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Vibrio cholerae detection: Traditional assays, novel diagnostic techniques and biosensors

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ABSTRACT

At present cholera remains a major public health problem. It is an acute worldwide diarrheal disease caused by the bacterium *Vibrio cholerae*. It affects in particular developing countries where people get infected by ingesting contaminated water and food. In this review, both internationally recognized conventional methods and new technologies in development for *V. cholerae* detection are reviewed. Currently, culture methods are the "gold standard" in cholera detection. Molecular techniques have a better sensitivity, but they require specific and expensive equipment. There is a need for fast, reliable, easy to use, sensitive, specific systems to use in the field during outbreaks as well as in the laboratory. Several prototype devices have been developed, but their sensitivity and specificity are low. To overcome these problems biosensors could become an attractive and efficient alternative, but they still have to be subjected to some modifications and improvements for their use in the field.

Contents

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1. Introduction

1.1. History in a nutshell

Cholera has been reported as far back as the 5th century BC when it was described in Sanskrit. It is well known that cholera spread out from the Indian continent over the centuries and there has been at least six cholera pandemics between 1817 and 1923. Another cholera pandemic began in Indonesia in 1961 and spread through Asia, Africa, Europe, and Latin America. Physician John Snow hypothesized, around 1849–1854, on the transmission of cholera via contaminated drinking water. In 1854, Filippo Pacini, an Italian anatomist, was the first to observe under a microscope the typical comma-shape form in cholera stool, and in 1884 Robert Koch isolated the bacterium in Egypt and India. In 1905 a new biotype, El Tor, was isolated in Egypt [1]. In 1992 the cholera epidemic was caused by yet another serotype, *Vibrio cholerae* O139 [2]. Currently cholera is endemic in Africa, South and East Asia, but sporadic cases have been observed also in other parts of the world, such as Haiti where it was absent prior to 2010 [3]. World Health Organization (WHO) estimates that there are 3–5 million cholera cases per year worldwide [4, 5] including more than 7,500 deaths in 2010.

1.2. Etiological agent

Cholera is an acute diarrheal disease caused by the Gram-negative, facultative anaerobe, bacterium *V. cholerae* [1, 6]. It is a member of the Vibrionaceae family and is classified into more than 200 serogroups based on the lipopolysaccharide O antigen, but only serotypes O1 and 0139 cause disease in humans [7, 8]. Serotype O1 is classified into two biotypes: classical and El Tor [1]. It includes also two major serotypes, Ogawa and Inaba. The other serogroups, termed non-O1/non-O139, have been associated with sporadic cases of diarrhea or extra-intestinal infection, but their pathogenesis has not been fully understood [9–12]. Cholera is best described as a waterborne pathogen, found in particular in coastal waters and estuaries.

1.3. Pathogenesis

People get infected by ingesting contaminated water or food. The majority of *V. cholerae* ingested are killed by gastric acid. The surviving organisms colonize the small intestine produce cholera toxin [1], the main virulence factor of the pathogenic strains, as well as, express the toxin co-regulated pilus [13], the second major virulence factor. Cholera toxin is a protein exotoxin composed by one A subunit and a B subunit pentamer. The B subunit binds the GM1 ganglioside on eukaryotic cells, thus enabling the A subunit to be subsequently translocated intracellularly.

1.4. Epidemiology

Cholera spreads rapidly due to its secretory nature and has a big epidemic potential. Epidemics are especially common in less developed areas where hygiene levels are lower with limited access to fresh, clean water and medical assistance. There is also a well-known and direct correlation of the appearance of cholera with the proliferation of phytoplankton and zooplankton, or existence of natural disasters, such as cyclones and earthquakes [14–16]. The transmission path can be from person-to-person or from environment or food-to-person. It has been observed that when *V. cholerae* originates from a human it is hyperinfective, with strength modulated by concentration of cholera in stool, it's presence as planktonic cells or stool aggregates, and the presence of lytic bacteriophage in stool or water. The infectious dose of *V. cholerae* O1 has been estimated to be 10^5 – 10^8 Vibrio cells in human, but may be as low as 10^3 in presence of achlorhydria [17]. The incubation period ranges from less than one day to 5 days [6, 18]. There are oral killed vaccines commercially available for *V. cholerae* O1 and O139, but they provide a limited 60–85% protective efficacy for 2–3 years and they are not yet part of cholera control programs outside of Vietnam [4, 19–21].

1.5. Diagnosis

Cholera is characterized by a fulminant disease course with severe secretory "rice water" diarrhea resulting in rapid dehydration and if not treated can result in death in 12- 24 hours post infection [1, 22]. Clinical manifestations are usually vomiting, diarrhea, abdominal discomfort, cramping, lethargy, sunken eyes, dry mouth, cold clammy skin, decreased skin turgor, wrinkled hands and feet [3, 23]. Treatment relies on rehydration, by replacing lost fluid and electrolytes, intravenous fluids, and occasionally antibiotics and zinc supplements [24]. Success of treatment relies mainly on timely diagnosis and rehydration. Delays in its diagnostic results in higher incidence and fatality rates. The use of antibiotics is an adjunctive therapy that may contribute to increase in antimicrobial resistance, but will help reduce the duration of diarrhea.

1.6. Why is monitoring needed?

Cholera diagnostics plays a pivotal part in the management of cholera disease. Indeed its severity and endemic nature are well known. If the disease is not treated properly, the mortality rate is very high (50–60%) and represents an enormous burden to the affected countries. It was estimated that from 2009 to 2010 the number of cases and deaths due to cholera has increased by about 50% [25]. If we take a look at the case of Haiti, the time occurred between the onset of symptoms and the death cases was only 12 hours [22]. This means that it is necessary to conceive a rapid and specific confirmation detection test as physicians usually can diagnose the disease fairly easily. Also useful would be the serotype of the etiological agent. For outbreak management it would be useful to have accurate, reproducible, and reliable answers to save time, improving turnaround times, decreasing costs and having a response in the event of a suspected outbreak, resulting in overall public health saving within the country [26]. Cholera diagnostics strongly relies on clinical evaluation and epidemiological factors that are still considered the most important initial diagnostic tools when approaching a suspected cholera case. Based on World Health Organization (WHO) [27] recommendations, cholera should be suspected when a patient aged 5 years or more develops severe dehydration or dies from acute watery diarrhea (both in endemic and non-endemic areas) or a patient in an endemic area, aged 2 years or more develops acute watery diarrhea [6, 28]. Clinical diagnostic while necessary is not enough to confirm cholera. Rapid tests, even though their use is limited because of their limited sensitivity (93–98%) and specificity (67–96%), can help in the setting of an outbreak of acute watery diarrhea to confirm if it is due to cholera or not. In general, if more than 10 people test positive for cholera, then a cholera outbreak is

Table 1
Summary of cholera biosensors

BIOSENSOR	TECHNOLOGY	TARGET	SENSITIVITY	PUBLICATIONS
Electrochemical	NALFI	DNA	5 ng	[101]
	NALFB	*ctxA* genomic DNA	1 pg	[97, 102]
		ctxA synthetic DNA	0.3 ng	
		ctxA	10 CFU/mL	
	Gold electrode in a capacitative flow cell	CT	1×10^{-14} M	[88]
	Nanocoxial-based electrochemical sensor	CT	2 ng/mL	[89]
	RTCA	CT	7 pg/mL (CT diluted in buffer)	[92]
			0.11 ng/mL CT diluted in stool specimens	
	Amperometric immunosensor	bacteria	1×10^{5} CFU/mL	[104]
		antibodies	50 ng/mL	[111, 112]
	Microcantilever surface	bacteria	1×10^{3} CFU/mL	[103]
	SPE with gold nanoparticles/	DNA (PCR products)	1 fM	[100]
	latex microsphere	DNA (LAMP products)	50 ng/mL	
	mLATE-PCR-coupled multiplex	wbeM	0.4 pg O1	[98]
	electrochemical genosensor	wbfR	0.5 pg O139	
		ctxA	~10 CFU/mL	
Optical	CdSe-Zn core-shell QDs	CT	10 ng/mL	[87]
	SPR	CT	1×10^{-11} M	[88]
		antibodies	4 pg/mL	[120]
	Optical fiber	antibodies IgG	1:13,107,200	[106]
			75 pg/mL	
		antibodies IgA	1:26,214,400	
		antibodies IgA	1:1,310,720 (luminol-coating buffer)	[107]
			1:2,621,440 (air)	
		antibodies	0.2 µg/mL (Pyrrol-biotin film)	[109]
	SERS (Surface enhanced Raman scattering)	CTB	1100 ng	[93]
	DLS (Dynamic light scattering light)	CTB	10 nM	[95]
	Fluoroimmunoassay	CT	1 nM	[94]
	Chemiluminescence transduction	antibodies	40 ng/mL	[115]
Electrochemical + optical	Fluorescence microfluidic detection	antibodies	6.6 ng/mL	[123]
	Electrochemical microfluidic detection		1 ng/mL	
	photoelectrochemical		0.5 µg/mL	[114]
	Optical fiber with electropolymerized film		1:1,200,000	[110]

confirmed [29]. The importance of monitoring cholera, for diagnostics or epidemiological reasons, is due to the fact that it is still an endemic scourge or pandemic in developing countries.

1.7. Future needs in cholera monitoring

Cholera is a historically important disease of an international nature that requires solutions, cooperation and assistance during epidemic outbreak investigation and expedited control from the international community [26]. Unfortunately current standards of care are insufficient, and ineffective [30], as they can only reduce the mortality down to less than 0.2% [1]. During an outbreak, medical personnel has to manage many patients at the same time, so it is necessary that a rapid, reliable, specific, and sensitive monitoring technology be at hand at the onset of the epidemic. In the last years, a rise in novel diagnostics brings rapid and accurate cholera biosensor devices to the field (Table 1, Table 2), even though early detection of cholera outbreaks still remains a challenge [26]. This review paper focuses on *V. cholerae* standard and novel diagnostic tools with a particular interest in biosensors.

2. Discussion

2.1. Standard diagnostics

2.1.1. Culture

Today, the gold standard of cholera diagnostics in outbreak management is still isolation of bacteria from stool samples on selective media followed by biochemical identification and serotyping with monoclonal antibodies [31]. WHO recommends the stool samples be transported to the laboratory preferably in Cary-Blair [32, 33] or other suitable media, such as alkaline peptone water (APW), BBL CultureSwab, Bile peptone, etc. to maintain the viability of *V. cholerae* [34], and it should be stored at 4°C if the specimens will be received by the laboratory within 48 hours. Stool samples are then inoculated onto selective media such as Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar [32, 35], taking approximately 8 days to confirm a cholera case [36]. Multiple steps are needed like enrichment media, such as APW, and incubation at 35–37°C for 18–24 hours (it is advisable to use more than one medium as that may allow convergence of the best results [37]). Then after incubation, yellow shiny colonies, 2 to 4 mm in diameter, on TCBS could be considered *V. cholerae* [38], and these suspicious cholera colonies are confirmed using biochemical tests, followed by serogroup confirmation, and determination of antibiotic resistance [39–43] or by PCR using primers developed to target O1 and O139 coding regions of the genomic DNA [37].

2.1.2. Microscopy

Early detection of *V. cholerae* is possible by dark field microscopic investigation of fresh human stool samples under 400x magnification. *V. cholerae* are seen as rod shaped, with a single polar flagellum and a typical shooting star motility [44].

2.2. DNA-based methods

Molecular characterization has become an essential support to *V. cholerae* phenotypic identification and its epidemiological investigation [45, 46]. Molecular diagnostics must at least identify nucleic acid sequences encoding both major virulence factors, cholera toxin (*ctxAB* genes) and toxin-coregulated pilus (*tcpA* gene) [26].

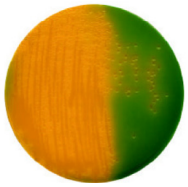
2.2.1. Amplification techniques

Nucleic acid amplification (PCR) is a well-established assay and continues to be widely used. Many authors developed conventional PCR for *V. cholerae* detection [47–50]. The *ctxA* gene, encoding the A subunit of cholera toxin, is the preferred target [51–54]. A multiplex

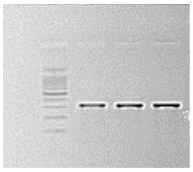
Table 2
Sample of conventional techniques and biosensors. **(a)** *V. cholerae* colonies on TCBS plate (Merck), ©Merck; **(b)** A representative agarose gel electrophoresis showing the amplification of *V. cholerae* amplicons. The size of the target amplicon corresponds to 333 bp, ©F. Cecchini; **(c)** *V. cholerae* biochemical test identification; **(d)** ELISA microplate, ©F. Cecchini; **(e)** Lateral flow by BioAssay Works, ©BioAssay Works; **(f-a)** black light-tight box connected to a computer: (**f1**) tube containing test sample, (**f2**) optical fiber that transmits the light from the bio-element to the Photo Multiplier Tube (PMT), (**f3**) fiber holder, (**f4**) case containing PMT; **(f5)** manual shutter to prevent light entry to the PMT when the door is opened; **(f6)** door**; (f-b)** structure of optical fiber and different kind of capture element that can be attached to the silica surface, ©Biosensorix Pte Ltd, Singapore; **(g)** Electrochemical lateral flow biosensor. The strip is made of a membrane that provides a solid support and enables the capillary flow along the strip; the conjugation pad is placed over and in contact with the membrane at one end of the strip and contains formulation of the adsorbed dehydrated labelled conjugate particles coated or filled with the electrochemically active component; the sampling pad provides adsorption of the sample; the screen-printed electrode is coated with the immobilized capture antibodies; the wick pad is placed on top and in contact with the SPE and provides absorption of excess reagents maintaining a lateral flow along the strip, ©Biosensorix Pte Ltd, Singapore; **(h)** SPR scheme: is a resonant oscillation of electrons between a negative and positive permitivity material stimulated by incident light, ©Ibrahim Abdulhalim; **(i)** Schematic drawing of the resonance frequency measurement by optical leverage method of atomic force microscope (AFM), ©Elsevier; **(l)** Nanocoxial array scheme: ELISA rationale in microtiter will have to be placed in nanocoxial array to make it one day a biosensor, CT: anti-cholera toxin, ALP: alkaline phosphatase secondary antibody, ©Elsevier.

CONVENTIONAL TECHNIQUES

(a) Culture method [126]



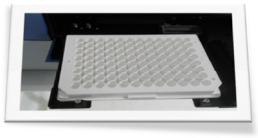
(b) Gel electrophoresis



(e) Lateral flow [128]

(c) Biochemical test identification [127]

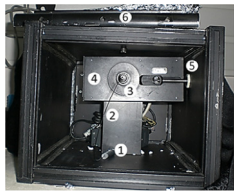
(d) ELISA



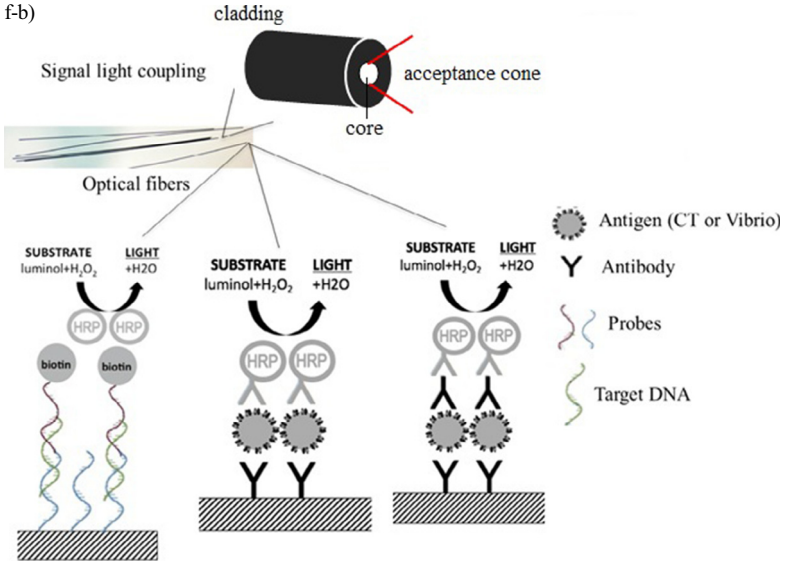
BIOSENSORS

(f) Optical fibers

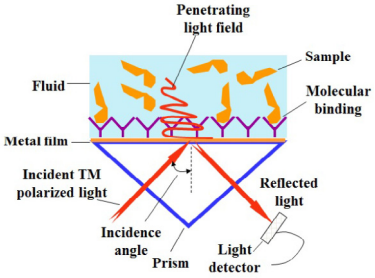
f-a)



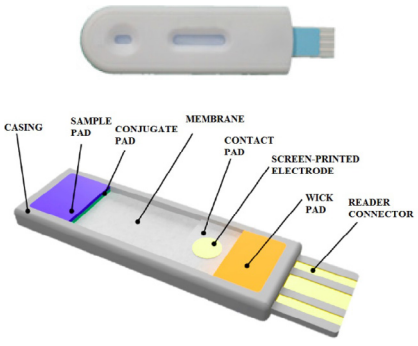
f-b)



(h) SPR

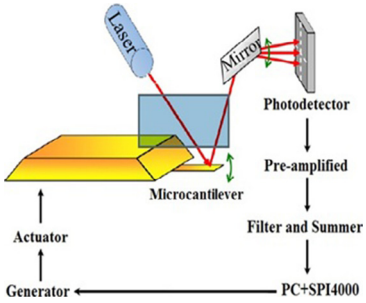
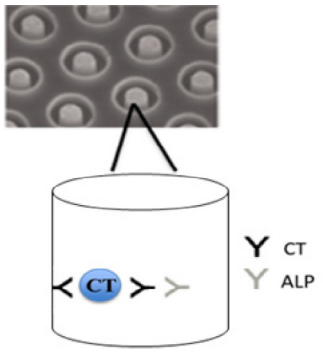


(g) Electrolateral flow [129]



(*continued on next page*)

Table 2 (*continued*)

BIOSENSORS	
(i) Microcantilever-based biosensor [103]	**(l) Nanocoxial array [89]**
	

PCR was developed by Keasler and Hall [52], enabling to detect simultaneously cholera toxin and toxin-coregulated pilus (TCP), and by Hoshino et al. [55] for the specific detection of O1 and O139 lipopolysaccharide (LPS). Lipp et al. [56] reported a high sensitivity multiplex end-point PCR analysis detecting 0.4 cells/mL after concentrating coastal waters and plankton samples.

An alternative PCR platform is Real Time PCR. Koskela et al. [57] optimized a Taq-man PCR detecting simultaneously cholera toxin gene, *tcpA* gene, and *toxR*. Their detection limit was 100 fg per reaction, corresponding to about 20 bacterial cells from purified *V. cholerae* DNA. Lyon [58] amplified the non-classical specific hemolysin (*hylA*) gene of *V. cholerae* O1, O139, and non-O1/O139. He reached 7.3 CFU per reaction as the lowest detection limit. Another quantitative TaqMan assay was developed for the detection of the *ctxA* gene with a sensitivity of <10 CFU per reaction by Blackstone et al. [59], and Gubala [7, 60] who reached 5 CFU per reaction as the lowest detection limit. Although the use of PCR to detect specific cholera genes is not validated as a gold standard, it does have the ability to detect low numbers of organisms [25]. At present in the market there are some commercial kits for PCR and real-time PCR including the DNA extraction. The sensitivity ranges between 10 to 10^2 CFU/mL (MolBio™ Himedia and Norgen) and from 10 to 10^3 copies/mL (Liferiver, Genesig, Techne, Qiagen) of DNA target respectively. The amplification product size ranges from 333 bp to 779 bp based on the kit. A quadruplex real-time PCR assay targeting four genes, cholera toxin gene (ctxA), hemolysin gene (hlyA), O1-specific rfb, and O139-specific rfb, was developed by Huang et al. [61]. The analytical sensitivity for detection of toxigenic *V. cholerae* O1 and O139 was 2 CFU per reaction, with cells from pure culture and 10 CFU/mL with inoculated water.

2.2.2. Genotyping techniques

For an accurate epidemiological investigation of toxigenic *V. cholerae*, genotyping techniques are useful [62–64], in particular when they are standardized and internationally validated for interlaboratory comparison of subtyping data. An example is pulsed-field gel electrophoresis (PFGE) performed according to methods standardized by the PulseNet International [65], supported by other subtyping methods such as PCR-based profiling methods, and DNA sequencing [66–68]. Bhattacharya et al. [69] using PFGE analysis discovered a novel mutation in *ctxB* in strains isolated from 2010–2014 during various outbreaks in India. They identified two main groups. The first one first one included strains isolated from 2010 and 2011 showing the classical *ctxB*. The second group represented a new Haitian variant that included all strains, isolated from 2012 to 2014. Another genotyping technique was developed by Awasthi et al. [70]. They reported an optimized PCR-RFLP assay able to differentiate three *chxA* variants, typically present in *V. cholerae* non-O1/non-O139, and they observed the presence of *chxA* gene also in *V. cholerae* O1 strains.

2.3. Novel microscopy techniques

Four microscopy culture-independent methods to evaluate the presence of *V. cholerae* were developed: fluorescent antibody coupled with direct viable count (FA-DVC), fluorescence *in situ* hybridization (FISH), indirect fluorescent antibody (IFA), and on-chip optofluidic system. FA-DVC [71] is a two-step method, cells are visualized under an epifluorescent microscope. The cells positive to DFA-DVC can be confirmed by PCR. By using FISH technique it is possible to detect serogroups O1 and O139 using a fluorescently-labeled oligonucleotide probe under epifluorescence or confocal laser scanning microscopy [37]. The IFA method is able to detect *V. cholerae* serogroup O1 by using antiserum specific for O1 somatic antigen, a method useful for detecting organisms in samples which gave negative results by culture [72]. Lastly, Liu et al. [73] described an innovative on-chip optofluidic imaging system to measure the biophysical features of *V. cholerae* considering their size (1.21 μm x 0.43 μm), shapes and refractive indices (1.365) from treated water sources. The optofluidic system was placed on an inverted microscope in phase contrast using a 60X objective lens and a digital CCD camera.

2.4. Rapid diagnostic tests (RDTs)

Since cholera frequently affects countries with limited laboratory infrastructure, rapid diagnostic tests (RDTs) represent an important resource to detect cholera in the field. An extensive review of the literature identified 24 rapid diagnostic tests (RDTs) developed for cholera since 1990 [74]. Most of the rapid tests are based on the use of monoclonal antibodies for *V. cholerae* O1 and O139 lipopolysaccharide detection and differentiation. Although their sensitivity (93–98%) and specificity (67–96%) are low [31], they can be sufficient to provide a clear indication of whether the outbreak of acute watery diarrhea is due to cholera [29]. Most of them capture a characteristic component of the bacterium on a solid surface producing a visual change.

One of the most recent cholera RDTs on the market is the Crystal CV®RDT (Span Diagnostic Ltd, Surat, India). It is a dipstick able to detect *V. cholerae* lipopolysaccharide (LPS) by using monoclonal antibodies in clinical and environmental samples. It is a one-step assay, based on vertical-flow immunochromatography principle and gold particles antibodies-conjugated [75]. The test shown sensitivity (up to 95.9%) suffers from low specificity (60–70%).The minimum detectable limit was 10^6 CFU/mL for *V. cholerae* O1 and 10^7 CFU/mL of *V. cholerae* O139. Page et al [25] observed an increase in specificity

up to 88.6%, when PCR was combined with culture results. It was verified that 24 hours of enrichment in APW further increases significantly the sensitivity [76, 77], while 6 hours allowed only a marginal increment [78]. All these studies demonstrated that this test can be valid for early outbreak detection or epidemiological studies even though it has some drawbacks such as the inability to detect viable but non-culturable (VBNC) bacteria [79], to differentiate toxigenic from non-toxigenic strains, and it requires filtration that is difficult to perform in some cholera endemic areas. Another five RDTs were evaluated under field conditions (COAT, IP cholera dipstick, IP dipstick, Medicos, and SMART), but they showed low specificity and sensitivity (some less than 90%) [74].

2.5. Biosensors

Current diagnostic methods are frequently faced with challenges such as limitations in specificity and sensitivity, a long turnaround time to receive results, and a high cost of equipment or need of specialization [80]. An attractive alternative to existing methods to overcome these limitations is represented by biosensors that can allow the creation of highly sensitive, low-cost devices for routine use, accurate, simple and affordable for the population for which they are intended, providing also qualitative, quantitative or semi-quantitative results in near-real time to institute effective treatment as they can be used directly in the field. Moreover, in the future, biosensors may be modulated to be part of an automated system, with either micro fabrication or miniaturization potential, allowing the construction of simple and portable equipment for fast analysis [81]. Indeed the goal of biosensor research is to provide an answer in reducing the time laps while preserving the precision and accuracy of laboratory analysis [82]. IUPAC defines a biosensor as a self-contained integrated device able to provide specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor) intimately immobilized to a transduction element [83]. Biosensors can be grouped based on the signal transduction and their bio-recognition elements. Indeed, biosensors involve bio-molecular receptors (usually antibodies, antigen, oligonucleotides or enzymes) immobilized via some chemical technique (covalent, physicochemical sorption, entrapment or other), and a transducer (optoelectronic, piezoelectric, amperometric or other) that converts the physical, chemical or biological signal, resulting from the interaction between the analyte and the bio-receptor into an electronic one. The signal is proportional to the measurement [84]. An electrochemical transducer is a device, which uses a chemical change to measure the input parameter; the output is a varying electrical signal proportional to the measurement. The acoustic transducer is an electrical device that converts sound wave vibrations into mechanical or electrical energy, and once a change in the surface occurs by the addition of a target analyte, then a measurable change is recorded. Optical transduction methods are based on the detection of changes in properties of light resulting from the analyte binding to the sensor surface [85]. Regarding cholera, biosensors developed until now are mainly electrochemical and optical.

2.5.1. Detection of cholera toxin (CT)

Cholera toxin (CT) is a key biomarker of *V. cholerae* whose detection is increasingly important because existing serological methods may not be able to detect any newly emerging serogroup. The lethal dose of CT in humans is relatively low (LD_{50} ~250 µg/kg) while the toxin targets the intestine, eventually being attached to it (the CTB part) and thus reducing the amount to be detected within stools unless shed with the intestinal cells [86]. Thus high sensitivity would be needed [80], as well as high specificity from anti-cholera toxin B subunit derived peptide antibodies which is required so as to avoid cross-reaction to other related toxins such as Shiga toxin, or staphylococcol enterotoxin.

Goldman et al. [87] developed a multiplexed fluoroimmunoassay using highly luminescent semiconductor nanocrystals (CdSe-Zn core-shell Quantum Dots-(QDs)) for single and simultaneous detection of CT, ricin, shiga-like toxin 1, and staphylococcal enterotoxin B. The selected emission for each toxin was 510, 555, 590, and 610 nm respectively. The CdSe-ZnS QDs were conjugated to a purification tool protein (MBP-zb) and to the antitoxin antibodies using an adaptor protein (PG-zb) as a bridge between the QDs and the antibodies. With the single-analyte assay the lowest detected CT level (LDL) at 510 nm was 10 ng/mL. QD-antibody reagent, in addition to antibody cross-reactivity, has another limitation such as nonspecific interaction. To overcome this drawback they added an excess of antibody so the majority of PG-zb that was not available to stick directly to the capture antibody.

A gold electrode, coated with monoclonal antibodies against CTB, was used by Labib et al [88] in a capacitive flow cell. Potential pulses of 50 mV at 50 Hz were transmitted through the flow cell and the capacitance value decreased when CTB bound the anti-CTB on the electrode. CT concentrations were tested in the range from $1x10^{-18}$ to $1x10^{-8}$ M, with the LOD found at $1x10^{-14}$ M. These results were compared in the same study to ELISA and surface plasmon resonance (SPR), with the LOD found at $1.2x10^{-12}$ M and $1x10^{-11}$ M respectively. This flow-injection capacitive immunosensor system proved to be more sensitive than the other two techniques tested.

Archibal et al. [89] developed a nanocoaxial-based electrochemical sensor to detect cholera toxin using an electrochemical readout and a miniaturized platform. They combined electrochemical enzyme-linked immunoassorbent assay (ELISA) and differential pulse voltammetry (DPV) or square wave voltammetry (SWV). The linear dynamic range of detection was 10 ng/mL -1 µg/mL, and the limit of detection (LOD) was 2 ng/mL. These were almost comparable results to the standard ELISA (LOD = 1 – 0.49 ng/mL) [90, 91]. This device is a potential POC candidate, but some modifications are necessary such as the immobilization of the capture antibody directly onto the coaxial electrode surface.

Another method was developed by Jin at al. [92]. The assay was tested both on isolates and spiked stool specimens by dynamic monitoring of the full production of the CT in a real-time cell analysis (RTCA). The RTCA uses an electric impedance sensor for dynamic real-time monitoring of the status of cells in response to either physiological or pathological elements. In this study they used the RTCA system for detecting and identifying CT. They compared their method with Real-Time PCR and Reverse passive latex agglutination (VET-RPLA). The sensitivity and specificity of the method testing isolates were 97.2 % and 100 % respectively, while 90% and 97.2 % when testing directly stool specimens. Based on their results the total sensitivity was lower than real time PCR (100%), but higher than VET-RPLA (71.6 %), while interestingly, the specificity was higher than Real time PCR (85.3%) and a bit lower than VET-RPLA (99.5 %). The advantage of this technique is that it works also on stool specimens without the need for strain isolation. The result can be achieved within 2 hours after inoculation. The detection limit was 7 pg/mL for toxin diluted in buffer, and 0.11 ng/mL for toxin diluted in stool specimens.

Surface enhanced Raman scattering (SERS) was used by Schmit et al. [93] to set up a novel sandwich assay comprising buoyant silica microspheres coated with antibodies and gold nanoparticles with a Raman reporter for the detection of the β subunit of CT. The detection limit for this technique was 1,100 ng. Singh et al [94] exploited the capability of cholera toxin to bind GM1 gangliosides developing a sensitive fluoroimmunoassay. Gangliosides are natural receptors for bacterial toxins included in liposomes. To induce in liposomes the capacity to generate a signal, fluorophore-labeled lipids were incorporated into the bilayer of liposomes. In this way a monoclonal antibody captures the toxin and then this complex is captured by ganglioside-bearing fluorescent liposomes. Using this system Singh et al were able to detect 1 nM of toxin. Schofield et al. developed a

colorimetric bioassay with a specifically synthesized lactose derivative, self-assembled onto gold nanoparticles. When cholera toxin (added as the B-subunit) (CTB) binds to the lactose derivative, it induces aggregation of the nanoparticles. Upon aggregation, the color solution shifts from red to deep purple color. The simple color change of the bioassay provides a selective means to detect and quantify the cholera toxin within 10 min. The theoretical limit of detection of the bioassay was determined to be 54 nM (3 μg/mL) for CTB.

Another colorimetric bioassay was performed by Khan et al [95] using antibody-conjugated gold nanoparticles based on a colorimetric and dynamic light scattering assay (DLS). The system is based on the distance dependent properties of the nanoparticles. When cholera toxin is present, they aggregate one to another and the color of the solution changes from red to purple to grey. With this assay it is possible to visualize the presence of CT at a concentration as low as 10 nM.

Yamasaki et al [96] constructed an immunochromatographic test strip employing polyclonal antibodies against whole toxin which contains both A and B subunit in isolates. The lower CT concentration detectable was 10 ng/mL. The limit of the system is that sometimes CT concentrations in most of the patient stools is lower than this value.

2.5.2. Detection of nucleic acids

In diagnostic research, the development of nucleic acid biosensors is gaining importance. PCR is a well established assay, but it requires instrumentation, skilled personnel and it is time-consuming, requiring agarose gel electrophoresis to visualize the result [97]. From the combination of nucleic acid amplification and portable multiplex electrochemical genosensor a new platform was developed by Yu et al. [98]. In their study they exploited the sensitivity of PCR and the microfabrication capability typical of electrochemical genosensors [99]. The system is based on the use of dry-reagent multiplex linear-after-the-exponential (mLATE)-PCR mix to reduce the risk of contamination and human errors due to pipetting steps. The amplification reaction requires only the addition of sterile water and DNA template. The electrochemical genosensor is able to detect directly the target DNAs without preliminary purification of the amplicons. During amplification the single strand DNA (ssDNA) is tagged with fluorescein label. Then ssDNA amplicons hybridize the thiol-modified capture probe attached to the printed gold electrode biosensors. This platform can be used as diagnostic tool for the detection of *V. cholerae* serogroups O1 and O139 showing a sensitivity and specificity of 100% in spiked stool samples. The limit of detection was 10 CFU/mL, and 0.4 pg and 0.5 pg of genomic DNA for *V. cholerae* O1 and O139, respectively.

Another electrochemical genosensor was developed by Liew et al. [100]. They used lyophilized gold nanoparticles/latex microsphere (AuNPs-PSA-avidin-reporter probe) on an SPE surface for detecting PCR and loop-mediated isothermal amplification (LAMP) products. The detection limit was 1 fM and 50 ng/μL respectively.

Chua et al. [101] developed a nucleic acid lateral flow immunoassay (NALFI) based on the detection of fluorescein in amplification products producing visual red lines. Primers forward and reverse are biotin and fluorescein labeled in 5' end respectively. Capture reagents are coated onto carrier beads and immobilized onto a glass fibre membrane. The accumulation of detector reagents produces a visual signal. The LOD was 5 ng. The major drawback of these devices is that they can give false positive results due to non-specific amplifications [97]. To overcome this limit, Ang et al. [97, 102] developed a two-step nucleic acid lateral flow biosensor (NALFB), sequence-specific cholera-toxin gene (*ctxA*) amplicons, at room temperature in a dry-reagent format. Capture probes were immobilized on the NALFB, binding specifically the target on the strip. The positive signal is achieved by visible red lines on the strip due to conjugated gold nanoparticles to the fluorescein label of the captured amplicons obtained by LATE-PCR. The sensitivity of this method is 1 pg with pure genomic DNA, 0.3 ng with synthetic DNA, and 10 CFU/mL.

2.5.3. Detection of the bacteria

The conventional techniques for detection and identification of bacterial pathogens relies on microscopic examination, plate counting and biochemical identification. These methods are of low cost, but they are time consuming and cannot detect viable but non-culturable forms. Sungkanak et al. [103] used a dynamic force microscopy within an atomic force microscope (AFM) to measure the cantilever's resonance frequency relating to the mass of *V. cholerae* O1 cells bound on the microcantilever surface using monoclonal antibodies. The lowest concentration detectable was $1x10^3$ CFU/mL and a mass sensitivity of ~146.5 pg/Hz. This result was confirmed by scanning electron microscopy. The assay showed a higher sensitivity than standard ELISA ($1x10^5$ CFU/mL) and an amperometric immunosensor ($1x10^5$ CFU/mL) [104]. Unfortunately, the instrumentation required is such that this work remains in the realm of the specialized laboratory.

Recently Chen et al. [105] optimized an immunochromatographic lateral flow device able to differentiate O1 serotypes (Ogawa and Inaba) in 5 minutes. He generated a new monoclonal antibody (McAb) pair, called IXiao3G6 and IXiao1D9, specific against *V. cholerae* O1 serotype Ogawa. The platform consists in an analyte adsorption pad made in a nitrocellulose membrane, and a wicking pad. Two bands appeared, one for the sample and the other for the control, if *V. cholerae* is present. The detection threshold for bacterial culture was $1x10^4$ CFU/mL showing higher sensitivity than most of the other RDTs (10^4 -10^7 CFU/mL).

2.5.4. Detection of cholera antitoxin antibodies

Another strategy for monitoring cholera lies in the detection of anti-cholera toxin immunoglobulins via the elaboration of electrochemical or optical immunosensors based on the immobilization of a pentameric cholera toxin B subunit protein (CTB), as the antigen bioreceptor. The superantigenicity of CTB enables it to capture a large number of types of elicited antibodies. The use of epitope peptides derived from CTB enables to lower cross-reaction with other related biotoxins, while cancelling the need for the isolation of a dangerous biotoxin in the process of making the diagnostic kits.

An optical immunosensor was developed to link covalently by silanization either cholera toxin B subunit or the synthetic peptide CTP3-BSA conjugated derived from it [106]. Sera obtained from humans exposed to live virulent *V. cholerae* O1, were analyzed. The device was a tapered optical fibre loop able to detect as lowest titer of 1:13,107,200 or 75 pg/mL of total IgG in human serum, and 1:26,214,400 for IgA. Two fluorophores were used to label the secondary immunoglobulins allowing the differentiation of both IgG and IgA and their measurement one after the other in the same sample. Another advantage of this device was the possibility to reuse the same loop for over 60 times.

The detection of jejunal cholera antitoxin IgA immunoglobulins was performed by Marks et al. [107] conjugating cholera toxin B subunit with the surface of the fiber and marking the analyte by a secondary antibody labeled with HRP (Horseradish peroxide). The lowest titer detected with this system was 1:1,310,720. A lower titer was achieved replacing the luminol-coating buffer solution by air (1:2,621,440).

Taking into account that the use of polymers aroused widespread attention in the design of biological sensors [108], the fixation of the safe part of this enterotoxin by specific interactions with polymerized films generated onto the sensor surface was privileged. Thus, an optical fiber was modified by a chemically polymerized poly(pyrrole-biotin) film. The latter allowed the successive binding of avidin and biotinylated cholera toxin B subunit through the well-known biotin-avidin affinity interactions (association constant $Ka = 10^{15}\ M^{-1}$). The detection of the target was performed by labeling the anti-cholera toxin antibody bound to the toxin receptor by a secondary antibody conjugated with a horseradish peroxidase. In

presence of luminol and hydrogen peroxide, a chemiluminescence reaction catalyzed by HRP, was achieved leading to a linear calibration curve between 0.3 and 1 μg/mL [109]. Such chemiluminescence optical fiber immunosensor exhibited a detection limit more sensitive than that of conventional ELISA test (0.2 μg/mL instead of 2 μg/mL). In addition, its performance could be further enhanced by improving the quality of the deposited polymer-cholera toxin coating in terms of homogeneity, absence of defects and spatial addressing. Moreover, the reproducibility of the chemical deposition of the biotinylated polymer remains difficult to control. This was attempted by using an electrochemical polymerization, which ensures better homogeneity, the absence of defect and control of film thickness. Silica optical fibers, which are not conductive, were thus modified by a conductive transparent and thin layer of indium tin oxide and then functionalized by electrochemical polymerization of pyrrole-biotin over its tip and end face. Biotinylated cholera toxin was immobilized onto the electropolymerized film via an avidin bridge, and following a similar labeling step by a secondary antibody-peroxidase, the chemiluminescence response of the resulting immunosensor presented a linear range for dilutions of antibody titers from 1:3,000 to 1:1,200,000 [110]. It should be noted that the detection limit was greatly improved from a dilution of 1:300,000 (0.2 μg/mL) for the optical fiber immunosensor based on chemically polymerized film to 1:1,200,000 for the optical fiber immunosensor based on an electropolymerized film.

Considering the attractive advantages of amperometric transduction as its ease of use in turbid samples, portability and low cost, the previous immunosensor configuration was studied on glassy carbon electrodes instead of optical fibers. The quantification of the antibody target was performed by the same labeling step, by adding compounds which are converted catalytically by peroxidase into electroactive species. The latter are amperometrically detected at the electrode surface after diffusion through the polymer coating. Taking into account that poly(pyrrole-biotin) films are hydrophobic and hence poorly permeable in aqueous media, the immobilization of the biotinylated cholera toxin was carried out via the electrochemical copolymerization of pyrrole-biotin and pyrrole-lactobionamide, a highly hydrophilic monomer, to increase the permeability of the resulting biotinylated copolymer. In the presence of hydrogen peroxide, hydroquinone and the HRP marker which is the final step in the immunoassay, the immunosensor was potentiostated at 0.2V vs SCE in order to perform the detection of antibody via the amperometric reduction of the enzymatically generated quinone. The resulting amperometric signal provided a linear calibration curve for anti-cholera toxin antibody over the range 1-200 μg/mL, which was associated with a very sensitive detection limit (50 ng/mL) [111, 112]. It should be noted that this detection limit is more sensitive than those recorded by a conventional spectrometric detection using the ELISA test, namely 100 ng/mL [113].

However, these chemiluminescent and amperometric transductions require an additional labeling step of the antibody target involving incubation and washing steps that drastically increase the response time and cost of the analysis. As a consequence, immunosensors based on a photoelectrochemical transduction of the immunoreaction were designed to quantify directly the target without a labeling step by a secondary antibody. The electrogeneration of a photosensitive biotinylated poly(pyrrole-ruthenium) film on electrode surfaces allowed the anchoring of biotinylated cholera toxin by avidin-biotin interactions. Moreover, the photoelectrochemical properties of the ruthenium film were strongly affected by the immunoreaction. In particular, the irradiation of this modified electrode potentiostated at 0.5V vs SCE, in the presence of pentaaminechlorocobalt(III) chloride as an oxidative quencher, led to a cathodic photocurrent. The anchoring of the antibody generates steric hindrances towards the permeation of the quencher inducing a decrease in the photocurrent intensity and hence a direct detection of the antibody target. The photocurrent decrease varies with increasing antibody concentration until 100 μg/mL and then reached a constant value for higher concentrations, the detection limit being 0.5 μg/mL [114]. Although this label-free immunosensor has attractive direct transduction of the immunological reaction, its sensitivity remains lower to that observed with the electrochemical transduction with enzymatic labeling.

Avidin-biotin interactions were widely used for biotinylated biomolecule immobilization onto biotinylated polymers via avidin-bridge. However, the formation of this additional protein layer may be a disadvantage for the transduction step, and therefore the immunosensor sensitivity. In order to improve the immunosensor performance, the avidin-biotin approach was replaced by an electrochemical photopatterning procedure consisting in the electrogeneration of a poly(pyrrole-benzophenone) film and the subsequent photografting of a cholera toxin by irradiation of the polymerized photoactive benzophenone groups at 345 nm. Such a photoelectrochemical procedure was applied to the biofunctionalization of the end-face of optical fibers by covalent binding of cholera toxin [115–117]. As previously reported, the resulting fiber immunosensors were used with a labeling step of the anti-cholera toxin antibody by a secondary antibody conjugate to a peroxidase. An amperometric or chemiluminescent transduction was reported by changing the substrate (luminol or hydroquinone) of the peroxidase reaction, leading to a detection limit of 40 and 80 ng/mL for the optical and electrochemical transduction, respectively [115]. With the aim to combine the advantages of the suppression of an avidin layer and those of the label-free detection based on photocurrent evolution, immunosensors were made with a copolymer exhibiting photochemical properties and allowing a direct anchoring of cholera toxin. The bifunctional polypyrene film was formed by oxidative electropolymerization of a pyrene-nitrilotriacetic acid (NTA) and a tris(bipyridine) ruthenium(II) complex modified by pyrene groups. After chelation of Cu^{2+} by the polymerized NTA groups, biotinylated cholera toxins were attached by coordination of the biotin groups on the chelated Cu^{2+} center [118]. Under irradiation of the copolymer in presence of ascorbate as a sacrificial donor, the detection principle was based on the photocurrent decrease induced by the binding of the anti-cholera toxin antibody. The resulting immunosensor exhibits a linear calibration curve between 0 and 8 μg/mL with a detection limit of 0.2 μg/mL [119].

More recently, the same immobilization procedure of cholera toxin was employed for the functionalization of a graphene monolayer deposited onto a thin gold film to develop a surface plasmon resonance label-free immunosensor [120]. The pyrene-NTA derivative was bound to graphene via $\pi - \pi$ interactions and then electropolymerized generating polypyrene oligomers that reinforce the attachment to the sensor surface. The sensitivity of surface plasmon resonance (SPR) based on light stimulated oscillation of electrons in the conduction band of gold deposits, was strongly improved by the coating of a single graphene sheet. This SPR signal is expressed in angle shift that reflects the thickness variation of the sensing layer. SPR is a well-known method for studying biological interactions without labeling step of the target. The immunoreaction between the immobilized biotinylated cholera toxin and the anti-cholera toxin antibody led to a linear calibration curve in the range 0.004–4 ng/mL with an extremely sensitive detection limit of 4 pg/mL.

It should be noted that all immunosensor configurations are based on the formation of a compact monolayer of cholera toxin as a sensing layer. To improve the performance of these optical or electrochemical immunosensors, three-dimensional bioconfigurations must be designed to increase the density of immobilized cholera toxin while preserving an excellent accessibility for the antibody target. In parallel to the use of carbon nanotube coatings for the functionalization of sensor surfaces, another possibility concerns the formation of highly porous polymers by the use of a temporary template during the

Table 3
List of techniques based on their function

Epidemiology	Detection	Diagnosis	Identification
To confirm which subtypes of *V. cholerae* are present	To confirm that *V. cholerae* sp. is present	To confirm patient is infected by *V. cholerae*	To confirm which *V. cholerae* is present
• Genotyping • Amplification techniques • Immunochromatographic lateral flow • Genosensors • Immunosensors	• Culture methods • Microscopic examination • Amplification techniques • Immunosensors • Genosensors • RDTs	• Clinical symptoms • Culture methods • RDTs	• Biochemical test • Serogroup confirmation • Determination of antibiotic resistance

electropolymerization process. This was recently illustrated by the electrogeneration of a polypyrrole film functionalized by a tris(bipyridine) ruthenium(II) complex on surfaces previously modified by polystyrene nanobeads. After dissolution of the polystyrene templates, different nanostructured Ru(II)-based polymers were obtained depending on the nanosphere diameter (100–900 nm) [121]. The photocurrent enhancement observed for a nanostructured polymer clearly indicates the benefits conferred by this porous structure for the diffusion of quenchers, a structure that should be promising for the permeability of antibodies [122].

From the combination of a microfluidic fluorescence platform, fabricated using soft lithography on polydimethylsiloxane (PDMS) and an electrochemical system [123] a device was developed to detect CTB. CTB-antibodies and ganglioside GM1 receptor were immobilized onto magnetic beads. After washing the beads, they were subjected either to fluorescence or electrochemical detection. The detection limit was 6.6 and 1 ng/mL respectively. A biological microchip was also developed for the detection of cholera antibodies by Utkin et al. [124]. On an activated slide they immobilized soluble antigen cholera agent. The biochip was tested also on blood sera of patients reaching the result in 2–3 hours.

3. Conclusion

Cholera continues today to be a plague inflicting high rates of mortality in developing countries [125]. Prevention and early response are necessary to contain and better understand a *V. cholerae* epidemic. Unfortunately current strategies are not effective enough to prevent cholera outbreaks (Table 3). At present, the gold standard to detect *V. cholerae* continues to be the bacterial culture method, which is laborious, time-consuming and lacks sensitivity as it does not include non-culturable but viable vibrios. Molecular techniques show higher sensitivity than other methods, but require sophisticated equipment and skilled personnel. New genomic platforms, genosensors, offer important advantages over the traditional amplification method. They are user-friendly, no trained personnel is required, and the result is obtained in a short time. As described by Dick [74] among the RDTs developed so far, only five were tested in the field, but their sensitivity and specificity are limited. For this reason efforts must be carried out in developing biosensor and point-of-care devices to allow for early outbreak detection. A good cholera diagnostic assay has to be field-friendly, rapid, low cost, easy to use, no particular required equipment, or supplies, sensitive and specific, with more than one year of shelf life, and storage without refrigeration. Continued efforts are necessary for early detection, early clinical treatment, and prevention by developing rapid and efficient diagnostic point-of-care devices.

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References

[1] D.A. Sack, R.B. Sack, G.B. Nair, A.K. Siddique, Cholera, Lancet 363 (9404) (2004) 223–233.
[2] T. Ramamurthy, S. Garg, R. Sharma, S.K. Bhattacharya, G.B. Nair, T. Shimada, et al., Emergence of novel strain of Vibrio cholerae with epidemic potential in southern and eastern India, Lancet 341 (8846) (1993) 703–704.
[3] J.B. Harris, R.C. Larocque, R.C. Charles, R.N. Mazumder, A.I. Khan, P.K. Bardhan, Cholera's western front, Lancet 376 (9757) (2010) 1961–1965.
[4] WHO, Cholera vaccines: WHO position paper, Wkly. Epidemiol. Rep. 85 (13) (2010) 117–128.
[5] WHO, Cholera 2010, Wkly. Epidemiol. Rec. 86 (2011) 325–340.
[6] J.G. Morris Jr., Cholera and other types of vibriosis: a story of human pandemics and oysters on the half shell, J. Commun. Dis. 37 (2) (2003) 272–280.
[7] A.J. Gubala, Multiplex real-time PCR detection of vibrio cholerae, J. Microbiol. Methods 65 (2) (2006) 278–293.
[8] R.C. Estelle, S.G. Jiang, V.R. Louis, A. Rublin, I.N.G. Rivera, N. Choopun, et al., Predictability of vibrio cholerae in Chesapeake Bay, Appl. Environ. Microbiol. 69 (5) (2003) 2773–2785.
[9] M. Dziejman, D. Serruto, V.C. Tam, D. Sturtevant, P. Diraphat, S.M. Faruque, et al., Genomic characterization of non-O1, non-O139 vibrio cholerae reveals genes for a type III secretion system, Proc. Natl. Acad. Sci. U.S.A. 102 (9) (2005) 3465–3470.
[10] A. Ghosh, D.R. Saha, K.M. Hoque, M. Asakuna, S. Yamasaki, H. Koley, et al., Enterotoxigenicity of mature 45-kilodalton and processed 35-kilodalton forms of hemagglutinin protease purified from a cholera toxin gene-negative vibrio cholerae non-O1, non-O139 strain, Infect. Immun. 74 (5) (2006) 2937–2946.
[11] R. Jørgensen, A.E. Purdy, R.J. Fieldhouse, M.S. Kimber, D.H. Bartelett, A.R. Merrill, Cholix toxin, a novel ADP-ribosylating factor from vibrio cholerae, J. Biol. Chem. 283 (2008) 10671–10678.
[12] S. Pukatzki, M.T. Ma, D. Sturtevant, B. Krastins, D. Sarracino, W.C. Nelson, et al., Identification of a conserved bacterial protein secretion system in vibrio cholerae using the Dictyostelium host model system, Proc. Natl. Acad. Sci. U.S.A. 103 (2006) 1528–1533.
[13] J.S. Matson, J.H. Withey, V.J. DiRita, Regulatory networks controlling vibrio cholera virulence gene expression, Infect. Immun. 75 (12) (2007) 5542–5549.
[14] R.R. Colwell, Global climate and infectious disease: the cholera paradigm, Science 274 (5295) (1996) 2025–2031.
[15] A.S. Jutla, A.S. Akanda, J.K. Griffths, R. Colwell, S. Islam, Warming oceans, phytoplankton, and river discharge: implications for cholera outbreaks, Am. J. Trop. Med. Hyg. 85 (2) (2011) 303–308.
[16] M. Pascual, X. Rodo, S.P. Ellner, R. Colwell, M.J. Bouma, Cholera dynamics and El Nino-Southern Oscillation, Science 289 (5485) (2000) 1766–1769.
[17] E.J. Nelson, D.S. Nelson, M.A. Salam, D.A. Sack, Antibiotics for both moderate and severe cholera, N. Engl. J. Med. 364 (1) (2011) 5–7.
[18] A.A. Weil, A.I. Khan, F. Chowdhury, R.C. Larocque, A.S. Faruque, E.T. Ryan, et al., Clinical outcomes in household contacts of patients with cholera in Bangladesh, Clin. Infect. Dis. 49 (10) (2009) 1473–1479.
[19] V.D. Thiem, J.L. Deen, S.L. Von, D.G. Canh, D.D. Anh, J.K. Park, et al., Longterm effectiveness against cholera of oral killed whole-cell vaccine produced in Vietnam, Vaccine 24 (20) (2006) 4297–4303.
[20] D.N. Taylor, V. Cardenas, J.L. Sanchez, R.E. Bengue, R. Gilman, C. Bautista, et al., Two-year study of the protective efficacy of the oral whole cell plus recombiannt B subunit cholera vaccine in Peru, J. Infect. Dis. 181 (5) (2000) 1667–1673.
[21] M.E. Lucas, J.L. Deen, S.L. Von, X.Y. Wang, J. Ampuero, M. Puri, et al., Effetiveness of mass oral cholera vaccination in Beira, Mozambique, N. Engl. J. Med. 352 (8) (2005) 757–767.
[22] WHO, Update: outbreak of cholera —Haiti, MMWR Morb. Mortal. Wkly Rep. 59 (48) (2010) 1586–1590.

[23] E.T. Ryan, U. Dhar, W.A. Khan, M.A. Salam, A.S. Faruque, G.J. Fuchs, et al., Mortality, morbidity, and microbiology of endemic cholera among hospitalized patients in Dhaka, Bangladesh, Am. J. Trop. Med. Hyg. 63 (1–2) (2000) 12–20.
[24] S.K. Roy, M.J. Hssain, W. Khatun, B. Chakraborty, S. Chowdhury, A. Begum, et al., Zinc supplementation plus oral rehydration salts compared with oral rehydration salts alone as a treatment for acute diarrhea in a primary care setting: a cluster randomized trial, Pediatrics 336 (7638) (2008) 266–268.
[25] A.L. Page, K.P. Alberti, V. Mondonge, J. Rauzier, M.J. Quilici, P.J. Guerin, Evaluation of a rapid test for the diagnosis of cholera in the absence of a gold standard, PLoS ONE 7 (5) (2012) 1–7.
[26] K.H. Keddy, A. Sooka, M.B. Parsons, B.M. Njanpop-Lafourcade, K. Fitchet, A.M. Smith, Diagnosis of vibrio cholerae O1 infection in Africa, J. Infect. Dis. 208 (1) (2013) 23–31.
[27] WHO, *The treatment of diarrhoea, a manual for physicians and other senior health workers* 2005.
[28] W.B. Greenough III, The human, societal, and scientific legancy of cholera, J. Clin. Invest. 113 (3) (2004) 334–339.
[29] CDC, *Rapid diagnostic tests for epidemic enteric diseases. Global disease detection manual*, 2012. CDC: Atlanta, GA (http://www.cdc.gov/cholera/laboratory.html).
[30] E.T. Ryan, The cholera pandemic, still with us after half century: time to rethink, PLoS Negl. Trop. Dis. 5 (1) (2011) e1003.
[31] M. Alam, N.A. Hasan, M. Sultana, A. Sadique, A.S. Faruque, H.P. Endtz, et al., Diagnostic limitations to accurate diagnosis od cholera, J. Clin. Microbiol. 48 (2010) 3918–3922.
[32] CDC, *Laboratory methods for the diagnosis of epidemic dysentery and cholera.* http://www.who.int/topics/cholera/publications/WHO_CDS_CSR_EDC_99_8_EN/en/index.html, 1999. Atlanta, GA: CDC.
[33] S.G. Cary, E.B. Blair, New transport medium for shipment of clinical specimens. I. Fecal specimencs, J. Bacteriol. 88 (1964) 98.
[34] E.S. Estrada, E. Jaramillo, M. Gaviria, S. Ospina, Comparative study of two transport systems for vibrio cholerae, Trop. Doct. 24 (1) (1994) 35–36.
[35] G.K. Morris, M.H. Merson, I. Huq, A.K. Kibrya, R. Black, Comparison of four plating media for isolating vibrio cholerae, J. Clin. Microbiol. 9 (1) (1979) 79–83.
[36] A.R. Pillot, S. Baron, J. Lesne, J.M. Fournier, M.L. Quilici, Improved specific detection of vibrio cholerae in environmental water samples by culture on selective medium and colony hybridization assay with an oligonucleotide probe, FEMS Microbiol. Ecol. 40 (1) (2006) 39–46.
[37] A. Huq, B.J. Haley, E. Taviani, A. Chen, N.A. Hasan, R.R. Colwell, Detection, isolation, and identification of vibrio cholerae from the environment, Curr. Protoc. Microbiol. 6A (5) (2012) 1–58.
[38] CDC, *Manual for the laboratory identification and susceptibility testing of bacterial pathogens of public health importance in the developing world.* http://www.who.int/drugresistance/publications/WHO_CDS_CSR_RMD_2003_6/en/, 2003. Atlanta, GA: CDC.
[39] F. Quadri, C. Wenneras, M.J. Albert, J. Hossain, K. Mannoor, Y.A. Begum, et al., Comparison of immune responses in patients infected with vibrio cholera O139 and O1, Infect. Immun. 65 (9) (1997) 3571–3576.
[40] C.M. O'Hara, E. Sowerse, C.A. Bopp, S.B. Duda, N.A. Strockbine, Accuracy of six commercially available sustems for identification of members of the family vibrionaceae, J. Clin. Microbiol. 41 (2003) 5654–5659.
[41] J.B. Kaper, J.G. Morris Jr., M.M. Levine, Cholera, Clin. Microbiol. Rev. 8 (1995) 48–86.
[42] P.A. Wayne, Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline, *second ed.*, Clinical Laboratory Standards Institute, 2011. Report no. M45-A2.
[43] D. Saha, M.M. Karim, W.A. Khan, S. Ahmed, M.A. Salam, M.L. Bennish, Single-dose azithromycin for the treatment of cholera in adults, N. Engl. J. Med. 354 (2006) 2452–2462.
[44] A.S. Benenson, M.R. Islam, W.B. Greenough III, Rapid identification of vibrio cholerae by darkfield microscopy, Bull. World Health Organ. 30 (1964) 827–831.
[45] A.R. Reimer, G. Van Domselaar, S. Stroika, M. Walker, H. Kent, C. Tarr, et al., V. cholerae *outbreak genomics task force*, comparative genomics of vibrio cholerae from Haiti, Asia, and Africa, Emerg. Infect. Dis. 17 (2011) 2113–2121.
[46] D. Talkington, C. Bopp, C. Tarr, M.B. Parsons, G. Dahourou, M. Freeman, et al., Characterization of toxigenic vibrio cholerae from Haiti, 2010–2011, Emerg. Infect. Dis. 17 (2011) 2122–2129.
[47] W.J. Le Roux, D. Masoabi, C.M. de Wet, S.N. Venter, Evaluation of a rapid polymerase chain reaction based identification technique for V. cholerae isolates, Water Sci. Technol. 50 (1) (2004) 229–232.
[48] E.M. Fykse, G. Sogan, W. Davies, J.S. Olsen, J.M. Blatny, Detection of vibrio cholerae by real-time nucleic acid sequence-based amplification, Appl. Environ. Microbiol. 73 (5) (2007) 1457–1466.
[49] P. Lalitha, M.N. Siti Suraiya, K.L. Lim, S.Y. Lee, A.R. Nur Haslindawaty, Y.Y. Chan, et al., Analysis of lolB gene sequence and its use in the development of a PCR assay for the detection of vibrio cholerae, J. Microbiol. Methods 75 (1) (2008) 142–144.
[50] K.F. Low, A. Karimah, C.Y. Yean, A thermostabilized magnetogenosensing assay for DNA sequence-specific detection and quantification of vibrio cholerae, Biosens. Bioelectron. 47 (2013) 38–44.
[51] P.I. Fields, T. Popovic, K. Wachsmuth, O. Olsvik, Use of polymerase chain reaction for detection of toxigenic vibrio cholerae O1 strains from the Latin American cholera epidemic, J. Clin. Microbiol. 30 (1992) 2118–2121.
[52] S.P. Keasler, R. Hall, Detecting and biotyping vibrio cholerae O1 with multiplex polymerase chain reaction, Lancet 341 (1993) 1661.
[53] D.V. Singh, S.R. Isac, R.R. Colwell, Development of a hexaplex PCR assay for rapid detection of virulence and regulatory genes in vibrio cholerae and vibrio mimicus, J. Clin. Microbiol. 40 (11) (2002) 4321–4324.
[54] Y.J. Cho, H. Yi, J.H. Lee, D.W. Kim, J. Chun, Genomic evolution of vibrio cholerae, Curr. Opin. Microbiol. 13 (5) (2010) 646–651.
[55] K. Hoshino, S. Yamasaki, A.K. Mukhopadhyay, S. Chakraborty, A. Basu, S.K. Bhattacharya, et al., Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic vibrio cholerae O1 and O139, FEMS Immunol. Med. Microbiol. 20 (3) (1998) 201–207.
[56] E.K. Lipp, I.N. Rivera, A.I. Gil, E.M. Espeland, N. Choopun, V.R. Louis, et al., Direct detection of vibrio cholerae and ctxA in Peruvian coastal water and plankton by PCR, Appl. Environ. Microbiol. 69 (2003) 3676–3680.
[57] K.A. Koskela, P. Matero, J.M. Blatny, E.M. Fykse, J.S. Olsen, L.O. Nuotio, et al., A multiplatform real-time polymerase chain reaction detection assay for vibrio cholerae, Diagn. Microbiol. Infect. Dis. 65 (2009) 339–344.
[58] W.J. Lyon, TaqMan PCR for detection of vibrio cholerae O1, O139, non-O1, and non-O139 in pure cultures, raw oysters, and synthetic seawater, Appl. Environ. Microbiol. 67 (10) (2001) 4685–4693.
[59] G.M. Blackstone, J.L. Nordstrom, M.D. Bowen, R.F. Meyer, P. Imbro, A. DePaola, Use of a real time PCR assay for detection of the ctxA gene of vibrio cholerae in an environmental survey of mobile bay, J. Microbiol. Methods 68 (2) (2007) 254–259.
[60] A.J. Gubala, D.F. Proll, Molecular-beacon multiplex real-time PCR assay for detection of vibrio cholerae, Appl. Environ. Microbiol. 72 (2006) 6424–6428.
[61] J. Huang, Y. Zhu, H. Wen, J. Zhang, S. Huang, J. Niu, et al., Quadruplex real-time PCR assay for detection and identification of vibrio cholerae O1 and O139 strains and determination of their toxigenic potential, Appl. Environ. Microbiol. 75 (22) (2009) 6981–6985.
[62] N.C. Leal, M. Sobreira, T.C. Leal-Balbino, A.M. de Almeida, M.J. de Silva, D.M. Mello, et al., Evaluation of a RAPD-based typing scheme in a molecular epidemiology study of Vibrio cholerae O1, Brazil, J. Appl. Microbiol. 96 (3) (2004) 447–454.
[63] J.H. Lee, K.H. Han, S.Y. Choi, M.E. Lucas, C. Mondlane, M. Ansaruzzaman, et al., Multilocus sequence typing (MLST) analysis of vibrio cholerae O1 El Tor isolates from Mozambique that harbour the classical CTX prophage, J. Med. Microbiol. 55 (2) (2006) 165–170.
[64] M. Scrascia, F. Maimone, K.A. Mohamud, F. Materu, F. Grimont, P.A.D. Grimont, et al., Clonal relationship among vibrio cholerae O1 El Tor strains causing the largest cholera epidemic in Kenya in the late 1990s, J. Clin. Microbiol. 44 (9) (2006) 3401–3404.
[65] F.L. Thompson, C. Thompson, A.C.P. Vicente, G.N.D. Theophilo, E. Hofer, J. Swings, Genomic diversity of clinical and environmental vibrio cholerae strains isolated in Brazil between 1991 and 2001 as revealed by fluorescent amplified fragment length polymorphism analysis, J. Clin. Microbiol. 41 (5) (2003) 1946–1950.
[66] K.L. Cooper, C.K. Luey, M. Bird, J. Terajima, G.B. Nair, K.M. Kam, et al., Development and validation of a PulseNet standardized pulsed-field gel electrophoresis protocol for subtyping of Vibrio cholerae, Foodborne Pathog. Dis. 3 (1) (2006) 51–58.
[67] M. Spagnoletti, D. Ceccarelli, M.M. Colombo, Rapid detection by multiplex PCR of Genomic Islands, prophages and integrative conjugative elements in V. cholerae 7th pandemic variants, J. Microbiol. Methods 88 (1) (2012) 98–102.
[68] M. Kotetishvili, O.C. Stine, Y. Chen, A. Kreger, A. Sulakvelidze, S. Sozhamannan, et al., Multilocus sequence typing has better discriminatory ability for typing vibrio cholerae than does pulsed-field gel electrophoresis and provides a measure of phylogenetic relatedness, J. Clin. Microbiol. 41 (5) (2003) 2191–2196.
[69] D. Bhattacharya, S. Dey, G.P. Pazhani, T. Ramamurthy, M.V. Parande, S.D. Kholkute, et al., Vibrio cholerae O1 El Tor variant and emergence of Haitian ctxB variant in the strains isolated from South India, Med. Microbiol. Immunol. (2015) [Epub ahead of print], doi:10.1007/s00430-015-0433-y.
[70] S.P. Awasthi, M. Asakura, S.B. Neogi, A. Hinenoya, T. Ramamurthy, S. Yamasaki, Development of a PCR-restriction fragment length polymorphism assay for detection and subtyping of cholix toxin variant genes of vibrio cholerae, J. Med. Microbiol. 63 (2014) 667–673.
[71] N. Binsztein, M.C. Costagliola, M. Pichel, V. Jurquiza, F.C. Ramírez, R. Akselman, et al., Viable but nonculturable vibrio cholerae O1 in the aquatic environment of Argentina, Appl. Environ. Microbiol. 70 (12) (2004) 7481–7486.
[72] J.A. Hasan, D. Bernestein, A. Huq, L. Loomis, M.L. Tamplin, R.R. Colwell, Cholera DFA: an improved direct fluorescent monoclonal antibody staining kit for rapid detection and enumeration of V. cholerae O1, FEMS Microbiol. Lett. 120 (1–2) (1994) 143–148.
[73] P.Y. Liu, L.K. Chin, E. Ser, T.C. Ayi, P.H. Yap, T. Bourouina, et al., An optofluidic imaging system to measure the biophysical signature of single waterborne bacteria, Lab Chip 14 (2014) 4237–4243.
[74] M.H. Dick, M. Guillerm, F. Moussy, C.L. Chaignat, Review of Two decades of cholera diagnostics – how far have we really come?, PLoS Negl. Trop. Dis. 6 (10) (2012) 1–8.
[75] F. Nato, A. Boutonnier, M. Rajerison, P. Grosjean, S. Dartevelle, A. Guénolé, et al., One-Step Immunochromatographic dipstick tests for rapid detection of vibrio cholerae O1 and O139 in Stool Samples, Clin. Diagn. Lab. Immunol. 10 (3) (2003) 476–478.
[76] S. Chakraborty, M. Alam, H.M. Scobie, D.A. Sack, Adaptation of a simple dipstick test for detection of vibrio cholerae O1 and O139 in environmental water, Front. Microbiol. 4 (2013) 405.

[77] X.Y. Wang, M. Ansaruzzaman, R. Vaz, C. Mondlane, M.E. Lucas, L. von Seidlein, et al., Field evaluation of a rapid immunochromatographic dipstick test for the diagnosis of cholera in a high-risk population, BMC Infect. Dis. 6 (2006) 17.
[78] C.M. George, M.U. Rashid, D.A. Sack, R.B. Sack, K.M. Saif-Ur-Rahman, A.S. Azman, et al., Evaluation of enrichment method for the detection of vibrio cholerae O1 using a rapid dipstick test in Bangladesh, Trop. Med. Int. Health 19 (3) (2014) 301–307.
[79] M. Alam, M. Sultana, G.B. Nair, A.K. Siddique, N.A. Hasan, R.B. Sack, et al., Viable but nonculturable vibrio cholerae O1 in biofilms in the aquatic environment and their role in cholera transmission, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 17801–17806.
[80] F. Ibrahim, T.H.G. Thio, T. Faisal, M. Neuman, The application of biomedical engineering techniques to the diagnosis and management of tropical diseases: a review, Sensors (Basel) 15 (3) (2015) 6947–6995.
[81] H.G. Schnerr, Food and Beverage Applications of Biosensor Technologies, in: R. Marks, D.C. Cullen, I. Karube, C.R. Lowe, H.H. Weetall (Editors), Handbook of Biosensors and Biochips, Wiley Online Library, 2007.
[82] D.J. Monk, D.R. Walt, Optical fiber-based biosensors, Anal. Bioanal. Chem. 379 (2004) 931–945.
[83] D.R. Thevenot, T. Koth, R.A. Durst, G.S. Wilson, Electrochemical biosensors: recommended definitions and classification, Biosens. Bioelectron. 16 (2001) 121–131.
[84] A. Sassolas, L.J. Blum, B.D. Leca-Bouvier, Immobilization strategies to develop enzymatic biosensors, Biotechol. Adv. 30 (3) (2011) 489–571.
[85] F.S. Ligler, D. Phil, Biosensors for Detection of Bioterrorist Threats, vol. 224, Springer Netherlands, Netherlands, 2006.
[86] D.M. Gill, Bacterial toxins: a table of lethal amounts, Microbiol. Rev. 46 (1) (1982) 86–94.
[87] E.R. Goldman, A.R. Clap, G.P. Anderson, H.T. Uyeda, J.M. Mauro, I.L. Medintz, et al., Multiplexed toxin analysis using four colors of quantum dot fluororeagents, Anal. Chem. 76 (3) (2004) 684–688.
[88] M. Labib, M. Hedström, M. Amin, B. Mattiasson, A capacitive immunosensor for detection of cholera toxin, Anal. Chim. Acta 634 (2009) 255–261.
[89] M.M. Archibald, B. Rizal, T. Connolly, M.J. Burns, M.J. Naughton, T.C. Chiles, A nanocoaxial-based electrochemical sensor for the detection of cholera toxin, Biosens. Bioelectron. 74 (2015) 406–410.
[90] Y. Uesaka, Y. Otsuka, M. Kashida, Y. Oku, K. Horigome, G. Balakrish Nair, et al., Detection of cholera toxin by a highly sensitive bead-enzyme linked immunosorbent assay, Microbiol. Immunol. 36 (1) (1992) 43–53.
[91] K.A. Edwards, J.C. March, GM1-functionalized liposomes in a microtiter plate assay for cholera toxin in vibrio cholerae culture samples, Anal. Biochem. 368 (1) (2007) 39–48.
[92] D. Jin, Y. Luo, M. Zheng, H. Li, J. Zhang, M. Stampfl, et al., Quantitative detection of vibrio cholera toxin by real-time and dynamic cytotoxicity monitoring, J. Clin. Microbiol. 51 (12) (2013) 3968–3974.
[93] V.L. Schmit, R. Martoglio, K.T. Carron, Lab-on-a-bubble surface enhanced raman indirect immunoassay for cholera, Anal. Chem. 84 (9) (2012) 4233–4236.
[94] A.K. Singh, S.H. Harrison, J.S. Schoeniger, Gangliosides as receptors for biological toxins: development of sensitive fluoroimmunoassays using ganglioside-bearing liposomes, Anal. Chem. 72 (24) (2000) 6019–6024.
[95] S.A. Khan, J.A. DeGrasse, B.J. Yakes, T.R. Croley, Rapid and sensitive detection of cholera toxin using gold nanoparticle-based simple colorimetric and dynamic light scattering assay, Anal. Chim. Acta 892 (2015) 167–174.
[96] E. Yamasaki, R. Sakamoto, T. Matsumoto, F. Morimatsu, T. Kurazono, T. Hiroi, et al., Development of an immunochromatographic test strip for detection of cholera toxin, Biomed Res. Int. 2013 (2013) 1–7.
[97] G.Y. Ang, C.Y. Yu, C.Y. Yean, Ambient temperature detection of PCR amplicons with a novel sequence-specific nucleic acid lateral flow biosensor, Biosens. Bioelectron. 38 (1) (2012) 151–156.
[98] C.Y. Yu, G.Y. Ang, K.G. Chan, K.K. Banga Singh, Y.Y. Chan, Enzymatic electrochemical detection of epidemic-causing vibrio cholerae with a disposable oligonucleotide-modified screen-printed bisensor coupled to a dry-reagent-based nucleic acid amplification assay, Biosens. Bioelectron. 70 (2015) 282–288.
[99] J. Wang, Electrochemical biosensors: towards point-of-care cancer diagnostics, Biosens. Bioelectron. 21 (10) (2006) 1887–1892.
[100] P.S. Liew, B. Lertanantawong, S.Y. Lee, R. Manickam, Y.H. Lee, W. Surareungchai, Electrochemical genosensor assay using lyophilized gold nanoparticles/latex microsphere label for detection of Vibrio cholerae, Talanta 139 (2015) 167–173.
[101] A. Chua, C.Y. Yean, M. Ravichandran, B. Lim, P. Lalitha, A rapid DNA biosensor for the molecular diagnosis of infectious disease, Biosens. Bioelectron. 26 (9) (2011) 3825–3831.
[102] G.Y. Ang, C.Y. Yu, K.G. Chan, K.K.B. Singh, C.Y. Yean, Development of a dry-reagent-based nucleic acid-sensing platform by coupling thermostabilised LATE-PCR assay to an oligonucleotide-modified lateral flow biosensor, J. Microbiol. Methods 118 (2015) 99–105.
[103] U. Sungkanak, A. Sappat, A. Wisitsoraat, C. Promptmas, A. Tuantranont, Ultrasensitive detection of vibrio cholerae O1 using microcantilever-based biosensor with dynamic force microscopy, Biosens. Bioelectron. 26 (2010) 784–789.
[104] V.K. Rao, M.K. Sharma, A.K. Goel, L. Singh, K. Sekhar, Amperometric immunosensor for the detection of vibrio cholerae O1 using disposable screen-printed electrodes, Anal. Sci. 22 (9) (2006) 1207–1211.
[105] W. Chen, J. Zhang, G. Lu, Z. Yuan, Q. Wu, J. Li, et al., Development of an immunochromatographic lateral flow device for rapid diagnosis of vibrio cholerae O1 serotype Ogawa, Clin. Biochem. 47 (6) (2014) 448–454.
[106] R.S. Marks, Z.M. Hale, M.M. Levine, C.R. Lowe, E.P. Payne, The single mode tapered optical fibre loop immunosensor: II. Assay of anti-cholera toxin immunoglobulins, SPIE 2131 (1994) 495–503.
[107] S.R. Marks, E. Bassis, A. Bychenko, M.M. Levine, Chemiluminescent optical fiber immunosensor for detecting cholera antitoxin, Opt. Eng. 36 (12) (1997) 3258–3264.
[108] S. Cosnier, M. Holzinger, Electrogenerated polymers for biological sensors, Chem. Soc. Rev. 40 (2011) 2146–2156.
[109] R.S. Marks, A. Novoa, D. Thomassey, S. Cosnier, An innovative strategy for immobilization of receptor proteins on to an optical fiber by use of poly (pyrrole-biotin), Anal. Bioanal. Chem. 374 (2002) 1056.
[110] T. Konry, A. Novoa, S. Cosnier, R.S. Marks, Development of an 'electroptode' immunosensor: indium tin oxide-coated optical fiber tips conjugated with an electro-polymerized thin film with conjugated cholera toxin B subunit, Anal. Chem. 75 (2003) 2633–2639.
[111] R.E. Ionescu, C. Gondran, L.A. Gheber, S. Cosnier, R.S. Marks, Construction of amperometric immunosensors based on the electrogeneration of a permeable biotinylated polypyrrole film, Anal. Chem. 76 (2004) 6808–6813.
[112] R.E. Ionescu, C. Gondran, S. Cosnier, R.S. Marks, Construction of amperometric immunosensors for the analysis of cholera antitoxin and comparison of the performances between three different enzyme markers. Compr. Anal. Chem. 49 (2007) 185–194.
[113] B. Leshem, G. Sarfati, A. Novoa, I. Breslav, R.S. Marks, Photochemical attachment of biomolecules onto fibre-optics for construction of a chemiluminescent immunosensor, Luminescence 19 (2004) 69–77.
[114] N. Haddour, J. Chauvin, C. Gondran, S. Cosnier, Photoelectrochemical immunosensor for label-free detection and quantification of anti-cholera toxin antibody, J. Am. Chem. Soc. 128 (2006) 9693–9698.
[115] T. Konry, K. Heyman, S. Cosnier, K. Gorgy, R.S. Mark, Characterization of thin poly (pyrrole-benzophenone) film morphologies electropolymerized on indium tin oxide coated optic fibers for electrochemical and optical biosensing, Electrochim. Acta 53 (16) (2008) 5128–5135.
[116] T. Konry, B. Hadad, Y. Shemer-Avni, S. Cosnier, R.S. Marks, ITO pattern fabrication of glass platforms for electropolymerization of light sensitive polymer for its conjugation to bioreceptors on a micro-array, Talanta 75 (2008) 564–571.
[117] P. Goldberg-Oppenheimer, S. Cosnier, R. Marks, O. Regev, Preparation and characterization of a novel poly-pyrrole benzophenone copolymerized silica nanocomposite as a reagent in a visual immunologic-agglutination test, Talanta 75 (2008) 1324–1331.
[118] J. Baur, M. Holzinger, C. Gondran, S. Cosnier, Immobilization of biotinylated biomolecules onto electropolymerized poly(pyrrole-nitrilotriacetic acid)-Cu2+ films, Electrochem. Commun. 12 (10) (2010) 1287–1290.
[119] W. Yao, A. Le Goff, N. Spinelli, M. Holzinger, G.W. Diao, D. Shan, et al., Electrogenerated trisbipyridyl Ru(II)-/nitrilotriacetic-polypyrene copolymer for the easy fabrication of label-free photoelectrochemical immunosensor and aptasensor. Application to the determination of thrombin and anti-cholera toxin antibody, Biosens. Bioelectron. 42 (2013) 556–562.
[120] M. Singh, M. Holzinger, M. Tabrizian, S. Winters, N. Berner, S. Cosnier, et al., Non-covalently functionalized monolayer graphene for sensitivity enhancement of surface plasmon resonance immunosensors, J. Am. Chem. Soc. 137 (2015) 2800–2803.
[121] A. Cernat, F. Haddache, B. Reuillard, A. Le Goff, K. Gorgy, C. Gondran, et al., Micro to nanostructured poly(pyrrole-nitrilotriacetic acid) films via nanosphere templates: applications to 3D enzyme attachment by affinity interaction, Anal. Bioanal. Chem. 406 (2014) 1141–1147.
[122] L. Fritea, F. Haddache, B. Reuillard, A. Le Goff, K. Gorgy, C. Gondran, et al., Electrochemical nanopatterning of an electrogenerated photosensitive poly-[trisbipyridinyl-pyrrole ruthenium(II)] metallopolymer by nanosphere lithography, Electrochem. Commun. 46 (2014) 75–78.
[123] N. Bunyakul, K.A. Edwards, C. Promptmas, A.J. Baeumner, Cholera toxin subunit B detection in microfluidic devices, Anal. Bioanal. Chem. 393 (2009) 177–186.
[124] D.V. Utkin, N.A. Osina, A.N. Spitsin, M.N. Kireev, O.V. Gromova, T.L. Zakharova, et al., The development of biochip to detect anti-cholera antibodies in human blood serum, Klin. Lab. Diagn. 60 (2) (2015) 50–53.
[125] A. Talavera, E.M. Perez, Is cholera disease associated with poverty?, J. Infect. Dev. Ctries. 3 (6) (2009) 408–411.
[126] Merck Microbiology manual 12th edition, <http://www.mibius.de/out/oxbaseshop/html/0/images/wysiwigpro/TCBS_Agar_110263_engl.pdf>, 2001.
[127] Dana Sinziana Brehar'Cioflec, <https://www.google.com.sg/search?q=biochemical+identification+cholera&espv=2&biw=1600&bih=799&source=lnms&tbm=isch&sa=X&ved=0CAYQ_AUoAWoVChMIuYGguvuYyQIV1wiOCh3wng6W#imgrc=rr850Kdbav1byM%3A>, 2015.
[128] BioAssay Works, <http://www.bioassayworks.com/researchassay.html>.
[129] R.S. Marks, L. Fajs, <www.biosensorix.com>, 2015.