

UNIVERSIDADE ESTADUAL DE CAMPINAS
SISTEMA DE BIBLIOTECAS DA UNICAMP
REPOSITÓRIO DA PRODUÇÃO CIENTÍFICA E INTELLECTUAL DA UNICAMP

Versão do arquivo anexado / Version of attached file:

Versão do Editor / Published Version

Mais informações no site da editora / Further information on publisher's website:

<https://www.sciencedirect.com/science/article/pii/S2666016422000044>

DOI: 10.1016/j.cscee.2022.100182

Direitos autorais / Publisher's copyright statement:

©2022 by Elsevier. All rights reserved.

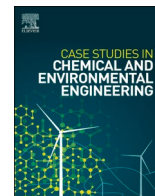
DIRETORIA DE TRATAMENTO DA INFORMAÇÃO

Cidade Universitária Zeferino Vaz Barão Geraldo

CEP 13083-970 – Campinas SP

Fone: (19) 3521-6493

<http://www.repositorio.unicamp.br>



Electrochemical point-of-care devices for monitoring waterborne pathogens: Protozoa, bacteria, and viruses – An overview

Alexsandra D. da Silva^a, Waldemir J. Paschoalino^a, Romeu C. Neto^b, Lauro T. Kubota^{a,*}

^a Department of Analytical Chemistry, Institute of Chemistry, University of Campinas, P.O. Box 6154, 13084-971, Campinas, SP, Brazil

^b Institute of Biology, University of Campinas, Campinas, SP, Brazil

ARTICLE INFO

Keywords:

Electrochemical point-of-care devices
Protozoa
Bacteria
Virus

ABSTRACT

Water is considered a natural resource of high importance to the society whose availability and quality are in great demand. The water that reaches homes may contain different species, among them microorganisms like bacteria, protozoa and viruses that can contaminate and compromise the health and safety of the population. Thus, frequent water analysis would allow the monitoring of potentially harmful and/or toxic species that can be found in water resources. The application of point-of-care devices (POCs) for monitoring water ensures faster on-site analysis. The use of electrochemistry as a detection technique for a specific analyte presents itself as a promising way, being able to serve different targets reporting fast, selective and reproducible responses. After the COVID-19 outbreak in the world, the necessity of the development of such devices that may monitor species quickly, even at low concentrations was evidenced. The conventional methods of water analysis are still the most widely employed, despite the existence of already well-established modes of detection of target species by electrochemical techniques allowing on-site detection with POCs. In the quest to elucidate such issues, this review aims to show some biosensors devices developed for the detection of bacteria, protozoa, and viruses, attempting to understand what would still be needed for the large-scale application of these devices in water monitoring, identifying the innovative features and shortcomings of POCs.

1. Introduction

According to data from the National Water Agency (NWA) [1] only 2.5% of all water available on Earth is freshwater. From this portion, almost 69% are found in glaciers, such as the Antarctic and Arctic regions, and on top of mountain ranges; 30% correspond to groundwater found mainly in aquifers and 1% is surface water represented by rivers, streams and freshwater lakes. Another point to be raised is that water is a finite natural resource, in contrast to the growing population, and its greater demand by the agricultural and industrial sectors registered over the years. Adding to them, the misuse, water losses and climate change cause water scarcity that reflects in periods of rationing, worsening in the quality of the water consumed, increasing the water treatment cost and water supply for the population [2].

The release of water and sewage into bodies of water in adequate quality conditions is important for the health and safety of humankind since the water cycle includes its quality control process and redistribution to the population. However, sanitation companies only monitor the main parameters regulated for quality verification, which

compromises the real assessment of water quality. Water scarcity in many regions of the world, including southeast Brazil, has motivated new studies which seek to increase the responsible use of water and also improve its quality. This water scarcity scenario is characterized by important chemical and biological changes (higher concentration in the water) increasing the complexity of the sample analysis [3]. New technologies, as molecular tools, in addition to detection systems, can characterize microbiological contamination in waters [4].

Physicochemical and microbiological parameters are monitored in water, however, other existing compounds like residues of pharmaceutical drugs and some microorganisms are neglected in routine analyzes and can cause health problems [5]. These analyses, most of the time, are carried out inside laboratories, which does not allow a quick understanding and decision-making about the quality of the sampled water. For this reason, point-of-care devices (POCs) emerge as a promising path for quick, cheap, selective, sensitive, reproducible responses [6].

Among the different detection modes that would be used in POCs devices for monitoring water quality, like colorimetry [7], fluorescence [8], SPR [9], electrochemiluminescence [10], optical techniques [11]

* Corresponding author.

E-mail address: kubota@unicamp.br (L.T. Kubota).

<https://doi.org/10.1016/j.csee.2022.100182>

Received 10 December 2021; Received in revised form 10 January 2022; Accepted 12 January 2022

Available online 20 January 2022

2666-0164/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

and others, electrochemical detection has advantages such as versatility perfectly adjustable and miniaturizable, cheap, sensitive with quick responses for the construction of compact POCs devices with low energy consumption [12]. A flow chart representing how electrochemical POC can be applied in water analysis is shown in Fig. 1. The importance of POC (bio)sensing for water is the quality assessment before drinking it or using it for agriculture, to avoid harmful contamination. Thus, a device able to analyze water easily just before using in any place could help minimize many problems, mainly in locations that do not have a complete water treatment system.

There is extensive literature that tries to elucidate the complex water security issue and how it directly reflects on the health of animals and humans [13]. As discussed, public awareness of the intelligent use of water is very important. The improvement and creation of public policies that establish regulations and the monitoring of more and more compounds that are potentially harmful and/or toxic to human beings are essential. The development of POCs for water monitoring allows quick, less costly, on-site assessments, enabling instantaneous actions, which would reflect on the quality of water supplied to the population.

Initially, this review addresses the issue of water quality in Brazil and worldwide and what are the main water contaminants. Next, the characteristics of POCs and how they work as devices for the analysis of bacteria, protozoa and viruses are presented. We also bring a critical point of view considering the use of electrochemical devices for the area of water quality monitoring and the small number of papers related to this theme. Finally, we summarize the challenges in the field and its future trends.

2. Water situation – overview

According to the World Health Organization (WHO) and the United Nations International Emergency Fund for Children (UNICEF), about 2 billion people depend on water contaminated with feces. Of the 842,000 deaths recorded from diarrheal diseases, almost 58% could be prevented by basic sanitation and hygiene [14]. The report concluded that the number of people who have access to basic drinking water services is growing, but this slight increase is far away from the ideal world. Another current problem is related to the scarcity of fresh water in the world. More than half of the world's population suffers from a medium to severe crisis of water scarcity, caused mainly by intense climatic changes and the amount of water available in sufficient quantity and quality to meet the high demand and satisfy the world population [15]. Given the questions presented, there is a need for measuring and practicing for the intelligent use of water resources, as well as care

concerning the quality of this resource, which has the most diverse destinations such as supplying homes, businesses and industries.

The improvements and developments presented in the plan, which should be implemented in the country by the year 2035, aim to preserve water in quantity and quality that can meet social, agricultural and industrial needs, in the same way, which it proposes actions to reduce losses and expenses caused by periods of drought and flooding. Given the evident importance of water for life, the United Nations General Assembly titled the 2018–2028 period the International Decade for Action, Water for Sustainable Development. The demand for clean, safe and available water increases the need for increasingly efficient analysis and treatment practices close to the sampling local.

To circumvent the low availability of fresh water in the world and the high demand by the population, solutions such as the reuse of water become subjects that are increasingly discussed. Water can serve as a means of transmission and circulation of different substances with harmful potential to human health, like chemical compounds and microorganisms [16].

3. POCs

POCs are devices for obtaining rapid and local sampling information about the intended analyte. The first concept of a miniaturized analytical device was developed in 1979 [17]. The device was used to separate gases from hydrocarbon mixtures following the premises of conventional gas chromatography. The system showed that it was possible to reduce the size of the sensors, which resulted in a smaller sample volume, lower analysis cost, and portability without loss of performance compared to laboratory analysis. This was the beginning of research aimed at portable devices, being applicable in the most diverse fields of study such as biological monitoring, environmental analysis and probes for difficult to access locals [18]. Another outstanding contribution was made in 1990 with the total miniaturization of chemical analysis, from working with the sample, through analysis, detection and adjustments for measurements [19]. The advantages of this operating system are many, such as the reduction of the volume of samples and reagents, lower analysis cost and short analysis time, maintaining good performance in sensitivity and selectivity. The possibility of analyzing different species and, of course, their portability, have made POCs gain more attention and much research has been carried out in recent decades to improve the devices and their use [20].

The term POC is usually applied to describe point-of-care devices for healthcare applications. The devices would be employed in the identification of a substance close to the patient requiring a short time for



Fig. 1. Representation of the use of an electrochemical POC for water analysis.

analysis. More broadly, the term point-of-need (PON) testing would be used to name on-site testing that would also include environmental, animal, and food sample analysis. The WHO has described the main aspects desired in POCs and PONs as being accessible, sensitive, specific, simple to use, robust and fast, of little equipment, and available where and to whom need it. Furthermore, POCs and PONs allow faster *in loco* analysis of some hazardous substances, helping the control of outbreaks and diseases [21].

Despite the numerous advantages discussed, when investigating practical use, few POCs are used in field analyses for human health monitoring and environmental monitoring. The hesitation regarding the effective use of POCs in routine analyzes may be related to a concern with obtaining reliable results and easy interpretation by users, taking into account sensitivity and selectivity parameters that the analysis instruments should present. When working with complex matrices, the need to pre-treat some samples, the lifetime of the devices and the quality of the tests compared to those performed in laboratories are pointed to be circumvented in the manufacture and application of the POCs [22]. Among the manufactured ones, the most consolidated system was the blood glucose evaluation system, developed by Clark and Lyons [23]. The device that has undergone many improvements since its creation, allows the patient to monitor their blood glucose level in a simple way and their own home, enabling the early discovery of diseases, which is essential in the treatment of diseases [18].

Still addressing the health issue, POCs can be used for water quality control, as they are portable and easy to operate at home, at water treatment plants, near rivers, lakes and seas, serving as a support for rapid analysis of the concentrations of species present in these environments. Even if they work only as screening devices, they would represent rapid and less expensive analyzes that would allow for *in loco* contaminant assessment and simultaneous decision-making. Fig. 2 summarizes the advantages of electrochemical POCs.

In the last two years, there has been a significant increase in the number of reviews and research publications addressing the development of POCs for health, water and wastewater quality monitoring. The fabrication of electrochemical devices is considered a promising analytical field due to the generation of rapid and highly sensitive responses, in addition to the portability and possibility of miniaturization that these systems offer combined with the Internet of Things (IoT) to

remote monitoring [25].

3.1. Application of POCs

Due to all the problems arising from the SARS-COVID-19 outbreak, recent review papers explore how electrochemical (bio)sensors can help in detecting microorganisms and streamline the understanding of the actual number of infected, providing better control of its spread and bringing greater safety to the population [26]. This tragic episode of the global pandemic brings out the need for research and development of devices that can serve the population of the most diverse parts of the world in an easy, safe and low-cost way, to identify, detect and/or quantify, in the early stages, any manifestation of illness. What can be inferred after a literature review is that the number of publications aiming to monitor and analyze water quality through POCs has been growing mainly in the latest two years. The same observation can be done for electrochemical systems as a technique to detect the target species. The main issues to be considered when integrating and implementing any detection platform include cost-effectiveness, real-time monitoring potential in terms of sensitivity and selectivity, and above all, simplicity of operation [27].

Among the main waterborne contaminants already mentioned, bacteria, protozoa, and viruses will be emphasized. These microorganisms have variants in their genetics, which makes their analysis and monitoring a complex task. At this point, the development of electrochemical POCs to monitor bacteria, protozoa and viruses in waters will be addressed.

3.1.1. Bacterias

Bacteria are single-celled, prokaryotic organisms with many different forms and can be found in the air, water, soil and other living things. They reproduce in a non-sexual manner, mainly by binary division, which, under favorable conditions, explains their rapid proliferation in infections [28]. A widely employed alternative in water analysis concerning fecal contamination is scanning the samples for one or more microorganisms. Often fecal indicator bacteria are monitored, including total coliforms, fecal coliforms, *Escherichia coli*, and enterococci. These indicators make it possible to predict the presence of bacterial, viral, and protozoan pathogens from fecal contamination [29]. Addressing the need for bacteria monitoring, researchers developed an amperometric detection immunosensor with the advantage of providing the separation between the biocomponents and the electrode, which prevents species from encrusting the recognition surface, increasing the durability of the device [30]. The design of the device is simple, consisting of an analyte capture and a detection system. The bacteria capture system is composed of immuno-functionalized magnetic particles (MPs), which enable a simple preconcentration of the target bacteria with the addition of providing a physical separation between the target bacteria and other constituents of the sample, improving the way that the sample is handled and decreasing the analysis time. The MPs were coupled to a microfluidic system that allowed the analysis of a small sample volume, with low consumption of reagents, low generation of discard and improvement in the conditions of immunocapture, reaction and electrochemical detection. The detection system used in this work, on the other hand, is based on chronoamperometric detection on the gold microband electrode where horseradish peroxidase (HRP) catalyzes the reaction of reducing hydrogen peroxide (H_2O_2) linked to the oxidation of hydroquinone (HQ) to benzoquinone (BQ). The enzymatic reaction takes place in a chamber separated from the working electrode, and the reaction product is pumped into the microchannel for detection.

The device in chips and flow cells created by the group illustrated in Fig. 3 brings characteristics intended in the field analysis, such as the application in a simple, reusable and portable way in the detection of pathogens. The MPs used to have a large surface area, which helps in target-antibody kinetics, providing analyzes within 1 hour, related to 40 min immunoassay, 2 washes of 5 min each, injection in the microfluidic

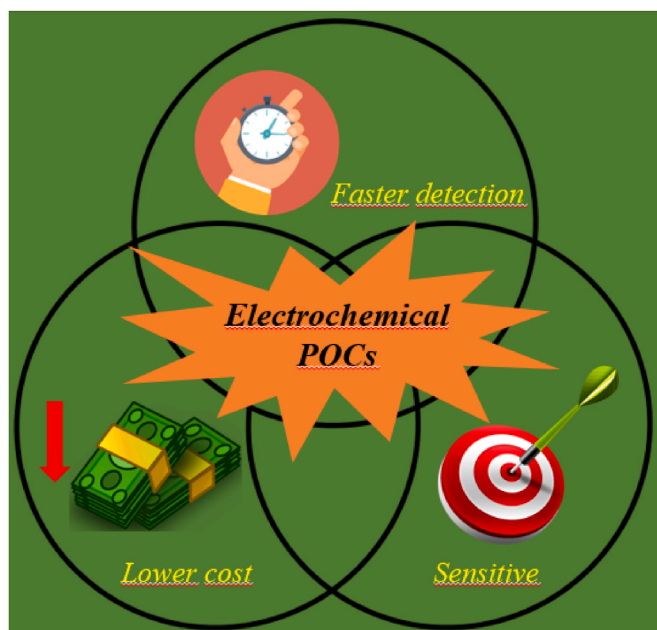


Fig. 2. Summary of the advantages of electrochemical POCs. Adapted from Ref. [24]. (Reprinted with permission from Ref. [24]).

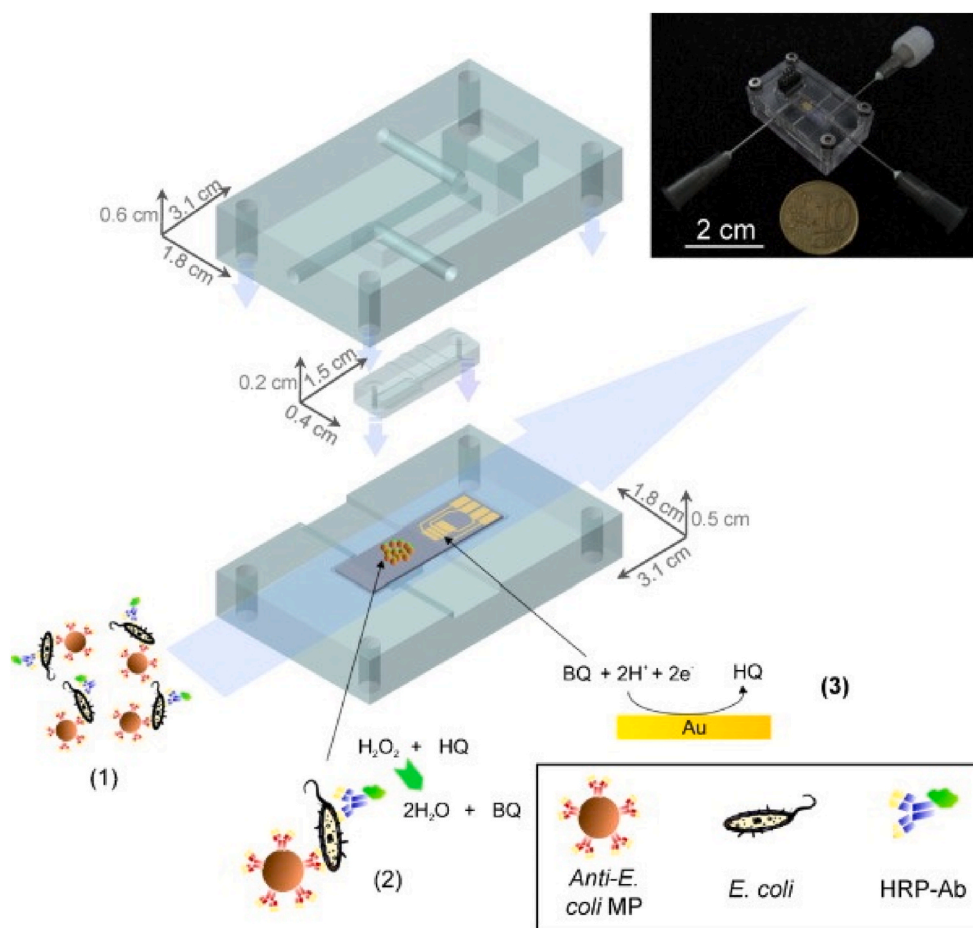


Fig. 3. Scheme of the microfluidic system. The bottom part contains the chip and two magnets that confine the MP upstream of the electrode. The upper part has fluid interconnections. The joint provides water-proof sealing and contains all microfluidic characteristics: a cavity 2 mm wide, 150 μm high and 5 mm long directly above the magnets, which is joined to a channel 500 μm wide, 150 μm high and 6.5 mm long which allows the solution pass through the electrodes. The electrodes of the gold micro band consist of three bands 500 μm wide and one band 1 mm wide, all parallel and separated from each other by 100 μm gaps. The widest band was used as an auxiliary electrode and the upstream micro band was used as the pseudo-reference to avoid potential deviations during measurements. The assay consists of the following steps: (1) immunocapture the target bacteria with MP, labeled with HRP-Ab, and injected into the microfluidic system. (2) Magnetic confinement of MP in the reaction chamber where the enzymatic reaction occurs. (3) Reduction of HRC produced by the enzyme in the gold electrodes, downstream. The different bio-components are not drawn to scale. (Reprinted with permission from Ref. [30]). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

system and enzyme monitoring.

A linear response was obtained for *Escherichia coli* concentrations ranging from 10^2 – 10^8 cells mL^{-1} , with LOD of 55 cells mL^{-1} in PBS, with no need for the *E. coli* pre-enrichment steps and where no significant interference in the answer. The microfluidic system helps to control the conditions of mass transport, influencing currents of greater intensity, protecting the electrode surface from fouling and passivation. Despite the numerous advantages exposed, a problem that must be solved before the application *in situ* of this system for monitoring *E. coli* in rivers and other waters is related to the working strip. According to updated resolution GM/MS No. 888, of May 4, 2021, referring to the potability standard and sampling plans for quality control of water intended for human consumption, after conventional treatment, the absence of *E. coli* cells per 100 mL of water should be verified [31]. This concentration is below the linear response range of the proposed system [30].

Subsequently, other research also developed an impedimetric detection of *E. coli* but using a smartphone-based microfluidic sensor [32]. The innovation, in this case, is the use of a smartphone, a portable device with easy and cheap access, and in the use of wireless connections, which is desired for analysis in the field, making it possible to monitor water in distant places from the laboratories. The combination of electrochemical impedance spectroscopy (EIS) as a detection method, which offers advantages such as high sensitivity, simplicity and cost-benefit, with microfluidic chips enabled greater analytical results for bacteria. Fig. 4 shows images of the device and the design of the created application.

The sensor consists of detection electrodes and a microfluidic detection chamber that is also used for the pre-concentration of bacteria in diluted samples. The developed Android application program (App) allows recording and viewing the EIS measurements and responses of

the tests, as well as enabling the control of the sensor electronics. The application also makes it possible to construct calibration curves, after measuring different known bacterial solutions to carry out the analysis of unknown samples in the next step. Real-time measurement data is transmitted to a smartphone via a Bluetooth circuit module. The authors used deionized water (DI) (18 M Ω) as an electrolyte and observed a decrease in the electron transfer resistance (R_{et}) as the concentration of bacteria increased in the solution.

The use of DI has the advantage of being similar to an analysis in the field, where pre-treatment steps and dilution in buffers would not be necessary. The dynamic working range of the sensor was 10 cells of *E. coli* mL^{-1} to 1000 cells mL^{-1} and LOD of 10 cells of *E. coli* per milliliter. Quickly and with a highly sensitive response, the authors were able to develop a promising device. There is also the prospect that this device can be adapted for the analysis of other pathogens. However, the proposed sensor does not meet the maximum allowable limit for *E. coli* in drinking water defined in the updated regulations.

Another example of the development of microdevices for microorganism monitoring has been proposed [33]. The authors were interested in building a multi-pathogen detection device, where *E. coli* and the gram-positive bacterium *Bacillus subtilis* were used as test organisms. When preparing the sample, the system operates with controlled temperature, which allows the denaturation of the target species and subsequent hybridization with the capture DNA. The sensor formed by a silicon glass micro-chamber and a matrix of indium and tin oxide (ITO) electrodes has a simple design and has great potential for water analysis at the research site. The electrodes proved to be very selective for the pathogen they were designed for, and even in the presence of other species in solution, the difference in responses was still significant. The linear response range achieved was between 10^2 – 10^5 cells sample $^{-1}$.

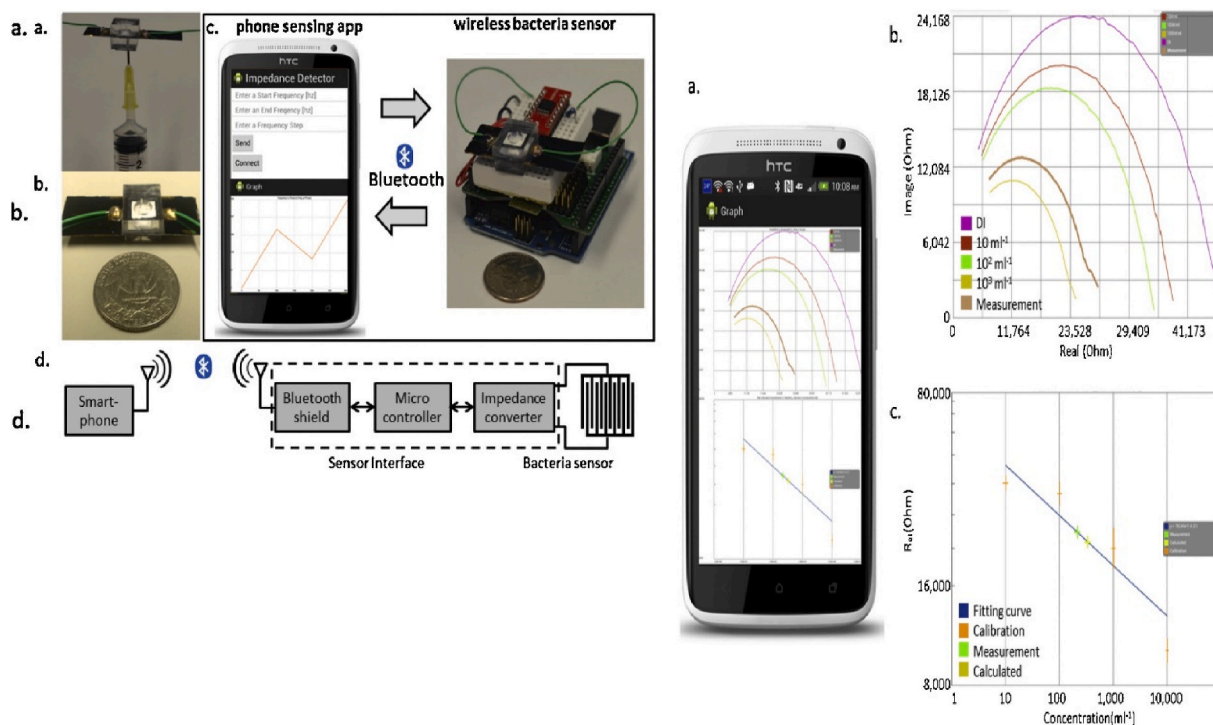


Fig. 4. (A) Bacteria detection system on wireless cell phones. (a) Image showing the syringe injection of test liquid in the sensor package; (b) close view of the EIS bacteria sensor package; (c) image showing the communication scheme between the smartphone detection application and the wireless bacteria sensor; (d) diagram of the wireless detection system. Calibration and sample measurement by the wireless cell phone bacteria detection system. (B) (a) Cellphone display with the results of the impedance measurement; (b) enlarged image of Nyquist graphs on the cell phone, with four solutions of known concentrations of bacteria used for sensor calibration, DI water and a sample solution to be tested; (c) R_{et} vs concentration batch and the equipped calibration curve. The *E. coli* concentration of the unknown sample was derived from adjusting the measurement results on the calibration curve and corresponding formula, as shown by the green cross; while yellow shows the calculated (real) concentration and the corresponding R_{et} . (Reprinted with permission from Ref. [32]). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

However, two main aspects must be improved to allow this device to be applied in the field. The first one concerns the sample preparation step, which involves the use of a buffer solution, temperature control and processing time. The second one is related to making the device more independent allowing untrained people in using it, becoming this device more accessible, dynamic and efficient.

A recent review lists some electrochemical biosensors that evaluate the detection of *E. coli* and other pathogens [34]. Each year the number of papers focusing on the development of simple and possibly portable electrochemical devices for monitoring bacterial pathogens in water grows. However, what we note is that this number is lower than the desired considering the great importance of an analysis aiming the human health. The ways of detecting bacteria in more widespread

waters are those that use microbiology and biotechnology to culture the species, however, a disadvantage in the application of these techniques is the difficulty in identifying a specific pathogen present in the water sample. Other methods that are most used to circumvent this limitation and accurately identify the genes of the pathogen are the virulence gene assays, multilocus sequence typing, enzyme-linked immunosorbent assays and chain reactions. quantitative polymerases [35]. However, the high cost and high time for analysis make it impossible to apply these methods to monitor water in the field, since the quick and less costly analysis of pathogens in water environments would make it possible to minimize the disease spread to consumers, avoiding cases of outbreaks and infection pandemics.

It is important to mention that electrochemical POCs act as a key role

Table 1

Recent work on potential POCs for electrochemical detection of waterborne bacteria.

Analyte	Sensor	Detection Method	Linear Range	Detection Limit	Incubation Time (min)	Sample Volume (μL)	Year	Ref.
<i>Escherichia coli B</i>	T4B-MES/Au	DPV	$1,9 \times 10^{-1} - 1,9 \times 10^9$ CFU/mL	14 ± 5 CFU/mL	20	Unspecified	2020	[36]
<i>Escherichia coli</i>	HRP-TMB/anti- <i>E. coli</i> /Au	Amperometric	$10^{-3} - 9,7 \times 10^7$ CFU/mL	50 CFU/mL	real	150	2018	[37]
<i>Escherichia coli</i>	CsWO ₃ -FCD/Si nanohybrid	EIS	$10^1 - 10^7$ CFU/mL	3,81 CFU/mL	real	Unspecified	2021	[38]
<i>Pseudomonas aeruginosa</i> (acyl homoserine lactones (AHLs))	GCE/TEMPO - ZnPc	Amperometric	2,32–39,9 μM	1,8 μM	0,083	Unspecified	2020	[39]
<i>Shigella dysenteriae</i> (<i>S. dysenteriae</i>)	aptamer/AuNP/GCE	EIS	10^1 a 10^6 CFU/mL	10^0 CFU/mL	30	Unspecified	2018	[40]
<i>Pseudomonas aeruginosa</i>	aptamer-NanoZyme	Amperometric	60,0 a $6,0 \times 10^7$ UFC/mL	~60 CFU/mL	10	40	2019	[41]

colony forming units (CFU); broad type T4 bacteriophage (T4B-MES); horseradish peroxides (HRP); 3,3', 5,5'-tetramethylbenzidine (TMB); cesium tungsten oxide (CsWO₃); electrochemically generated fluorescent carbon dots (FCDs); 2,2,6,6-tetramethyl-1 piperidinoyloxy zinc(II) phthalocyanine (TEMPO-ZnPc).

in solving the obstacles existing in the usual monitoring methods, allowing an easier, faster and cheaper way for the population. Table 1 summarizes data obtained from recent work using electrochemical techniques for the detection of waterborne bacteria. The listed works are promising research for the development of POCs that have had their application evaluated in water samples.

Some factors may be considered to the low practical implementation of electrochemical POCs in monitoring bacteria in water. A challenge may be to find elements of capture that are selective and/or specific to a given pathogen. In addition, the devices should be able to distinguish the variants of each pathogen even at low levels, favoring the observation of microbiological changes in the water in the initial stage. As the electrochemical POCs platforms are generally built not only with selective and/or specific capture species but from the combination of different materials with properties such as conductivity and large surface area, adsorption of species can occur on the electrode surface, which can lead to changes in the responses observed in the system causing false-positive signals in the analysis. Thus, a throughout the study of the interactions that the electrode could carry out with the species in the water sample should be developed, but keeping in mind that *in loco* analysis of a complex matrix is difficult for the sensor's operating way [42].

3.1.2. Protozoa

Protozoa are eukaryotic, unicellular, heterotrophic beings. Among the existing protozoa, *Cryptosporidium* spp. is the most common waterborne parasite and is the main cause of mortality associated with gastrointestinal diseases due to water contamination. Identified by Tyzzer in 1907, *Cryptosporidium* spp. is an intracellular coccidian that localizes in the outer region of the cytoplasm [43]. This parasite has a monoxenous lifecycle, inhabiting a single host, in epithelial cells of the gastrointestinal and respiratory tracts of humans and some animals. It is estimated that 1×10^6 to 2×10^6 oocysts g^{-1} of infective oocysts are released in the feces of hosts [44].

In the genus *Cryptosporidium*, almost 30 species and more than 40 genotypes have been identified. In humans, the species *C. hominis*, *C. parvum* are the most reported in problems associated with an intestinal infection. The infection starts with direct contact with an infected person and/or animal, or indirect contact by ingesting contaminated food or water. Cryptosporidiosis is ranked as the 4th leading cause of death in children under 5 years of age. Alarming numbers of contamination, as well as cases of diarrhea and deaths associated with contamination by species of the genus *Cryptosporidium*, have been announced in the USA and Africa [45]. A characteristic of *Cryptosporidium* that makes water decontamination difficult is the resistance of the oocysts to chlorine and other chemicals traditionally used in water treatment. The oocysts are usually found in the concentration range of 0.01–150 oocysts L^{-1} of surface water [46]. Some obstacles can be pointed out in the determination of these parasites as their low number in the samples, the great variability of species and genotypes observed for *Cryptosporidium*, besides the complexity of the water matrix itself. Because of these points discussed, there is a need for the development of determination methods with high sensitivity and specificity to distinguish the existing different species and genotypes [44].

EPA Method 1623.1 (USA) [47] is a revision of EPA Method 1623 (USEPA 2005) and is being used in an attempt to recover, detect, and control *Cryptosporidium* and *Giardia* oocysts in water samples. Being able to use some alternative technologies, this method involves different steps, where the recovery is performed by particle concentration, separation of the protozoa and the identification and numbering of the genera [48]. The detection step, on the other hand, can be applied using PCR [49], microscopy [50], and immunofluorescence techniques [51]. These techniques, although widely used, have some disadvantages, such as unspecific or using antibodies, which makes the techniques less robust [44]. The use of electrochemical analysis techniques could overcome these difficulties. The research was conducted to develop a label-free capacitive biosensor for the detection of *Cryptosporidium*

oocysts in water samples [52]. The used anti-*Cryptosporidium* antibody allowed to accurately distinguish different numbers of captured cells and densities on the biosensor surface. The biosensor can operate in a linear detection range between 15 and 153 cells mm^{-2} with a LOD of 40 cells mm^{-2} .

Assays based on the application of aptamers as recognition elements are reported as more sensitive, thermostable, selective and robust than those described above. They allow in some cases the differentiation of host species, genotype and even sub-genotype of the parasite [44]. In the literature, there is research that has developed probes for the detection of protozoa. A study of DNA aptamer by microplate fluorescence to evaluate how it could be used to detect *C. parvum* and other *Cryptosporidium* species in wastewater samples was reported [53]. The aptamer Min_Crypto2 showed a LOD of 5 *C. parvum* oocysts in 300 μL of wastewater, exhibiting potential for use in sensors for local application. In other work, the group developed an aptasensor to detect *C. parvum* oocysts in drinking water and recreational sources [54]. The electrochemical aptasensor enabled rapid, target-specific detection through changes in the electrical signal. The aptamer-coated probes had a detection limit of 50 oocysts/50 μL phosphate solution. Its effective performance even when applied to raw water makes it feasible to use in field samples without relying on monoclonal antibodies and microscopy, and with the advantage of requiring minimal technical knowledge.

At this point, one can point out some challenges encountered by researchers when developing POCs for the detection of protozoa in water. Under environmental conditions, *Cryptosporidium* exists as a spherical oocyst about 5 μm in diameter [49], while *G. lamblia* has oocysts of $\sim 14 \mu m$ in diameter [55]. This small size influences the electrochemical behavior of the biosensor depending on the used method. The protozoa will cover less of the electrode, having a relatively minor effect on charge transfer at the electrode-electrolyte interface [34].

Another example of a lab-on-chip device based on a three-dimensional TAS microchip (μ TAS 3D) was applied as a DNA biosensor for rapid and highly selective detection of *Cryptosporidium* [56]. The electrochemical signals were recorded by differential pulse voltammetry (DPV) and impedance and the results were compared to those of a common commercial screen-printed electrode (SPE). The detection device (Fig. 5), obtained excellent responses.

The μ TAS 3D DNA biosensor showed a LOD of 1.8 ng mL^{-1} and sensitivity of 12.844 $\mu A/(\mu g mL^{-1})$ over a linear range from 2.5 ng mL^{-1} up to 0.1 $\mu g mL^{-1}$. The biosensor achieved a 6-fold lower LOD and 23-fold higher sensitivity than the commercial SPE over a similar linear response range. The high selectivity of the microchip fabrication enhances the use of this system in POCs. Table 2 provides a list of more recent work on potential POCs where electrochemical techniques have been applied to the detection of waterborne protozoa.

For the case of the electrochemical hybridization system of immobilized DNA linked to an enzyme for detection of *Cryptosporidium parvum* in water. The response range obtained for *C. parvum* was 5–50 $\mu g mL^{-1}$ with a LOD of 2 $\mu g mL^{-1}$ (or 146 nM) of the target DNA. The assays demonstrated high selectivity for *C. parvum*. In the interference studies, the biosensor showed no signal for the synthetic DNA of *Escherichia coli*, *Giardia lamblia*, *Salmonella typhimurium* and *Listeria monocytogenes*. The proposed biosensor also had a much shorter analysis time than the immunofluorescence detection of the EPA methods (from 4 days to less than 20h), reducing processing steps and making the analysis faster [58]. The development of an ELISA electrode-based potentiometric sensor for the detection of *Cryptosporidium parvum* oocysts has also been reported [59]. After optimization of the analysis parameters, the researchers were able to detect 5×10^2 oocysts of *C. parvum* per ml in 60 min. Here in the same as the bacteria section, we think POC devices could play an important role in local measurement/detection of protozoa, especially protozoa is one of the main reasons for a child under 5 years of death worldwide [44].

Concerning the detection of protozoa, the same difficulties are observed as with the detection of bacteria. Ensuring the construction of

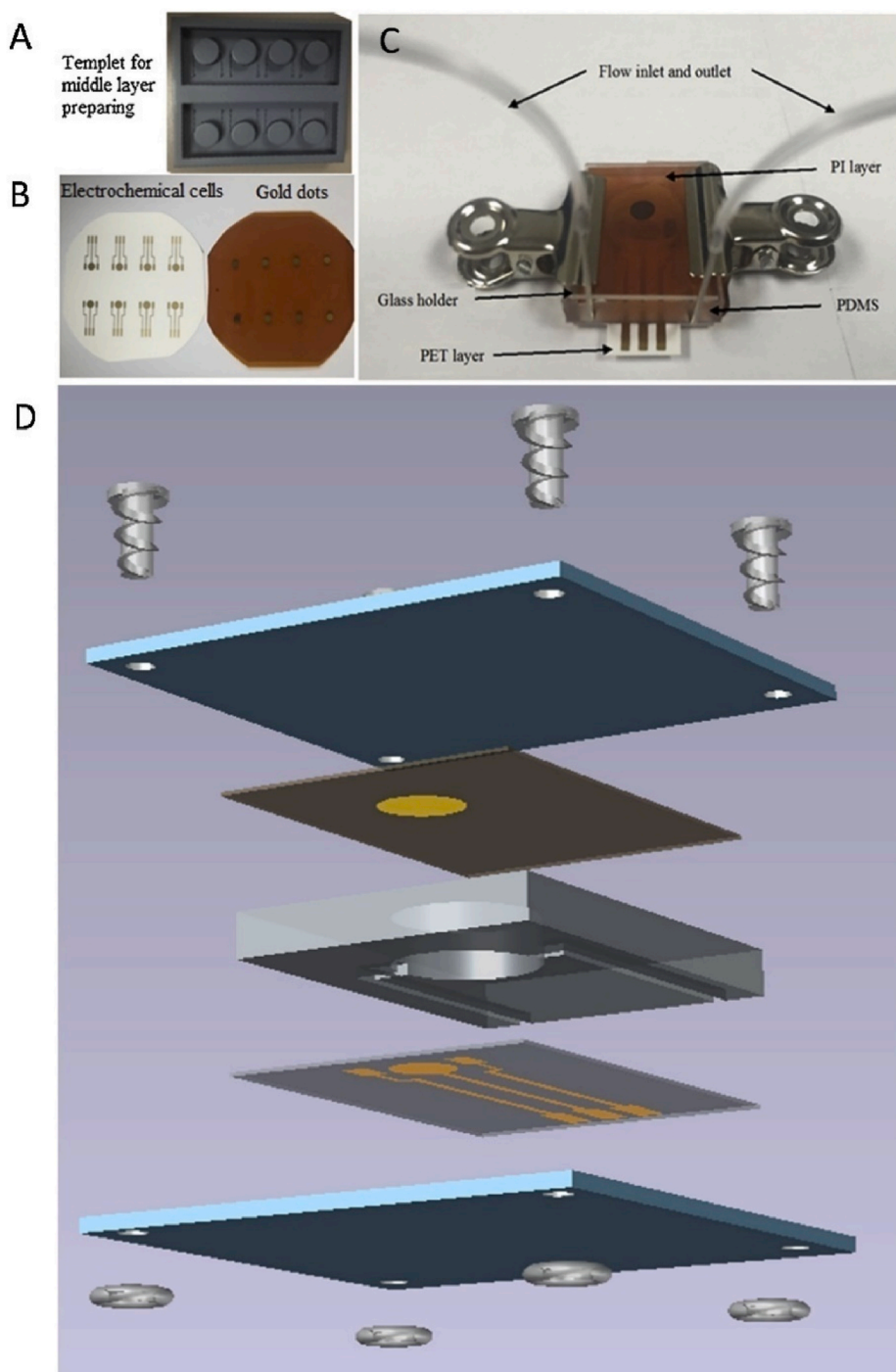


Fig. 5. Electrochemical μ TAS 3D biosensor used as a detection device. (A) Plastic model to prepare the media layer. (B) Layer one, electrochemical cells, and layer 3, gold dots. (C) The final fabricated μ TAS 3D electrochemical biosensor. (D) The schematic structure of the designed μ TAS 3D electrochemical biosensor. (Reprinted with permission from Ref. [56]). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

portable systems that maintain high sensitivity and robustness even when applied to complex matrices is still a challenge. Understanding and solving these problems requires a lot of study and research time, which delays the manufacturing of POCs.

3.1.3. Virus

Due to all the current problems regarding the contamination by SARS-COVID-19, there is a need to monitor other microorganisms in addition to bacteria and protozoa, such as viruses. Viruses are formed by the genome (DNA or RNA), protein capsid (for protection of nucleic acid) and in some viruses, there is also a lipid envelope that covers the

capsid [60]. Although the definition of the term virus is still a subject that generates different interpretations, it is known that viruses have a life cycle and reproduction divided into two well-defined stages. The first happens inside the host cell where the virus reprograms the cell to produce viral particles, called virions. The second is an extracellular stage, where virions come out of the cell that was generated and spread at the extracellular environment [61].

A recent work [62] evaluated how a paper-based platform could be used as a rapid mechanism for the identification of SARS-COVID-19 in wastewater. Based on the previous knowledge that water contaminated with feces and urine of people infected with the virus had biomarkers for

Table 2

Recent work on potential POCs for electrochemical detection of waterborne protozoa.

Analyte	Sensor	Detection Method	Linear Range	Detection Limit	Incubation Time (min)	Sample Volume (μL)	Year	Ref.
<i>Cryptosporidium</i> spp.	aptamer R4-6 3'-biotinylated/GNP-SPCE	SWV	50 a 900 oocysts/50 μL PBS solution	50 oocysts/50 μL PBS solution	120	50	2019	[54]
<i>Cryptosporidium</i>	Interdigitated microelectrode array/Au	Capacitance (EIS)	15 a 153 cells/ mm^2	40 cells/ mm^2	20	5	2019	[52]
<i>G. lamblia</i> and <i>E. histolytica</i>	PGE with MDZ printing	DPSV	1,6 a $1,6 \times 10^7$ CFU/mL	2,0 CFU/mL	Unspecified	Unspecified	2014	[57]
<i>Cryptosporidium</i>	DNA μTAS 3D	DPV	2,5 ng/mL a 0,1 μg /mL	1,8 ng/mL	60	10	2019	[56]

Screen-printed carbon electrode modified with gold nanoparticles (GNP-SPCE); molecularly printed polymer-modified graphite electrode (PGE); metronidazole (MDZ); differential pulse redissolution voltammetry (DPSV); three-dimensional micro total analysis system (μTAS 3D).

the disease, the device on paper was evaluated and presented potential use in the detection of SARS-COVID-19. Its simple testing process, smaller size, low cost, compared to a polymerase chain reaction (PCR) assay, in addition, portability and easy storage confirmed the ability to use paper devices to analyze even in complex matrices such as sewage and wastewater.

In contrast to the main case of the new coronavirus that is transmitted by air, several enteric viruses can be transmitted mostly in water, which again brings the need for devices that can assess water quality in the most diverse regions. An example is the hepatitis E virus (HEV) which, according to data collected in Africa and Asia, it is estimated that in 2005 around 20 million people were infected and 70 000 died [63]. HEV is an RNA virus, without an envelope, that can manifest itself through outbreaks or in discontinuous episodes, being transmitted mainly by water contaminated by urine or feces of sick people. It is associated with HEV mainly acute liver diseases, but also other chronic diseases, not only restricted to the liver but also affecting problems in the neurological and renal systems [64].

Of the eight existing genotypes for HEV, G1 and G2 are the genes that only infect humans via the fecal-oral route. The physical-chemical treatment of water would allow the removal and inactivation by disinfection of the HEV from the water [65]. Contamination cases are more

prevalent in underdeveloped and/or developing countries where there is little or no basic sanitation for the population, leading to the contamination of food and water. The detection of HEV is usually performed by molecular techniques such as the quantitative retrotranscription polymerase chain reaction (RT-qPCR), being considered a very sensitive test in the analysis of low levels of RNA, in addition to being a quick and easy handle test [64]. However, the efficiency of RT-qPCR is dependent on the type and mode of conservation of the studied RNA genome, and has the disadvantage of requiring high-cost reagents and equipment [66]. Thus, analysis alternatives with lower added value that can serve underdeveloped or developing countries are necessary.

In the literature, works can be found reporting the use of electrochemical techniques as a means of detection and quantification of viruses, mainly of blood and other biofluids transmission and those of respiratory transmission [60]. As is the case [67] where the group developed an aptamer-based biosensor in a sandwich structure for detection by differential pulse voltammetry (DPV) of hepatitis B virus (HBV) [68] combined a genomagnetic assay with DPV detection for HBV analysis. After optimizing the detection process, the researchers reached a detection limit of $74.8 \text{ fmol mL}^{-1}$ of target concentration in the 50 μL samples, a limit below that found in previous studies. Already [69] manufactured a disposable paper platform for HBV detection, shown in

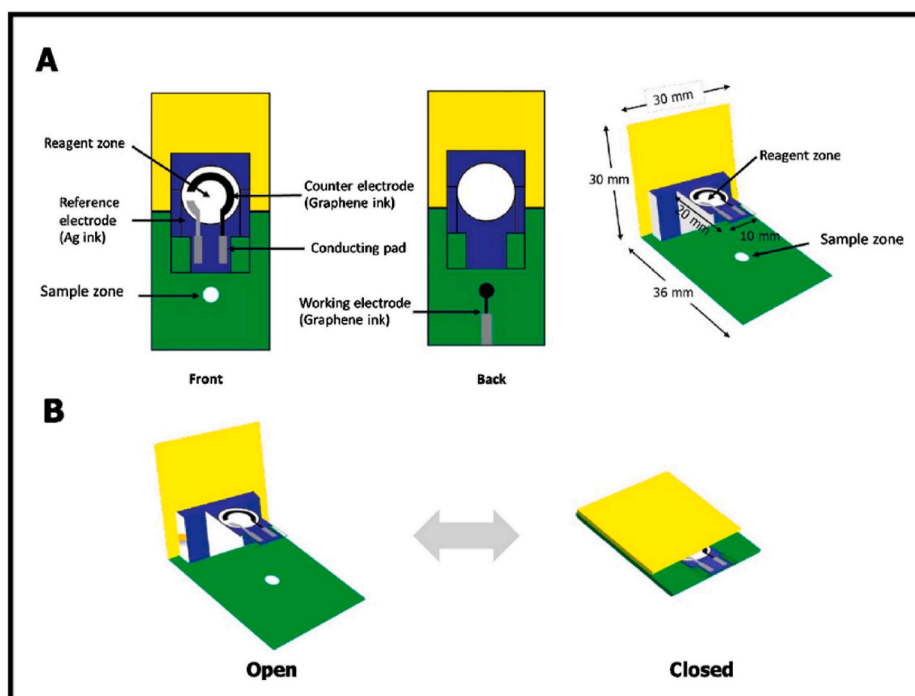


Fig. 6. Representation of the developed sensor (A) Compartment of a PAD DNA pop-up sensor. (B) Schematic of the PAD DNA pop-up sensor in the “open” and “closed” formats. (Reprinted with permission from Ref. [69]).

Fig. 6.

This sensor was designed in a way to allow some stages of the detection process to be controlled, such as the fluid path, the incubation time and electrical connectivity, in addition to being able to be used in the field. The review by Ref. [60] provides an extensive list of biosensors manufactured for the detection of respiratory viruses, including electrochemical detection.

A work aiming the detection of Hepatitis A virus (HAV) with potential application in water samples was developed [70]. The screen-printed gold electrode used was able to analyze HAV by cyclic voltammetry at a linear response range of 10 fg/ μ L to 0.1 ng/ μ L, achieving a LOD of 0.65 pM. This work demonstrated the possibility of building sensitive and robust systems for the detection of species found in low concentrations, such as viruses. In the literature, it is possible to find research that has developed (bio)sensors for virus detection with application in food, clinical and biological samples. However, the construction of electrochemical devices for application in water samples is still an area in need of development.

Although there are several advantages described in this review regarding the application of electrochemical techniques as a way of detecting target species, what would be desired would be more POCs already in operation in virus monitoring in water. About this, some existing barriers can be pointed out. First of all, the choice of the recognition element. Its immobilization on the electrochemical platform, without damage to the element-target interaction, as well as long-term stability and lifetime, are complex questions to be analyzed [71]. Having answered these questions, it should be borne in mind that, for the case of adenovirus and calicivirus viruses found in sewage and infected waters, there are no standard methods for cultivating and detecting these species, which makes it difficult to develop new detectors without at the end of the development is possible to validate the results found. Even for enteroviruses, a species of the virus also found in water contaminated with the feces of infected people, where protocol methods already exist, differentiating the type of enterovirus present in the samples is a major obstacle for the analyzes [72].

Another difficulty that arises in the search for the development of new methods of detection of these microbiological species is in the fact that the viruses can mutate in the DNA or RNA gene [72], which limits and makes difficult the type of recognition means to be used in electrochemical devices that can differentiate or recognize the different forms of the same viral species. The need for preparation and pre-concentration of the samples containing the viruses, and the existence of natural interferences in the aquatic matrices, add to the set of considerations to be made of why the electrochemical POCs for aquatic dissemination viruses are still not so widespread [73].

In a recent review by Ref. [24], the authors describe strategies in the development of biosensors and immunosensors for the detection of viruses and viral diseases. With a focus on recent work, the researchers report the modes of immobilization of probes observed, as well as the types of assays, modes of detection and results found. As in this study [24], it is also considered that the technology coming through the use of electrochemical POCs would be the solution for the formation of faster, miniaturized and more sensitive systems. But the authors also point out what they think is the obstacle to the implementation of this mode of monitoring, highlighting, in addition to the reasons already reported in this review, the difficulty of entering these devices in the commercial market, which initially requires a specific demand for electrochemical devices by market share for technology to be implemented and approved in industries. However, after the outbreak of the new coronavirus, it is believed that studies to identify potential pathogens, including viruses, protozoa and bacteria, in a faster, safer and cheaper way, will be a field of study of global interest, opening up possibilities for the construction of portable electrochemical analytical systems.

4. Conclusions and future perspectives

A challenge, especially when working with devices that use biomolecules as a recognition element, such as DNA, aptamers and antibodies, is the use of such molecules in complex matrices, such as rivers, lakes and sewage water. These sensors are developed to operate under specific conditions, such as optimum pH to remain active and maximize their detection efficiency. Thus, developing systems that can operate in complex matrices without losing performance is the crucial point. As already mentioned, the method of construction of POCs and PONs, combining the properties of different materials, demands the understanding of the different interactions and signals that may be generated during the analysis, which makes the development of these devices a hard and meticulous work. The sensor's manufacturing method, its precursor materials, size, selectivity, as well as its correct use and storage directly influence the performance, reproducibility, durability and stability guaranteeing safe and reliable results.

For DNA/RNA monitoring of bacteria, protozoa and viruses, the construction of oligonucleotide-based biosensors is the most interesting strategy. The specific bonds and high-affinity constant values that arise from the probe-target interaction allow the detection of genes of microorganisms in a highly specific way with or without sample preparation, even at low concentrations and in complex matrices. Researches dedicated to the study of the cell wall composition of the pathogens would allow a better evaluation of the best recognition element to be used in the development of each POC. However, concerning the electrode and electrochemical techniques, one point to consider is that their performance is affected by the electrode surface condition and the non-specific adsorption of compounds in biological samples. These conditions are unique to each matrix to be analyzed and are difficult to predict and regenerate for all analyses/samples [74].

As perspectives for the near future, there is a perceived demand for more portable multi-target systems. To meet the growing need for routine monitoring of specific species in water resources aiming at improving the quality of life and health of the population, POCs could perform simultaneous analysis of different species, optimizing the analysis time and providing information on the conditions of consumption and use of the sampled water. Another system emerging with promising characteristics for application in POCs is the printable electrodes, which its low cost allows the modification of its surface, increasing selectively for the analyte of interest.

The use of POC and PON in high-traffic environments can prevent the spread of infectious diseases, contaminated water due to the rapidity of analysis, allowing a high frequency of analysis. In addition, the minimal sample volumes needed in the measurements and incubation times for the targeted species, as pointed out in the tables, are features for practical on-site analysis. From the point of view of water monitoring, the use of POC and PON plays an important role and the devices can help review the processes and modes of water treatment, guiding to greater adherence and optimization in the processes employed. In the future, POCs and PONs may be used to test, correct, improve, and/or develop new ways of water treatment, playing a key role in ensuring that water resources are free of substances and microorganisms that are harmful to health.

Author contributions

ADS and WJP wrote the manuscript under the supervision of RCN and LTK. All authors contributed to the discussions and corrections.

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.

Acknowledgments

The authors would like to thank the National Institute of Science & Technology in Bioanalytics (INCTBio), National Council for Scientific and Technological Development (CNPq: 434303/2016–0, ADS 131574/2020–5), and São Paulo Research Foundation (FAPESP: 2014/50867–3, 2013/22127–2, WJP 2017/05213–3).

References

- ANA, Situação da Água no Mundo 2020. <https://www.ana.gov.br/panorama-das-aguas/agua-no-mundo/agua-no-mundo>, 2020. (Accessed 17 September 2020).
- M.P. Rodrigues, J. Ebdon, S. Purnell, H. Taylor, Potential microbial transmission pathways in rural communities using multiple alternative water sources in semi-arid Brazil, *Int. J. Hyg. Environ. Health* 224 (2020) 113431, <https://doi.org/10.1016/j.ijheh.2019.113431>.
- A. Lass, B. Szostakowska, K. Korzeniewski, P. Karanis, Detection of *Giardia intestinalis* in water samples collected from natural water reservoirs and wells in northern and north-eastern Poland using LAMP, real-time PCR and nested PCR, *J. Water Health* 15 (2017) 775–787, <https://doi.org/10.2166/wh.2017.039>.
- A. Efstratiou, J.E. Ongerth, P. Karanis, Waterborne transmission of protozoan parasites: review of worldwide outbreaks - an update 2011–2016, *Water Res.* 114 (2017) 14–22, <https://doi.org/10.1016/j.watres.2017.01.036>.
- P. Saingam, B. Li, T. Yan, Fecal indicator bacteria, direct pathogen detection, and microbial community analysis provide different microbiological water quality assessment of a tropical urban marine estuary, *Water Res.* 185 (2020) 116280, <https://doi.org/10.1016/j.watres.2020.116280>.
- V. Sagan, K.T. Peterson, M. Maimaitijiang, P. Sidike, J. Sloan, B.A. Greeling, et al., Monitoring inland water quality using remote sensing: potential and limitations of spectral indices, bio-optical simulations, machine learning, and cloud computing, *Earth Sci. Rev.* 205 (2020) 103187, <https://doi.org/10.1016/j.earscirev.2020.103187>.
- S. Dey, A. Kumar, A. Mahto, I.H. Raval, K.M. Modi, S. Haldar, et al., Oxalix[4] arene templated silver nanoparticles as dual readout sensor: developing portable kit for rapid detection of methylmercury and its speciation, *Sensor. Actuator. B Chem.* 317 (2020) 128180, <https://doi.org/10.1016/j.snb.2020.128180>.
- A. İncel, O. Akin, A. Çağır, Ü.H. Yıldız, M.M. Demir, Smartphone assisted detection and quantification of cyanide in drinking water by paper-based sensing platform, *Sensor. Actuator. B Chem.* 252 (2017) 886–893, <https://doi.org/10.1016/j.snb.2017.05.185>.
- 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, *Biosens. Bioelectron.* 32 (2012) 89–95, <https://doi.org/10.1016/j.bios.2011.11.037>.
- J. Xu, Y. Zhang, L. Li, Q. Kong, L. Zhang, S. Ge, et al., Colorimetric and electrochemiluminescence dual-mode sensing of lead ion based on integrated lab-on-paper device, *ACS Appl. Mater. Interfaces* 10 (2018) 3431–3440, <https://doi.org/10.1021/acsami.7b18542>.
- B.A. Bergamaschi, J.A. Fleck, B.D. Downing, E. Boss, B. Pellerin, N.K. Ganju, et al., Methyl mercury dynamics in a tidal wetland quantified using in situ optical measurements, *Limnol. Oceanogr.* 56 (2011) 1355–1371, <https://doi.org/10.4319/lo.2011.56.4.1355>.
- M. Díaz-González, M. Gutiérrez-Capitán, P. Niu, A. Baldi, C. Jiménez-Jorquera, C. Fernández-Sánchez, Electrochemical devices for the detection of priority pollutants listed in the EU water framework directive, *TrAC Trends Anal. Chem.* (Reference Ed.) 77 (2016) 186–202, <https://doi.org/10.1016/j.trac.2015.11.023>.
- L. Bijlsma, R. Bade, F. Been, A. Celma, S. Castiglioni, Perspectives and challenges associated with the determination of new psychoactive substances in urine and wastewater – a tutorial, *Anal. Chim. Acta* 1145 (2021) 132–147, <https://doi.org/10.1016/j.aca.2020.08.058>.
- United Nations Children's Fund (UNICEF) and World Health Organization (WHO), Progress on Household Drinking Water, Sanitation and Hygiene 2000–2017. Special Focus on Inequalities, UNICEF, WHO, New York, 2019, 2019.
- M.M. Mekonnen, A.Y. Hoekstra, Four billion people facing severe water scarcity, *Sci. Adv.* 2 (2016) 1500323, <https://doi.org/10.1126/sciadv.1500323>.
- A.B. Müller, T. Avellán, J. Schanze, Risk and sustainability assessment framework for decision support in “water scarcity – water reuse” situations, *J. Hydrol.* 591 (2020) 125424, <https://doi.org/10.1016/j.jhydrol.2020.125424>.
- S.C. Terry, J.H. Jerman, J.B. Angell, A gas chromatographic air analyzer fabricated on a silicon wafer, *IEEE Trans. Electron. Dev.* 26 (1979) 1880–1886, <https://doi.org/10.1109/T-ED.1979.19791>.
- B. Naseri, N. Soleimani, N. Rabiee, A. Kalbasi, M. Karimi, M.R. Hamblin, Point-of-care microfluidic devices for pathogen detection, *Biosens. Bioelectron.* 117 (2018) 112–128, <https://doi.org/10.1016/j.bios.2018.05.050>.
- A. Manz, N. Graber, H.M. Widmer, Miniaturized total chemical analysis systems: a novel concept for chemical sensing, *Sensor. Actuator. B Chem.* 1 (1990) 244–248, [https://doi.org/10.1016/0925-4005\(90\)80209-1](https://doi.org/10.1016/0925-4005(90)80209-1).
- D.R. Reyes, D. Iossifidis, P.A. Auroux, A. Manz, Micro total analysis systems. 1. Introduction, theory, and technology, *Anal. Chem.* 74 (2002) 2623–2636, <https://doi.org/10.1021/ac0202435>.
- R. Derda, J. Gitaka, C.M. Klapperich, C.R. Mace, A.A. Kumar, N. Lieberman, et al., Enabling the development and deployment of next generation point-of-care diagnostics, *PLoS Neglected Trop. Dis.* 9 (2015), 0003676, <https://doi.org/10.1371/journal.pntd.0003676>.
- J. Woo, V. Arboleda, O.B. Garner, Point-of-Care testing for group A Streptococcus infection and influenza, *Clin. Microbiol. Newsl.* 39 (2017) 151–157, <https://doi.org/10.1016/j.clinmicnews.2017.09.004>.
- L.C. Clark Jr., C. Lyons, Electrode systems for continuous monitoring in cardiovascular surgery, *Ann. N. Y. Acad. Sci.* 102 (1962) 29–45, <https://doi.org/10.1111/j.1749-6632.1962.tb13623.x>.
- L.C. Brazaca, P.L. dos Santos, P.R. de Oliveira, D.P. Rocha, J.S. Stefano, C. Kalinke, et al., Biosensing strategies for the electrochemical detection of viruses and viral diseases – a review, *Anal. Chim. Acta* 1159 (2021) 338384, <https://doi.org/10.1016/j.aca.2021.338384>.
- X. Li, J. Ping, Y. Ying, Recent developments in carbon nanomaterial-enabled electrochemical sensors for nitrite detection, *TrAC Trends Anal. Chem.* (Reference Ed.) 113 (2019) 1–12, <https://doi.org/10.1016/j.trac.2019.01.008>.
- V.V. Tran, N.H.T. Tran, H.S. Hwang, M. Chang, Development strategies of conducting polymer-based electrochemical biosensors for virus biomarkers: potential for rapid COVID-19 detection, *Biosens. Bioelectron.* 182 (2021) 113192, <https://doi.org/10.1016/j.bios.2021.113192>.
- N. Baig, M. Sajid, Applications of layered double hydroxides based electrochemical sensors for determination of environmental pollutants: a review, *Trends Environ. Anal. Chem.* 16 (2017) 1–15, <https://doi.org/10.1016/j.teac.2017.10.003>.
- R.S. Gupta, The natural evolutionary relationships among prokaryotes, *Crit. Rev. Microbiol.* 26 (2000) 111–131, <https://doi.org/10.1080/10408410091154219>.
- D.M. Silva, L. Domingues, On the track for an efficient detection of *Escherichia coli* in water: a review on PCR-based methods, *Ecotoxicol. Environ. Saf.* 113 (2015) 400–411, <https://doi.org/10.1016/j.ecoenv.2014.12.015>.
- O. Laczka, J.M. Maesa, N. Godino, J. del Campo, M. Foug-Hansen, J.P. Kutter, et al., Improved bacteria detection by coupling magneto-immunocapture and amperometry at flow-channel microband electrodes, *Biosens. Bioelectron.* 26 (2011) 3633–3640, <https://doi.org/10.1016/j.bios.2011.02.019>.
- Portaria GM/MS No888, de 4 de Maio de 2021, Diário Oficial da União, Brasil, <https://www.in.gov.br/en/web/dou/-/portaria-gm/ms-n-888-de-4-de-maio-de-2021-318461562>, 2021. (Accessed 19 June 2021).
- J. Jiang, X. Wang, R. Chao, Y. Ren, C. Hu, Z. Xu, et al., Smartphone based portable bacteria pre-concentrating microfluidic sensor and impedance sensing system, *Sensor. Actuator. B Chem.* 193 (2014) 653–659, <https://doi.org/10.1016/j.snb.2013.11.103>.
- S.W. Yeung, T.M.H. Lee, H. Cai, I.M. Hsing, A DNA biochip for on-the-spot multiplexed pathogen identification, *Nucleic Acids Res.* 34 (2006) 118, <https://doi.org/10.1093/nar/gkl702>.
- E. Cesewski, B.N. Johnson, Electrochemical biosensors for pathogen detection, *Biosens. Bioelectron.* 159 (2020) 112214, <https://doi.org/10.1016/j.bios.2020.112214>.
- K.C.B. Krishna, A. Sathasivan, M.P. Ginige, An assessment of the persistence of putative pathogenic bacteria in chloraminated water distribution systems, *Water Res.* 190 (2021) 116677, <https://doi.org/10.1016/j.watres.2020.116677>.
- J. Xu, C. Zhao, Y. Chau, Y.K. Lee, The synergy of chemical immobilization and electrical orientation of T4 bacteriophage on a micro electrochemical sensor for low-level viable bacteria detection via Differential Pulse Voltammetry, *Biosens. Bioelectron.* 151 (2020) 111914, <https://doi.org/10.1016/j.bios.2019.111914>.
- Z. Altintas, M. Akgun, G. Kokturk, Y. Uludag, A fully automated microfluidic-based electrochemical sensor for real-time bacteria detection, *Biosens. Bioelectron.* 100 (2018) 541–548, <https://doi.org/10.1016/j.bios.2017.09.046>.
- A.I. Robby, S.G. Kim, U.H. Lee, I. In, G. Lee, S.Y. Park, Wireless electrochemical and luminescent detection of bacteria based on surface-coated CsWO₃-immobilized fluorescent carbon dots with photothermal ablation of bacteria, *Chem. Eng. J.* 403 (2021) 126351, <https://doi.org/10.1016/j.cej.2020.126351>.
- S.M. Ozcan, N.C. Sesal, M.K. Şener, A. Koca, An alternative strategy to detect bacterial contamination in milk and water: a newly designed electrochemical biosensor, *Eur. Food Res. Technol.* 246 (2020) 1317–1324, <https://doi.org/10.1007/s00217-020-03491-2>.
- S.S. Zarei, S. Soleimani-Zad, A.A. Ensafi, An impedimetric aptasensor for *Shigella dysenteriae* using a gold nanoparticle-modified glassy carbon electrode, *Microchim. Acta* 185 (2018) 538, <https://doi.org/10.1007/s00604-018-3075-0>.
- R. Das, A. Dhiman, A. Kapil, V. Bansal, T.K. Sharma, Aptamer-mediated colorimetric and electrochemical detection of *Pseudomonas aeruginosa* utilizing peroxidase-mimic activity of gold NanoZyme, *Anal. Bioanal. Chem.* 411 (2019) 1229–1238, <https://doi.org/10.1007/s00216-018-1555-z>.
- H. Yu, W. Guo, X. Lu, H. Xu, Q. Yang, J. Tan, et al., Reduced graphene oxide nanocomposite based electrochemical biosensors for monitoring foodborne pathogenic bacteria: a review, *Food Control* 127 (2021) 108117, <https://doi.org/10.1016/j.foodcont.2021.108117>.
- E.E. Tytzer, A sporozoan found in the peptic glands of the common mouse, *Exp. Biol. Med.* 5 (1907) 12–13, <https://doi.org/10.3181/00379727-5>.
- E.M. Hassan, B. Örmeci, M.C. DeRosa, B.R. Dixon, S.A. Sattar, A. Iqbal, A review of Cryptosporidium spp. and their detection in water, *Water Sci. Technol.* 83 (2021) 1–25, <https://doi.org/10.2166/wst.2020.515>.
- C. Troeger, M. Forouzanfar, P.C. Rao, I. Khalil, A. Brown, R.C. Reiner, et al., Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the Global Burden of Disease Study 2015, *Lancet Infect. Dis.* 17 (2017) 909–948, [https://doi.org/10.1016/S1473-3099\(17\)30276-1](https://doi.org/10.1016/S1473-3099(17)30276-1).
- M.L. Power, N.C. Sangster, M.B. Slade, D.A. Veal, Patterns of Cryptosporidium oocyst shedding by eastern grey kangaroos inhabiting an Australian watershed,

- Appl. Environ. Microbiol. 71 (2005) 6159–6164, <https://doi.org/10.1128/AEM.71.10.6159-6164.2005>.
- [47] Environmental Protection Agency, USEPA Method 1623.1: Cryptosporidium and Giardia in Water by Filtration/IMS/FA, EPA-816-R-12-001, 2012a, Office of Water, Washington DC, 2012, pp. 1–61.
- [48] Expedited approval of alternative test procedures for the analysis of contaminants under the safe drinking water act; analysis and sampling procedures, in: <https://www.federalregister.gov/documents/2012/06/28/2012-15727/expedited-approval-of-alternative-test-procedures-for-the-analysis-of-contaminants-under-the-safe>, 2012 (Accessed 19 June 2021).
- [49] S. Jain, T.G.C. Melo, S.S. Dolabella, J. Liu, Current and emerging tools for detecting protozoan cysts and oocysts in water, TrAC Trends Anal. Chem. 121 (2019) 115695, <https://doi.org/10.1016/j.trac.2019.115695>.
- [50] A. Fall, R.C.A. Thompson, R.P. Hobbs, U. Morgan-Ryan, Morphology is not a reliable tool for delineating species within Cryptosporidium, J. Parasitol. 89 (2003) 399–402, [https://doi.org/10.1645/0022-3395\(2003\)089\[0399:MINART\]2.0.CO;2](https://doi.org/10.1645/0022-3395(2003)089[0399:MINART]2.0.CO;2).
- [51] F. Lora-Suarez, R. Rivera, J. Triviño-Valencia, J.E. Gomez-Marin, Detection of protozoa in water samples by formalin/ether concentration method, Water Res. 100 (2016) 377–381, <https://doi.org/10.1016/j.watres.2016.05.038>.
- [52] G. Luka, E. Samiei, S. Dehghani, T. Johnson, H. Najjaran, M. Hoorfar, Label-free capacitive biosensor for detection of Cryptosporidium, Sensors 19 (2019) 258, <https://doi.org/10.3390/s19020258>.
- [53] E.M. Hassan, B.R. Dixon, S.A. Sattar, A. Stalker, B. Örmeci, M.C. DeRosa, Highly sensitive magnetic-microparticle-based aptasensor for Cryptosporidium parvum oocyst detection in river water and wastewater: effect of truncation on aptamer affinity, Talanta 222 (2021) 121618, <https://doi.org/10.1016/j.talanta.2020.121618>.
- [54] A. Iqbal, J. Liu, B. Dixon, B. Zargar, S.A. Sattar, Development and application of DNA-aptamer-coupled magnetic beads and aptasensors for the detection of Cryptosporidium parvum oocysts in drinking and recreational water resources, Can. J. Microbiol. 65 (2019) 851–857, <https://doi.org/10.1139/cjm-2019-0153>.
- [55] R.D. Adam, Biology of Giardia lamblia, Clin. Microbiol. Rev. 14 (2001) 447–475, <https://doi.org/10.1128/CMR.14.3.447-475.2001>.
- [56] H. Ilkhani, H. Zhang, A. Zhou, A novel three-dimensional microTAS chip for ultra-selective single base mismatched Cryptosporidium DNA biosensor, Sensor. Actuator. B Chem. 282 (2019) 675–683, <https://doi.org/10.1016/j.snb.2018.11.120>.
- [57] E. Roy, S.K. Maity, S. Patra, R. Madhuri, P.K. Sharma, A metronidazole-probe sensor based on imprinted biocompatible nanofilm for rapid and sensitive detection of anaerobic protozoan, RSC Adv. 4 (2014) 32881, <https://doi.org/10.1039/C4RA04868G>.
- [58] Z.P. Aguilar, I. Fritsch, Immobilized enzyme-linked DNA-hybridization assay with electrochemical detection for Cryptosporidium parvum hsp70 mRNA, Anal. Chem. 75 (2003) 3890–3897, <https://doi.org/10.1021/ac026211z>.
- [59] O. Laczka, L. Skillman, W.G. Ditcham, B. Hamdorf, D.K.Y. Wong, P. Bergquist, et al., Application of an ELISA-type screen printed electrode-based potentiometric assay to the detection of Cryptosporidium parvum oocysts, J. Microbiol. Methods 95 (2013) 182–185, <https://doi.org/10.1016/j.mimet.2013.08.014>.
- [60] B.V. Ribeiro, T.A.R. Cordeiro, G.R. Oliveira e Freitas, L.F. Ferreira, D.L. Franco, Biosensors for the detection of respiratory viruses: a review, Talanta Open 2 (2020) 100007, <https://doi.org/10.1016/j.talo.2020.100007>.
- [61] J.M. Claverie, Viruses take center stage in cellular evolution, Genome Biol. 7 (2006) 110, <https://doi.org/10.1186/gb-2006-7-6-110>.
- [62] K. Mao, H. Zhang, Z. Yang, Can a paper-based device trace COVID-19 sources with wastewater-based epidemiology? Environ. Sci. Technol. 54 (2020) 3733–3735, <https://doi.org/10.1021/acs.est.0c01174>.
- [63] D.B. Rein, G.A. Stevens, J. Theaker, J.S. Wittenborn, S.T. Wiersma, The global burden of hepatitis E virus genotypes 1 and 2, Hepatology 55 (2012) 988–997, <https://doi.org/10.1002/hep.25505>, 2005.
- [64] H. Fenaux, M. Chassaing, S. Berger, C. Gantzer, I. Bertrand, E. Schvoerer, Transmission of hepatitis E virus by water: an issue still pending in industrialized countries, Water Res. 151 (2019) 144–157, <https://doi.org/10.1016/j.watres.2018.12.014>.
- [65] A. Goel, R. Aggarwal, Hepatitis E: epidemiology, clinical course, prevention, and treatment, Gastroenterol. Clin. N. Am. 49 (2020) 315–330, <https://doi.org/10.1016/j.gtc.2020.01.011>.
- [66] R.F. Franco, R.M. Montenegro, A.B.M.P. Machado, F. de Paris, D.S. Menezes, R. C. Manfro, Evaluation of diagnostic tests for cytomegalovirus active infection in renal transplant recipients, Brazilian J. Nephrol. 39 (2017) 46–54, <https://doi.org/10.5935/0101-2800.20170008>.
- [67] F. Zhao, Y. Bai, L. Cao, G. Han, C. Fang, S. Wei, et al., New electrochemical DNA sensor based on nanoflowers of Cu₃(PO₄)₂-BSA-GO for hepatitis B virus DNA detection, J. Electroanal. Chem. 867 (2020) 114184, <https://doi.org/10.1016/j.jelechem.2020.114184>.
- [68] A. Erdem, D. Ozkanarikoşsal, H. Karadeniz, P. Kara, A. Sengonul, A. Sayiner, et al., Electrochemical genomagnetic assay for the detection of hepatitis B virus DNA in polymerase chain reaction amplicons by using disposable sensor technology, Electrochem. Commun. 7 (2005) 815–820, <https://doi.org/10.1016/j.elecom.2005.05.006>.
- [69] C. Srisomwat, P. Teengam, N. Chuaypen, P. Tangkijvanich, T. Vilaivan, O. Chailapakul, Pop-up paper electrochemical device for label-free hepatitis B virus DNA detection, Sensor. Actuator. B Chem. 316 (2020) 128077, <https://doi.org/10.1016/j.snb.2020.128077>.
- [70] M. Manzano, S. Viezzi, S. Mazerat, R.S. Marks, J. Vidic, Rapid and label-free electrochemical DNA biosensor for detecting hepatitis A virus, Biosens. Bioelectron. 100 (2018) 89–95, <https://doi.org/10.1016/j.bios.2017.08.043>.
- [71] M.Z.H. Khan, M.R. Hasan, S.I. Hossain, M.S. Ahommed, M. Daizy, Ultrasensitive detection of pathogenic viruses with electrochemical biosensor: state of the art, Biosens. Bioelectron. 166 (2020) 112431, <https://doi.org/10.1016/j.bios.2020.112431>.
- [72] C.M.A.P. Franz, H.M.W. den Besten, C. Böhnlein, M. Gareis, M.H. Zwietering, V. Fusco, Reprint of: microbial food safety in the 21st century: emerging challenges and foodborne pathogenic bacteria, Trends Food Sci. Technol. 84 (2019) 34–37, <https://doi.org/10.1016/j.tifs.2019.01.009>.
- [73] Y.C. Shieh, T.L. Cromeans, M.D. Sobsey, VIRUSES | hepatitis viruses transmitted by food, water, and environment, in: C.A. Batt, M.L.B.T. E Tortorello (Eds.), Food Microbiol., Elsevier, Oxford, 2014, pp. 738–744, <https://doi.org/10.1016/B978-0-12-384730-0.00350-5>.
- [74] H.O. Kaya, A.E. Cetin, M. Azimzadeh, S.N. Topkaya, Pathogen detection with electrochemical biosensors: advantages, challenges and future perspectives, J. Electroanal. Chem. 882 (2021) 114989, <https://doi.org/10.1016/j.jelechem.2021.114989>.