microchip with screen-printed graphene-silver electrodes (GSE), which were designed for the detection of Zika Virus (ZIKV) [25]. The virus was isolated from biological samples and labeled using antibodies and platinum nanoparticles (PtNPs). The complex formed (ZIKV-PtNPs) was subsequently lysed to release electrically charged molecules that caused a change in the solution's conductivity. This change was measured on the electrodes on the paper microchips. Sensitivity and specificity were tested in an assay with the target ZIKV and non-target viruses such as *dengue virus* (DENV) type-1 and -2, and *cytomegalovirus* (CMV), among others. The detection limit of the microfluidic device was 10^1 copies/μL. Furthermore, the device was also tested in more complex samples, such as urine, semen, and human plasma, with detection limits of 10^2 particles/μL.

Another paper-origami LAMP device was developed to detect three sexually transmitted bovine diseases in semen: *Bovine Herpes virus-4* (BoHV-1), *Brucella*, and *Leptospira* [104]. The device was composed of five panels in which DNA was extracted, washed, and eluted to the LAMP reaction chamber. The five LAMP reaction chambers available in the device allowed the testing to be done with two controls, and three primers (one for each pathogen). Once the amplification finished, the results could be visualized with a UV flashlight. When pyrophosphate was present, it became excited under UV light, causing green emissions. To evaluate the device's sensitivity and limit of detection, different concentrations of the pathogens were introduced in healthy semen samples. Detection limits of 0.5 pg/μL for *Leptospira* and *Brucella*, and of 25 fg/μl for BoHV-1 were achieved.

Multifunctional paper devices have also been developed. These are especially useful to detect common and endemic diseases in resource-constrained areas. For instance, an all-in-one paper chip designed by Ref. [76] detected zika, dengue, and chikungunya simultaneously. The device can carry out the sample treatment, RNA extraction, automatic flow control, and reverse transcription LAMP. This lab-on-paper had three main sections: Serum treatment, Fluidic flow of target RNA, and RNA amplification. First, serum was injected into the loading pad, activating the dried lysis buffer. Then viral RNAs were released, followed by the purification and subsequent transport of genetic material to the amplification zone through the lateral flow on a chitosan-modified pad. Then, a vertical flow took RNAs to the four reaction pads, where dried re-agents were activated, causing an RT-LAMP amplification. Finally, the hydroxy naphthol blue (HNB) produced a fluorescence signal. The device could detect the three viruses in 60 min by adding RT-LAMP re-agents with specific primers to cause a target-specific reaction. No exact quantification was reported by this biosensor.

Aptamer-based biosensors have emerged as an option for virus detection due to their high specificity. An aptamer is an unnatural short-stranded nucleic acid sequence, DNA or RNA, selected from a library of oligonucleotides. The selection of the aptamer is based on its affinity to a target molecule. Therefore, aptamer-based sensors tend to be highly selective and specific. A specific biosensor for murine norovirus was engineered with gold nanoparticles catalytic activity and aptamers as the main elements [92]. The enzyme-mimicking catalytic activity of gold particles converted a colorless substrate into a colored product, which in this case was blue. The aptamer was adsorbed to the surfaces of gold nanoparticles causing the loss of both the enzymatic activity and the blue color. In presence of the target virus, the aptamer was removed from the nanoparticle's surface and attached to murine norovirus capsid. This reactivated the enzyme, causing the oxidation of the substrate

and, as a consequence, the emission of the blue color. The aptamers were also applied in an Avian influenza virus sensor, in which an aptamer-based hydrogel worked as the coating material of a quartz resonator [91].

2.1. Economic considerations for the implementation of biosensing devices in WBE

Besides providing feasible and straightforward results, biosensors must also be scalable and affordable in order to implement them in low-middle income countries (LMICs). Additionally, for regions where screening is challenging, detection procedures must be kept as simple as possible to allow unskilled personnel to carry out the tests and interpret the results. The biosensors described in Table 2 were further analyzed according to the matrix tested, equipment needed to visualize results, and the sample treatment requirements, in order to evaluate their feasibility in scarce-resource areas. The following devices arose as possible choices: NanoZyme aptasensor, Zika's paper microchip, BoHV's paper microfluidic device, Plasmonic enzyme-linked immunosorbent (ELISA) assay, and Malaria's paper microfluidic device. All devices that required laptops or smartphones were discarded for economic reasons. Biosensors were also discarded if they required any extra step to interpret results, which could hinder implementation by non-specialized personnel.

Manufacturing costs were compared to find the most suitable biosensors for LMICs. Manufacturing costs were estimated according to information provided by each author as well as prices shown in the provider's websites. Some of the websites belonged to Xerox, Sigma-Aldrich, and Thermo Scientific. The NanoZyme aptasensor was not considered for this analysis since no information was provided by the author. Table 3 shows a detailed description of the costs and materials needed for the construction of each of the selected devices.

The construction of each biosensing device required a variety of materials, which do not represent a significant expense when compared to printers or laser machines. Laser system prices tend to be remarkably more expensive than printers or ELISA kits; consequently, the Zika paper microchip was discarded. The remaining alternatives (BoHV paper microfluidic device, Plasmonic enzyme-linked immunosorbent (ELISA) assay, and Malaria's paper microfluidic device) suit the economic and pragmatic needs of developing countries. Further research of each biosensor is required in order to determine their potential to detect target viruses in wastewater matrices.

Even though the biosensors proposed still require economic investment, complementing their use with WBE is a viable alternative to traditional surveillance tools. Conventional monitoring systems applied during the COVID-19 pandemic have proven to be ineffective or unsuitable for LMICs. For instance, massive testing strategies are ideal, however, instrumental costs and the lack of skilled personnel and infrastructure limit its implementation in resource-constrained regions [40].

Case-based routine surveillance has been implemented in LMICs for both suspected and confirmed cases. When applied with no other strategy, this technique eases disease outbreaks even though no early detection measures are implemented whatsoever [40]. However active surveillance could be an interesting alternative, as it traces the pathogen to identify potential infections, allowing for preventative measures. This procedure has been successfully applied in China, Europe, and the USA, nonetheless, it relies heavily on technological developments that are not widely available in LMICs [46].

Even though there are several surveillance techniques, most of them are difficult to apply in LMICs due to technological and economical limitations. WBE and biosensors could represent a cost-effective, reliable, and practical alternative to conventional surveillance systems; however, the challenges discussed throughout the next section need to be worked on in order to achieve an optimal implementation.

Considering the issues discussed in this section, Fig. 2. Below shows an ideal schematic of an appropriate biosensing device for its implementation in WBE. The design integrates a series of steps that aim to ensure that the retrieved samples are processed as simply as possible, as well as to enhance the sample's concentration and nucleic acid extraction from a matrix as complex as sewage.

3. Challenges of biosensors applied to WBE

Different factors need to be considered to successfully implement wastewater-based epidemiology, including aspects of sampling and sample quantification. Some of the most relevant steps in WBE include: (1) applying appropriate spatial and temporal sampling procedures in order to ensure proper sample representativeness; (2) simple and efficient sample concentration and pretreatment processes that maintain the sample integrity; and (3) an accurate quantification of results.

3.1. Sampling process: spatial and temporal representativeness

The representativeness of a WBE study is directly affected by the spatial and temporal aspects of the sampling process [68,98]. The particularities of the sewage systems must be considered when establishing a WBE protocol. Additionally, models of viral decay and wastewater flow rates throughout the sewage system should be considered to accurately estimate viral concentration within the catchment population. Furthermore, the time of sampling is also another important factor; for example, in some sewage systems, there may be a specific period of time that takes for water to travel from the households to the centralized WWTPs. Autosamplers can be used to obtain more representative samples over specific periods of time [68]. Nonetheless, this may not be economically feasible for many LMICs and other resource-constrained regions.

Grab samples are commonly used since they represent a more practical and cost-effective sampling method. Plastic or glass containers are usually used to transport the samples to centralized laboratories where the analytical studies take place [94]. However, besides the lack of accessibility to centralized laboratories and skilled personnel in certain regions, grab sampling and the transportation of the samples pose a potential risk to those participating in the sampling process, since they may be exposed to multiple infectious agents present in sewage [56,65,74].

3.2. Sample concentration and pretreatment processes

The detection of viral pathogens requires the concentration of the samples into smaller volumes to improve detection limits [68]. Currently, membrane filtration is the most common primary sample concentration and virus recovery method for water matrices; e.g., virus adsorption-elution (VIRADEL) and crossflow ultrafiltration (CFUF). Even though these recovery techniques have been useful for viral detection in water samples, the variability and complexity of sewage composition usually result in low and poorly reproducible recoveries. Different types of molecules that are suspended or dissolved in wastewater are likely to foul the filters, limit recovery yields, and potentially interfere with downstream assays. Moreover, some filters may require preconditioning steps to facilitate adsorption; this is time-consuming and can limit the final sample volume, which is disadvantageous, especially if

Table 2
Biosensing techniques for the detection of pathogen's biomarkers in complex matrices.

Disease/ pathogen	Biomarker	Device/Assay	Sample pre-treatment	Visualization of results	Equipment required for results visualization	Limit of detection (LOD)	Matrix tested	Reference
Avian influenza virus (H5N1)	hemagglutinin (HA) protein	Electrochemical biosensor with an electrode modified with porous Au nanoparticles	NA	Cyclic voltammogram	Versastat 3 potentiostat	1 pM	Chicken serum	[49]
Zika Virus	Virus particles	Electrochemical biosensor based on nanocarbon composites that measures changes in electrical signal when changing the virus concentration.	The only preparation was diluting the stock to the desired concentrations, for example, 1% and 10% serum	Nyquist plot	A computer or software to generate the plots and Autolab PGSTAT302 N	Dilution of 10% of serum concentration: 250 RNA copies/mL. Dilution of 1% of serum concentration: 10 RNA copies/mL	PBS buffers and serum	[84]
Hepatitis E virus (HEV)	Virus particles	Impedimetric sensing based biosensor complemented with induced pulses to achieve lower detection limits	NA	Nyquist plot	Not specified but a computer might be required.	96.7 RNA copies/mL	Human serum	[16]
Zika virus	RNA (NS5 protein)	Label free impedimetric electrochemical DNA biosensor	Not specified but it is remarked that the label free feature simplifies sample preparation when compared to colorimetric biosensors. .	Nyquist plot	A computer to use OriginPro8G software and an Autolab PGSTAT 302 potentiostat	25 nM	DNA samples	[28]
Zika virus	ZIKV NS1	Graphene biosensor based on Field Effect Biosensing (FEB)	Not specified	Different plots such as percent change in capacitance against time.	Agile R100 system and a computer	0.45 nM	PBS and dilutions of simulated serum	[1]
SARS-CoV-2	SARS-CoV-2 spike protein	Field-effect transistor-based biosensor with graphene as sensing material. Graphene sheets were coated with SARS-CoV-2 spike antibody	None	Plot of time against normalized current	Not specified but a computer might be required	culture medium: 1.6×10^1 pfu/mL clinical samples: 2.42×10^2 copies/mL	Clinical samples (nasopharyngeal swab)	[75]
HIV-1	Capsid antigen p24	Plasmonic enzyme-linked immunosorbent (ELISA) assay induced by gold nanoparticles	None	Visually, red or blue color.	None	$1x10^{-18}$ g/mL	Whole serum	[23]
Malaria	DNA	Diagnostic platform that uses paper folding and vertical flow to integrate preparation steps required for LAMP	None	Visually, a single red line for negative results, and multiple red lines for positive cases.	None	level of sensitivity of 10^5IU/mL for *P. falciparum*	Whole blood	[70]
BoHV-1	DNA	Paper origami DNA microfluidics device with integrated LAMP amplification and fluorescence detection	None	Fluorescence. Green color for positive cases.	UV flashlight	0.5 pg/μLfor *Leptospira* and *Brucella*, and of 25 fg/μL for BoHV-1.	Fresh semen	[104]
All-in-one diagnosis of zika, dengue and chikungunya	RNA	Lab-on-paper device combination of 3D lateral and vertical flow stacking structure and integrated ready-to-use NAT process from dried reagents through specific RT-LAMP for target RNA	Included in the device	Fluorescence	Chemi-Doc system used for measuring fluorescence intensity.	No limit of detection was reported but fluorescence intensity was analyzed for different virus concentrations in the serum. This range goes from 5 to 5000 copies of zika virus	Human serum	[76]
Zika	Envelope protein	Paper microchip with screen-printed GSE	Plasma: 10 mL of fresh whole blood was centrifuged for 15 min.	Electrical conductivity measurement through	An LCR Meter for recording impedance magnitudes.	10^1 copies/μL in a specificity and selectivity test that included non-target virus.	PBS, urine, plasma and semen	[25]

Table 2 (*continued*)

Disease/ pathogen	Biomarker	Device/Assay	Sample pre-treatment	Visualization of results	Equipment required for results visualization	Limit of detection (LOD)	Matrix tested	Reference
				impedance spectroscopy		10^2 particles/μL in complex biological samples, such as human plasma, urine, and semen.		
West Nile	Virus particles	Microfluidic paper-based analytical device with integrated microwire Au electrodes (Electrochemical impedance spectroscopy).	NA	Measure of impedance	CH660 potentiostat	LOD: 10.2 particles in 50 μL of cell culture	Viral samples	[15]
Norovirus	Capsid protein VP1	Wax printed microfluidic paper analytical device. The microfluidic design was printed on a nitrocellulose paper.	Known norovirus concentrations were spiked in environmental water samples	An image processing algorithm detects aggregated particles.	Two devices were tested: A benchtop fluorescence microscope along with an imaging software. Smartphone complemented with an external fluorescence microscope.	LOD: 1 copy/μL in deionized water and 10 copies/μL in reclaimed wastewater.	Reclaimed wastewater, deionized water, and drinking tap water	[19]
Avian influenza virus, H5N1	AIV H5N1 surface protein	ssDNA crosslinked polymeric hydrogel based QCM aptasensor. The hydrogel worked as a coating material for the quartz resonator. This coating is what causes virus specificity.	Inactivated AIV H5N1 with a titer of 10-fold dilutions.	Change in resonance frequency.	A QCA922 quartz crystal analyzer for recording frequency variations.	Three different coating materials were developed, hydrogel III proved to be the most sensitive one, with a detection limit of 0.128 HAU	Viral samples	[91]
Murine norovirus	MNV AG3 aptamer	NanoZyme Aptasensor. Gold nanoparticles (GNPs) catalytic activity converts a TMB (3,3′,5,5′-tetramethylbenzidine) substrate to a blue colored product. Aptamers bind to GNPs' surface causing loss of both Nanozyme activity and, in consequence, the blue color.	NA	In presence of target virus, aptamers leave GNPs surface in order to bind to MNV capsid. This desorption allows gold particles to oxidize the substrate into a blue color.	None, the blue color produced shows the analyte presence	LOD: 30 viruses/mL	Human serum, shellfish homogenate, and viral samples	[92]
General	DNA	Poly-silicon wire coated with a nanocomposite for DNA label free detection	The DNA was purified through high performance liquid chromatography (HPLC)	Current changes measured before and after applying the sample	Semiconductor parameter analyzer Agilent 4156C	LOD: 0.3 fM	Synthetic DNA samples	[97].
Influenza virus A (H1N1) and Norovirus	DNA	Gold/Iron oxide nanoparticles magnetically aligned to an electrode. A DNA probe was attached to the nanoparticles' surface.	NA	Electrical conductivity changes of the nanoparticles.	Potentiometer to measure electrical conductivity and resistance changes.	LOD for influenza virus and norovirus were calculated to be approximately 8.4 pM and 8.8 pM, respectively	DNA samples	[48]
General	DNA	DNA/chitosan/multi-walled carbon nanotube nanocomposite with a probe DNA modified electrode.	NA	Complementary sequence hybridization was examined by calculating the difference between the electron transfer resistance before and after exposing the electrode to the sample.	IM6 impedance analyzer and IM6 THALES software	LOD: DNA was detected as low as 0.01×10^{-12} M	DNA sequences in PBS solution	[89]

Abbreviations: AIV: avian influenza virus; **EIS**: electrochemical impedance spectroscopy; **GSE**: graphene-silver electrode; **LAMP**: loop-mediated isothermal amplification; **MNV**: murine norovirus; **NA**: Not applicable; **NAT**: nucleic acid testing; **QCM**: Quartz crystal microbalance.

Table 3
Cost comparison of each potential biosensing solution to be employed in resource-constrained regions as a complementary tool to WBE.

Biosensor	Material required for manufacturing	Estimated cost (USD) Last verified on May 25, 2021	Provider
Zika's paper microchip	Chromatography cellulose paper- Whatman 3 MM Chromatography paper, Whatman™ 30,306,185	$26.72 - Pack of 100 sheets	Whatman plc
	Screen printing film - Mask-ease, Blick Art Materials; 44,908-1003	$18.17	Blick Art Materials
	Desktop laser system - Laser Cutting, VLS2.30 from Universal Laser System	Price not found but it has been reported that desktop laser systems can be afforded for $7600-$15,000 price range	
	Transparency film - CG5000 - Dual-Purpose Transparency Film	$2.96	ebay
	Graphene conductive dispersion - Graphene Supermarket, UHC-NPD-100ML	Price not found	
	Silver ink - Engineered Conductive Materials, CI-1001	Price not found	
BoHV paper microfluidic device	Black Ink - Xerox, cat# 108R00935	$169.90	Xerox
	Wax Xerox printer -Xerox ColorQube 8570	Product no longer available at Xerox but versalink c500 is available for $481.62	Xerox
	Optical Adhesive film - MicroAmp® Optical Adhesive Film, Thermo Scientific	$371.19for 100 covers -	Thermo Scientific
	Glass Microfiber Filter - GFF, Whatman	$131.87 for 100	Sigma-Aldrich
Malaria's paper microfluidic device	Wax Xerox printer - Xerox ColorQube 8570	Product no longer available at Xerox but versalink c500 is available for $481.62	Xerox
	Black Ink - cat# 108R00935; Xerox	$169.90	Xerox
	Chromatography cellulose paper - 3 mm in diameter; Whatman	$33.88	Sigma-Aldrich
Plasmonic enzyme-linked immunosorbent (ELISA) assay	No information provided by the author.	No cost or exact materials are shown but ELISA kits for human serum are available for $484.80	Sigma-Aldrich

Costs information was retrieved from the following providers [13,26,79–81,85,99–101].

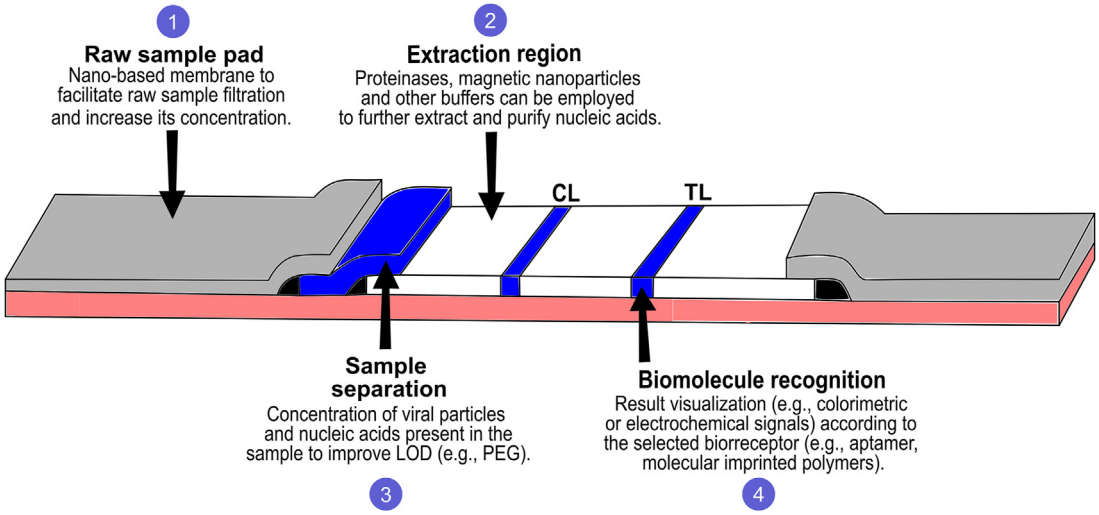

Fig. 2. Scheme of an ideal biosensor for WBE. (1) Nano-based membrane facilitates raw sample retrieval and minimizes its processing. (2–3) Sample separation and nucleic acid extraction in order to improve the limits of detection (LOD) and purify the sample to enhance biomolecule recognition. (4) According to the type of biological recognition mechanism employed, the last step of the test should aid unskilled personnel to rapidly interpret the results onsite. Control and Test lines guarantee the proper use of the test and are marked with CL and TL respectively. Additionally, these sensors are based on visual identification.

collected samples need to be transported for their processing. Preconditioning processes may also have a negative impact on the integrity of the target pathogen, hindering the accurate assessment of the sample [78].

There are several secondary concentration methods that have been designed and optimized for non-enveloped viruses, such as combinations of filtrations, ultracentrifugation, polyethylene glycol (PEG) precipitations, and other purification steps. However, it is important to consider that the application of such methods on enveloped viruses is less suitable due to their structural outer lipid layer, which makes them more vulnerable to temperature and pH changes, as well as to organic solvents, among other solutions [68].

3.3. Viral quantification process: analytical considerations

Finally, viral quantification is the main goal of WBE allowing the assessment of potential disease outbreaks. For RNA viruses, WBE is usually carried out through reverse transcription PCR (RT-PCR) and RT-real time PCR (RT-qPCR). However, the quantification step may be hindered due to the presence of PCR inhibitors in wastewater samples (e.g., fats, proteins). In certain cases, inhibition can be minimized by performing dilutions or using quenching agents [68]. Nonetheless, in regions where there is a lack of centralized laboratories or specialized equipment, the use of ASSURED devices is key to interpreting WBE results accurately.

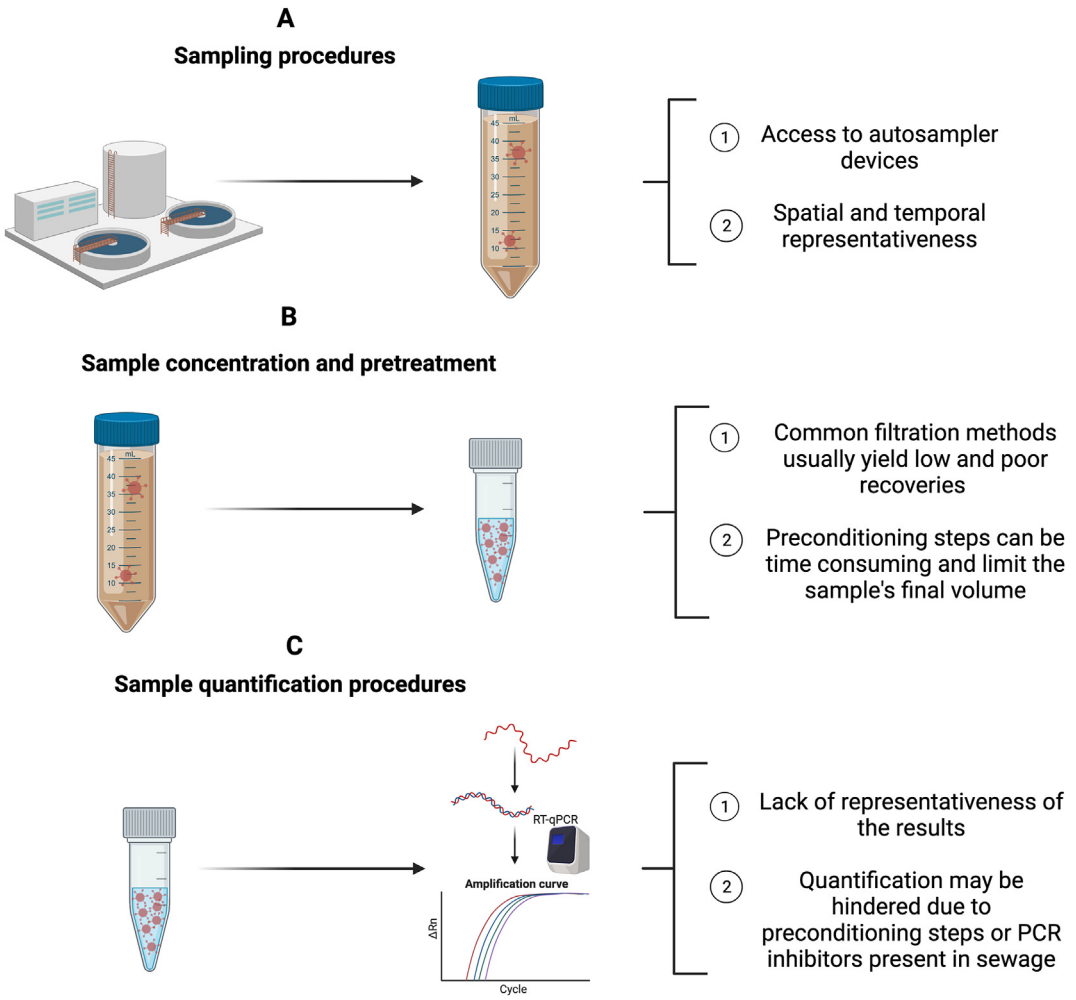

Fig. 3. Main challenges in sampling and quantification for wastewater-based epidemiology: (A) appropriate spatial and temporal sampling procedures, (b) efficient sample concentration and pretreatment methods, and (c) accurate quantification of the results. Created with BioRender.com.

Due to their enhanced properties, the use of nanomaterials to develop biosensing devices is a promising analytical approach [33]. The use of nanoparticles as labels for optical and biochemical biosensors enhances their biocompatibility, bioconjugation, and sensitivity while using less sample volume due to their optical and electronic properties, as well as their large surface area-to-volume ratio [33,64]. Metal nanoparticles, such as gold and silver nanoparticles, offer excellent optical absorption and emission properties. Magnetic; and magnetic nanoparticles, while other metal nanoparticles (e.g., iron, nickel, manganese) are capable of improving the biomolecule separation and purification processes, which results useful for the substitution of certain filtration and centrifugation steps during sample preparation. Graphene has gained great attention in developing biochemical sensors due to its electronic conductivity and large surface area [33].

The factors described earlier need to be thoroughly considered to successfully implement biosensors in WBE. As shown in Fig. 3, the concentration and pretreatment of samples are vital preconditioning steps to accurately detect viral pathogens. Appropriate spatial and temporal sampling, along with simple and efficient concentration steps are required to avoid the interference of other molecules in the detection and quantification of pathogens. Fortunately, the importance of developing cost-effective devices with minimum sample preparation for biomarker detection in complex matrices is already being addressed by the scientific community. Nonetheless, it is important to emphasize the need to integrate quantitative and qualitative measurements that can be easily interpreted by unskilled personnel when designing biosensors, as well as taking into account other important factors like their standardization and scalability.

Efficient concentration and virus recovery methods are a challenge that remains unsolved, and the successful implementation of WBE with or without the use of biosensors will depend on the development of better extraction methods. Additionally, further research should focus on testing the technologies in environmental samples, like wastewater.

4. Conclusions and future perspectives

Eighteen recently developed biosensing devices for the detection of infectious disease causative pathogens were reviewed as possible approaches for the detection of viral pathogens in sewage. The inclusion criteria involved their recency, as well as their focus on detecting pathogen biomarkers in complex matrices with minimum sample preparation steps. In general, most biosensing approaches found in the literature use clinical samples and aim to reduce their processing steps and the costs of the conventional nucleic acid tests. While multiple literature reviews did acknowledge the need for diagnostic tools that could be employed in wastewaters, they too referred to clinical sample

sensing devices. Furthermore, most mentions of potential sewage biosensors for SARS-CoV-2 and other viral pathogens described paper-based biosensors as the most suitable devices for under-resourced regions.

It is important to consider that resource-constrained municipalities need an evidence-based decision-making tool, that is standardized, scalable, cost-effective, and capable of achieving very low levels of detection, in order to be able to carry out WBE and monitor the evolution of infectious diseases. Therefore, further studies should focus on the design and validation of easy-to-standardize, scalable, sensitive devices that can be employed in complex matrices, specifically, sewage. Simple paper-based microfluidic devices and nano-based sensing technology are promising alternatives to be explored.

Overall, biosensors have the potential to be used in wastewater as a public health tool to assess the presence of viral pathogens. Their use in WBE could have a positive economic and social impact, providing information to decision-makers so that early and effective interventions are put in place. This would be particularly useful for resource-constrained communities in Latin America, where affordable and sensitive detection methods can play a key role in the well-being of the population, as this region remains the most affected by the ongoing pandemic.

Credit author statement

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Declaration of competing interest

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

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