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► To cite this version:

Elise Da-Silva, Julia Baudart, Lise Barthelmebs. Biosensing platforms for *Vibrio* bacteria detection based on whole cell and nucleic acid analysis: A review. *Talanta*, 2018, 190, pp.410-422. 10.1016/j.talanta.2018.07.092 . hal-03943931

HAL Id: hal-03943931

<https://hal.science/hal-03943931>

Submitted on 17 Jan 2023

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Biosensing platforms based on whole cell and nucleic acid detection for
***Vibrio* bacteria analysis : a review**

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Abstract:

Vibrio related illnesses are increasing worldwide in humans and marine animals. The detection of these bacteria is still mainly performed using traditional microbiological methods based on culture grown on differential agar media which are labor intensive, time consuming and unsuitable for in-field and high-throughput analysis. To overcome these limitations, biosensing platforms have emerged as promising alternative tools for rapid, sensitive, and real-time detection of *Vibrio* species in clinical, food and environmental samples. In this review, we will focus on strip test devices, and on optical and electrochemical biosensors developed for *Vibrio* analysis. Particular attention is given to sample preparation techniques.

Keywords : *Vibrio* detection ; strip tests ; optical biosensors ; electrochemical biosensors ; sample preparation.

1. Introduction

Due to the increased prevalence of well-known pathogens and the emergence of novel pathogenic microorganisms in aquatic sources, global incidence of water-borne diseases is currently rising. This phenomenon results from a combination of complex human factors including inadequate water supply, sanitation, and hygiene issues as well as the intensification of continental run-off discharging pathogens into coastal waters. Environmental factors such as climate changes and extreme weather events could also induce the persistence of pathogens in water sources [1].

Among emerging and reemerging infectious agents, *Vibrio* bacteria are widely distributed in coastal and estuarine environments where they are found as free-living cells and also closely associated with marine animals, plankton, algae or abiotic surfaces [2].

At least twelve *Vibrio* species, including *V. cholerae* O1 and O139 serogroups, responsible for cholera outbreaks, and *V. parahaemolyticus* and *V. vulnificus* involved in seafood-borne infections, have been described as human pathogens [3]. Cholera disease remains a major threat to public health, especially in countries without improved sanitation facilities. According to the World Health Organization [4], 1.3 to 4.0 million cholera cases, leading to 21 000 to 143 000 deaths, occur each year in the world. However, the global impact is still largely underestimated because the majority of cases is not reported [5]. Furthermore, in the last decades, an increase in human *Vibrio* infections has been observed worldwide, mainly in Africa and in Asia, but also in USA [6]. As an example, the number of vibriosis excluding *V. cholerae* O1 and O139 serogroups, had regularly increased between 2007 and 2014 from 549 to 1252 in this country. The most frequently reported species during this seven-year analysis was *V. parahaemolyticus*, isolated from 39 to 51% of patients, followed by *V. alginolyticus* and *V. vulnificus* isolated from 16 to 19% and 10 to 14% of the patients, respectively.

Several pathogenic *Vibrio* species are also involved in mass mortalities of marine animals, leading to important economic losses in aquaculture. This is well illustrated by *V. anguillarum* and *V. salmonicida*, two major pathogens involved in outbreaks in eel and salmon, respectively [7], by *V. splendidus* and *V. aestuarianus* associated with summer mortality events affecting oyster farming in Europe [8], and by *V. harveyi* and *V. nigripulchritudo*, the main infectious agents of farmed shrimps [9,10].

Traditionally, *Vibrio* detection in clinical, food and environmental samples is assessed by microbiological methods based on culture grown on differential agar media followed by colony counting and phenotypic identification of isolated strains. However, these approaches suffer

1 from inherent and well-known drawbacks since they are time-consuming and require a delay of
2 up to a week for species identification. Furthermore, it has been shown that *Vibrio* bacteria are
3 able to modify their metabolic and enzymatic activities in response to environmental changes,
4 inducing phenotypic and biochemical variabilities, and leading to a low efficiency of these
5 approaches for an accurate identification [11,12]. Culture-based methods also fail to detect cells
6 induced, by environmental stresses, to enter a viable but-non culturable (VBNC) state
7 [13,14,15,16,17].

8 It appears that the surveillance programs suffer from a lack of rapid and sensitive tools to
9 detect pathogenic *Vibrio* strains in various matrix samples. In order to ensure relevant diagnosis,
10 alternative methods to culture ones have to meet several criteria. Ideally, they must be accurate
11 and reliable, rapid and low cost, automated or integrated into systems to provide high-
12 throughput analysis. They should also be suitable for a broad range of applications and offer
13 simultaneous detection of multiple targets.

14 During the last decades, biosensing platforms, mainly represented by biosensors, have been
15 proposed as attractive and effective alternatives to detect microorganisms for clinical diagnosis,
16 food analysis and environmental monitoring [18,19,20].

17 Biosensors are analytical devices typically comprising (i) a bioreceptor (i.e., an antibody for
18 lipopolysaccharide in the outer membrane, an aptamer selected to recognize the whole cell, a
19 DNA sequence that is complementary to *Vibrio* DNA) which corresponds to the biological
20 recognition element able to detect the target analyte and (ii) a transducer (i.e., optical,
21 electrochemical, mechanical) that converts the biochemical reaction induced by the
22 bio-recognition event into a measurable signal (**Fig. 1**). Compared to current diagnostic
23 methods, biosensors offer several advantages since they are highly sensitive, low-cost, easy-to-
24 use and could allow real-time analysis. Optical and electrochemical transduction techniques
25 have been prominently applied for *Vibrio* detection. Optical biosensors include a large number
26 of subclasses based on absorption, reflection, refraction, dispersion, infrared,
27 chemiluminescence, and fluorescence. These devices provide advantages of compactness,
28 flexibility, and resistance to electrical noise (Narsaiah et al., 2012 ; Ahmed et al., 2014 ; Yoo
29 and Lee, 2016). Electrochemical biosensors can be classified into amperometric/voltammetric,
30 potentiometric, impedimetric and conductimetric, based on their observed parameters such as
31 current, potential, impedance and conductance, respectively. They are fast, easy to use, cheap,
32 small-sized and easily miniaturized (Ivnitski et al., 2000 ; Palchetti and Mascini, 2008 ; Ahmed
33 et al., 2014).

1 More recently, the use of paper-based platforms, especially strip tests has also been
2 intensively reported for *Vibrio* analysis. These devices are based on the analyte migration, under
3 capillary action, through different zones of a membrane, on which bioreceptors (i.e., antibodies,
4 aptamers, DNA probes) are immobilized. The analyte recognition leads to a response on the test
5 line that can be assessed by eye or using a reader. A response on the control line validates the
6 proper migration of the sample through the membrane (**Fig. 2**). Strip tests don't require
7 laboratory infrastructure or technical expertise and thus, constitute interesting screening tools
8 for a rapid analysis to be performed at the point of care (Cheng et al., 2014 ; Kim et al., 2015).

9 Thus, the purpose of this review is to examine the current status of biosensing platforms for
10 *Vibrio* bacteria detection. In order to allow bacteria sensing, two main strategies can be
11 considered: the direct detection of whole bacterial cells or the detection of specific nucleic acid
12 targets after the bacterial lysis. Toxin detection is not addressed in this article. For each of these
13 strategies, strip tests, and biosensors either optical or electrochemical, targeting *Vibrio* are
14 presented. A special emphasis is placed on sample preparation techniques used prior analysis.
15 Further, current trends, advantages and disadvantages and future prospects of these methods
16 are discussed.

17 **2. Sample preparation before analysis**

18 Due to their influence on the performance of the analytical methods for bacteria
19 determination, techniques applied to sample preparation are of great importance.

20 Depending on the bioreceptor, biosensors and strip tests designed for bacteria analysis are
21 able to detect either whole cells, using antibodies or aptamers, or nucleic acids, using
22 complementary DNA sequences as probes. Furthermore, these tools are, in principle, dedicated
23 to real sample analysis. Thus, sample preparation has to be adapted not only to the type of
24 biosensor but also to the kind of sample. In the case of *Vibrio* monitoring, commonly analyzed
25 matrices are food (shrimps, oysters, mussels, clams), clinical (stool and blood), and
26 environmental samples (seawater, ground water, tap water). Since these complex matrices could
27 contain some compounds interfering with the response signal, such as fats, polysaccharides,
28 proteins, minerals and even some antimicrobial preservatives, a preliminary treatment step is
29 generally needed prior analysis. Besides, *Vibrio* cells are usually present at low levels in
30 environmental samples such as water, requiring the use of concentration techniques to allow
31 their detection and avoid false negative results. The different sample preparation methods

reported in the literature for *Vibrio* analysis by biosensor and strip test technologies are detailed below.

2.1. Whole cell preparation

To validate the biosensing tools targeting whole cells, *Vibrio* strains are cultivated in an appropriate medium, the cells are then harvested by centrifugation, washed and resuspended at different concentrations in saline buffer or nutritive medium. The obtained suspensions are then directly used for analysis [21,22,23,24,25,26] or added to real samples, often pretreated, to evaluate their matrix effect. Liquid samples, such as water, are usually sterilized by a filtration through 0.2 or 0.45 μm pore size membranes [24,25]. For solid samples, such as seafood, a mass of product is mixed and homogenized in buffer or in alkaline peptone (APW) [22,27]. A filtration step can be added to remove the large debris [25,28,29].

Similar protocols are applied to *Vibrio* cells from naturally contaminated samples, which are, in most of cases, stools from patients presenting cholera symptoms. After a simple dilution step in appropriate buffer, liquid stool samples are usually directly analyzed [30,31,32,33,34,35]. A pre-enrichment step can also be carried out in APW for 4 to 6h at an appropriate growth temperature to increase the bacterial biomass [35,36,37]. In order to determine the concentration of cells, and/or to check the presence of *Vibrio* in samples, culturable cells are always enumerated in parallel by classical culture-based methods.

2.2. Nucleic acid preparation

Nucleic acid extraction is a critical step to obtain a positive signal in biosensor analysis. The selected method must allow to obtain pure and concentrated nucleic acid extracts. Among the numerous methods available for this purpose, the boiling lysis method is mainly used for DNA extraction from pure culture [38,39,40,41]. Cells are boiled for 10 min in saline solution, the lysate is centrifuged and the released DNA is recovered from the supernatant. Commercial DNA extraction kits such as QIAamp® DNA minikit from Qiagen, and Nucleospin® Tissue from Macherey-Nagel, are also used to extract and purify DNA from pure cultures in 30 to 45 minutes with a high-throughput [42,43,44].

In the case of nucleic acids extraction from real samples, the boiling lysis method can also be applied [45,46,47], with slight modifications such as the addition of a chemical lysis before boiling for shrimp and oyster samples [42,43,48,49]. Nucleic acids can also be extracted using the phenol chloroform method, in order to complex and eliminate polysaccharides from the samples [50,51]. Nevertheless, this method is laborious and uses toxic compounds. As a good

alternative to those methods, several companies have developed kits to decrease extraction time and to obtain nucleic acids without inhibitors. Surprisingly, no publication concerning *Vibrio* detection using biosensing platforms has reported the use of such kits.

Recently, an automated method was reported for DNA extraction from pure cultures and spiked tap water samples. This step is incorporated into a rotary microfluidic system, using glass microbeads as solid phase matrix allowing a strong DNA adsorption in presence of chaotropic salts [52].

After DNA extraction, direct quantification of the extracted nucleic acids is rarely performed since the majority of the assays used an amplification step of DNA by PCR before analysis. The obtained PCR products are often subjected to gel electrophoresis for quality checking [38,39] and their concentration is measured by spectrophotometry at 260 nm [44]. The double-stranded PCR products can be denatured by a heating step at 95°C during few minutes, sometimes followed by a cooling step on ice to obtain single stranded-DNA (ssDNA) for the hybridization reaction with DNA probes [45]. An asymmetric PCR, or a linear after the exponential PCR (LATE-PCR), can also be applied to directly generate ssDNA amplicons [39,42,45,53].

Interestingly, in some recent reports, direct nucleic acid detection was performed without any amplification step by PCR. Nucleic acid concentration, purity and integrity were assessed using either Nanodrop spectrophotometer [54,55,56], Eppendorf Biophotometer [57], or Agilent 2100 Bioanalyzer [51]. Prior to biosensor analysis, a pretreatment step was nevertheless needed to improve the recognition between probes and targets. For DNA samples, this pretreatment consisted in a denaturation at 92°C for few minutes immediately followed by an ice cooling [57]. The heating step could also be combined with an ultrasonication fragmentation [54,55]. For RNA samples, a chemical fragmentation step was applied [51].

3. Biosensing platforms for the detection of whole bacterial cells

One of the most important factors in the set-up of biosensing platforms for whole bacterial cells is to define a suitable targeted molecule linked to the bacterial cell surface, such as outer membrane lipopolysaccharide, a virulence factor in pathogenic *Vibrio* species. This targeted molecule is then used to produce specific bioreceptors (i.e., antibodies, aptamers), which will be integrated into biosensing platforms to allow a direct and rapid detection of bacteria with high affinity and specificity.

3.1. Strip test devices

1 The strip tests developed for the detection of whole *Vibrio* cells are summarized in **Table 1**.
2 All these devices are based on a lateral flow immunochromatography assay (LFA) using
3 antibodies as presented in **Fig. 2**.

4 Since cholera mainly affects developing countries with poor resources and limited access to
5 laboratory facilities, strip test devices appear as interesting tools to detect this disease. Thus,
6 numerous strip tests have been developed for the detection of *V. cholerae* species in stool
7 samples (**Table 1**). The most widely used is the Pasteur Institut dipstick, nowadays
8 commercially available as Crystal VC[®] RDT (Span Diagnostics Ltd., India). This test is able to
9 detect the lipopolysaccharide (LPS) antigen of *V. cholerae* O1 and O139 using specific
10 monoclonal antibodies (MAb) in about 10 min. Several studies have evaluated the
11 performances of both the prototype and the commercial version. Most of these studies used
12 culture on solid medium as reference method. The sensitivity corresponds to the proportion of
13 patients with positive strip tests among patients diagnosed positive by a reference method
14 whereas the specificity corresponds to the proportion of patients with negative strip tests among
15 patients diagnosed negative by a reference method. A first evaluation on frozen stool samples
16 showed high sensitivity and specificity ranging from 94.2 to 100% and from 84 to 100%,
17 respectively [58]. These observations were in accordance with the results obtained for rectal
18 swab analysis with sensitivity and specificity ranging from 93-96% and 92-98%, respectively
19 [36]. However, two studies performed on bulk stools during cholera outbreaks showed lower
20 specificities ranging from 71% to 77% and from 77% to 97% [31,59]. In order to increase the
21 sensitivity of the assay and to obtain performances similar to that of culture [37], an enrichment
22 of samples in APW was often performed prior analysis. Recent studies have shown that a 24h
23 enrichment step in APW significantly increased the sensitivity compared to a direct testing [60]
24 whereas after 6h of enrichment, the sensitivity was only marginally increased [35]. The
25 importance of the reference method was also underlined by several reports [32,34] since a
26 significant increase in the assay specificity was observed when both culture and PCR were used
27 as references. Some authors have also highlighted the impact of the technician skill level on the
28 test specificity [30,61] that can vary, for example, from 67% when the assay was performed by
29 field paramedics to 76% when the users were lab technicians [30].

30 Strip test devices were also proposed for the rapid and specific detection of *Vibrio cholerae*
31 O1 serotype Ogawa [62]. The test was based on a LFA using MAb highly specific to the Ogawa
32 serotype and colloidal gold particles-antibodies conjugates. The limit of detection (LOD) of the
33 developed device was 10⁴ CFU mL⁻¹ and showed 100% sensitivity and selectivity compared to
34 standard culture method. Others LFAs, were developed for the detection of *V. cholerae* O139

1 alone. These tools showed sensitivities ranging from 10^4 [22] to 10^6 CFU mL⁻¹ [21] and were
2 highly specific. Although strip tests can be used for early detection of cholera, all those studies
3 reveal some limitations since the tools can fail to detect *V. cholerae* leading to false negative
4 results [32,33,58].

5 More recently, two similar assays have been described for the detection of other *Vibrio*
6 species. The first one, targeting *V. parahaemolyticus*, showed an LOD of 1.2×10^3 CFU mL⁻¹
7 using pure cultures and no cross reaction within a panel of 32 species [26]. Furthermore, when
8 performed on hepatopancreas and fecal samples from shrimp, and on human fecal samples, the
9 assay demonstrated 100% sensitivity and specificity. In the second one, specifically designed
10 for *V. anguillarum* detection, the sensitivity of the assay was increased using upconversion
11 fluorescent materials, able to convert near infrared light into visible light, instead of standard
12 gold nanoparticles reporters [23]. This assay demonstrated an LOD of 10^2 CFU mL⁻¹ and no
13 cross-reaction with 8 other pathogenic species (*V. alginolyticus*, *V. harveyi*, *V.*
14 *parahaemolyticus*, *V. vulnificus*, *Escherichia coli*, *Edwardsiella tarda*, *Streptococcus iniae*, and
15 *Staphylococcus aureus*).

16 3.2. Optical biosensors

17 Comparatively to LFA, few optical immunosensors were proposed for the detection of whole
18 *Vibrio* cells as reported in **Table 2**.

19 The first optical immunosensor was applied to *V. cholerae* detection and was based on
20 surface plasmon resonance (SPR) detection technique. A detection range of 10^5 - 10^9 cells mL⁻¹
21 and an LOD of 10^5 cells mL⁻¹ were obtained using MAb against LPS as bioreceptors [63]. An
22 interesting study showed that, by targeting the outer membrane protein OmpW instead of the
23 LPS, linear range and LOD were significantly improved to 10^2 - 10^5 cells mL⁻¹ and 43 cells mL⁻¹,
24 respectively [64].

25 An immunosensor with 1-pyrenebutyric acid as a fluorescent reporter system grafted onto
26 multi-walled carbon nanotubes (MWCNT) was developed to target *V. alginolyticus* [28]. The
27 fluorescence intensity was correlated with culturable cell concentrations in the range 9.0×10^2
28 to 1.5×10^6 CFU mL⁻¹ and the LOD was 2.8×10^2 CFU mL⁻¹ when tested with pure bacterial
29 culture. The sensor showed no cross reaction with the 4 other tested bacterial species (*E. coli*,
30 *Pseudomonas aeruginosa*, *S. aureus* and *Bacillus subtilis*) and demonstrated recovery rates in
31 the range of 89.4-94% and 95.9-102.3% when tested with shrimp and fishpond water samples,
32 respectively.

1 A stationary liquid phase lab-on-chip (SLP-LOC) was recently described for *V.*
2 *parahaemolyticus* detection [27] (**Fig. 3**). The device is based on a sandwich immunoassay
3 using magnetic nanoparticles (NP) functionalized with antibodies as capture particles and silica
4 NP coated with horseradish peroxidase (HRP) and antibodies as labeling particles. *V.*
5 *parahaemolyticus* was detected in the range 10^2 - 10^6 CFU mL⁻¹ with an LOD of 100 cells mL⁻¹.
6 The method was then successfully applied to the analysis of spiked oyster samples.

7 Alternatively to antibodies, aptamers constitute an interesting strategy for whole cell
8 detection. These short oligonucleotides selected through an *in vitro* process (SELEX), are able
9 to bind with high affinity and specificity to their target by a conformational change of their
10 structure. Several aptamer-based assays were developed for *V. parahaemolyticus* detection.

11 The first assay, reported by Duan *et al.* [29], was based on a fluorescent “signal off” system.
12 The aptamers were first mixed with the targeted bacteria, followed by the addition of a
13 complementary DNA (cDNA) and a sensitive fluorescent dsDNA dye (AccuBlue®). The
14 hybridization occurring between free aptamers and cDNA formed a double-stranded DNA
15 (dsDNA) revealed by the insertion of AccuBlue®, leading to the generation of a fluorescent
16 signal. The linear dynamic range of detection was 50 to 10^6 CFU mL⁻¹ with an LOD of 35 CFU
17 mL⁻¹. No cross reaction with *Salmonella typhimurium*, *S. aureus*, *E. coli* and *Listeria*
18 *monocytogenes* was observed. When tested with spiked shrimp samples, results were similar to
19 those obtained with plate counting method showing the usefulness of the developed aptasensor
20 for real sample analysis.

21 In a colorimetric assay, aptamers immobilized on magnetic NP were employed as capture
22 probes whereas gold NPs coated with other aptamers and HRP were used as signal probes [24].
23 The aptasensor responses were proportional to the target concentration in the range of 10 - 10^6
24 CFU mL⁻¹, with an LOD of 10 CFU mL⁻¹. No cross reactivity with other *Vibrio* and non-*Vibrio*
25 strains was observed. The sensor validated with spiked water samples offered recoveries values
26 between 92 and 102%.

27 An aptamer-based assay using surface-enhanced Raman scattering (SERS) detection
28 technique was also developed [25]. A first aptamer was immobilized on SiO₂ functionalized
29 with gold NP (SiO₂@Au NPs) to capture the targeted bacteria. The captured cells were then
30 revealed with a second aptamer conjugated to the Cyanine 3 fluorescent dye (**Fig. 4**). A linear
31 relationship between the SERS signal and the target concentration was obtained in the same
32 range as described above [24], and with the same LOD. The developed sensor exhibited no
33 cross reaction and good recovery rates ranging from 91 to 101% for spiked shrimp and water
34 samples, respectively.

3.3. Electrochemical biosensors

The different electrochemical immunosensors reported for the detection of whole *Vibrio* cells are listed in **Table 3**. No electrochemical aptasensor has been published so far for *Vibrio* detection.

The first electrochemical biosensor has been developed by Rao *et al.* [65]. This tool was based on the indirect sandwich ELISA principle, with the use of polyclonal antibodies (PAb) directed towards the pathogen species *V. cholerae*, that were adsorbed on the surface of a screen printed electrode (SPE). Antibodies conjugated to alkaline phosphatase (ALP) were used as enzyme tracer to reveal the bacterial cell binding, and 1-naphtyl phosphate as the enzyme substrate, giving an electroactive product that can be detected *via* an amperometric measurement. The data showed that no cross reaction was observed with the 3 other Gram negative strains tested and that 10^5 cells mL^{-1} could be detected in only 55 minutes. This amperometric immunosensor was then applied to the analysis of spiked water samples detecting as few as 8 CFU mL^{-1} in seawater and 80 CFU mL^{-1} in tap water after an enrichment step [66].

A similar amperometric immunosensor allowing the detection of total *Vibrio* using a biotinylated PAb, immobilized on neutravidine modified surface of SPE was described [67]. An LOD of 4.0×10^2 cells mL^{-1} was obtained without any enrichment step, in a total analytical time of 60 min.

Interestingly, two one-step label-free immunosensors were recently developed. The first one, designed for *V. cholerae* O1 detection, used antibodies covalently immobilized on a CeO_2 nanowire-modified sensor surface of a microelectrode to capture the targets. The resulting complex was detected by impedance analysis with $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as the redox probe [68]. After optimization of experimental parameters an LOD of 1×10^2 CFU mL^{-1} was obtained. Specificity was only assessed with few strains (*E. coli* and *Salmonella* spp.). The second one targeting *V. parahaemolyticus* was applied to real samples [69] (**Fig. 5**). The electrode surface was modified with a magnetic graphene oxide to immobilize antibodies, and an electrochemiluminescence detection was performed, using N-(4-aminobutyl)-N-ethylisoluminol as label, to detect the targeted cells. The proposed immunosensor exhibited ultrasensitive, specific and rapid detection of *V. parahaemolyticus* in seawater (LOD of 5 CFU mL^{-1}), and in seafood (5 CFU g^{-1}).

4. Biosensing platforms for nucleic acid detection

1 Nucleic acid detection is mainly based on the natural affinity of either ssDNA or RNA to its
2 complementary strand, allowing to target specific genes. This strategy based on hybridization
3 has been extensively assessed for DNA microarrays development in biotechnology and clinical
4 diagnostics, and is nowadays transferred towards strip test devices and biosensors, referred as
5 genosensors.

4.1. Strip test devices

Contrary to strip tests based on whole cell detection, mainly used for *V. cholerae* analysis, those based on nucleic acid detection have been developed for 5 well studied pathogen species of *Vibrio* (Table 4).

For all of these reported strip test devices, an amplification step is systematically applied on the extracted nucleic acids to generate DNA amplicons that were always labelled before analysis. As an example, *lolB* amplicons from *V. cholerae* were double tagged in 5' ends with biotin and fluorescein (FITC), and then captured on a glass-fiber membrane through biotin/streptavidin affinity [70]. The FITC was then recognized by anti-FITC antibodies coated with gold NPs to generate visual red lines. This LFA offered a detection sensitivity of 5 ng of PCR products, and a good specificity evaluated using 174 stool samples spiked with *V. cholerae* strains and other enteric bacteria.

In another study, the *ctxA* gene of *V. cholerae* was amplified by a LATE-PCR [45]. The amplicons were labeled with FITC and then bound to capture probes immobilized on the membrane. Hybridization revelation was performed using gold NPs coated with anti-FITC antibodies. This LFA showed sensitivities of 1 pg of pure genomic DNA and 10 CFU mL⁻¹ of toxigenic *V. cholerae*. The biosensor demonstrated 100% specificity using spiked stool and a complete agreement with the results of the ELISA method.

Loop-mediated isothermal amplification (LAMP) coupled with a LFA using the *rpoX* gene as amplification target was described for *V. alginolyticus* detection [43]. The biotinylated amplicons were first hybridized to a FITC labeled probe recognized by gold-conjugated anti-FITC antibodies. This complex was then captured on the membrane using streptavidin. LODs of the assay were 1.8 x 10² CFU mL⁻¹ and 2 x 10³ CFU g⁻¹ for pure cultures and spiked shrimp samples, respectively. The same method coupling LAMP with LFA was applied to the detection of other *Vibrio* species. In pure culture, the LODs obtained for *V. alginolyticus*, *V. parahaemolyticus* [48] and *V. harveyi* [49] were in the range of 10² CFU mL⁻¹, which was ten-fold lower than that observed for *V. vulnificus* [42]. A similar ratio between the LODs was also observed for real samples spiked with the same *Vibrio* strains.

In order to increase the sensitivity of the detection, an isothermal amplification technique, the cross-priming amplification, using multiple cross linked primers was combined with vertical flow assay to target the *tlh* gene of *V. parahaemolyticus* [41]. Amplicons were doubled tagged with biotin and FITC, and hybridization was performed as described above, leading to very interesting LODs of 1.8 CFU mL⁻¹ and 18 CFU g⁻¹ for pure culture and spiked oyster

1 samples, respectively. In another study, the *toxR* gene of *V. parahaemolyticus* was detected by
2 using the same lateral flow principle coupled with a multiple cross displacement amplification,
3 a novel isothermal amplification technique that yield amplicons as low as 3 bacterial cells [71].
4 The test allowed the detection as low as 10 fg of DNA and 4.2×10^2 CFU mL⁻¹ for pure cultures
5 and spiked oyster samples, respectively. Among the 143 tested strains, no cross-reaction was
6 observed with other *Vibrio* and non-*Vibrio*.

7 A more innovative procedure was proposed by Park *et al.* for *V. parahaemolyticus* detection,
8 using an microfluidic system integrating DNA extraction, LAMP amplification and LFA [52]
9 (**Fig. 6**). Amplicons doubled-tagged with biotin and digoxigenin were immobilized on strips
10 using anti-digoxigenin. Visual purple lines were obtained by addition of streptavidin coated
11 gold NPs. The assay exhibited an LOD of 10⁴ CFU mL⁻¹. By incorporating the extraction,
12 amplification and detection steps in only one device, this rotary microfluidic system offers a
13 rapid and a high- throughput analysis as well as multiplexing possibilities.

14 4.2. Optical biosensors

15 Few optical biosensors have been developed for the detection of nucleic acids from *Vibrio*
16 species (**Table 5**). The first one, proposed by Lee *et al.* in 2003, was based on multiplex PCR
17 followed by a colorimetric sandwich hybridization assay, allowing the simultaneous detection
18 of *V. vulnificus*, *V. cholerae*, and *V. parahaemolyticus* [72]. After amplification, amplicons
19 were hybridized between a phosphorylated capture probe immobilized on the surface of
20 functionalized microplate and a biotinylated signal probe recognized by a ALP-avidin
21 conjugate. When tested with oyster tissue homogenates, the assay allowed the detection as low
22 as 10² CFU g⁻¹ for each pathogen.

23 These last years, other optical genosensors that did not require PCR amplification before
24 nucleic acid analysis were developed. A label-free photoluminescent device, based on target
25 DNA hybridization with complementary DNA probes coated onto nanostructured magnesium
26 oxide NPs, was designed for *V. cholerae* detection [50]. Photoluminescence signal was
27 proportional to the target DNA concentration in the range 100-500 ng μ L⁻¹ with an LOD of
28 3.133 ng μ L⁻¹. More recently, the detection of *V. cholerae ompW* gene was achieved using a
29 sandwich hybridization format device in which the target was recognized by both capture
30 probes coated on magnetic NPs and FAM labeled probes immobilized on gold NPs [56]. The
31 reported device exhibited linear relationship between fluorescence emission and target DNA
32 concentration in the range 10 to 250 ng mL⁻¹, with an LOD value of 2.34 ng mL⁻¹.

Due to its high copy number in prokaryote genomes, the presence of highly conserved regions and its *a priori* single stranded form, the rRNA molecule has been intensively used in genosensors for pathogen detection [73]. However, only one study reported the detection of *Vibrio* using rRNA instead of DNA molecules [51]. In this system, the target was bound between an immobilized capture probe and a labelled signal probe and hybridization event was revealed by chemiluminescence (**Fig. 7**). The described assay showed high specificity and an LOD of 5 ng μL^{-1} of RNA. *Vibrio* detection was achieved using pure cultures as well as spiked and real environmental samples. The study demonstrated that the microplates could be coated beforehand with capture probes and stored at 4°C for at least 1 month, in order to significantly reduce analysis time.

We could notice that the LOD values reported for these optical biosensors were not converted into CFU mL^{-1} which makes comparison with other methods difficult.

4.3. Electrochemical biosensors

The different electrochemical genosensors reported for the detection of nucleic acids from *Vibrio* bacteria are summarized in **Table 6**.

The first electrochemical genosensor was reported in 2008, and used amperometry for the detection of PCR amplicons from *lolB* gene of *V. cholerae* [38]. Amplicons were labelled both by biotin for the immobilization on streptavidin modified surface of a carbon SPE, and by fluorescein. They were then detected with HRP conjugated anti fluorescein antibodies, followed by the addition of 3,3',5,5'-Tetramethylbenzidine and H_2O_2 . The oxidation signal was analyzed using intermittent pulse amperometry, achieving an LOD of 10 CFU mL^{-1} of *V. cholerae* under the optimum conditions, and a good specificity. This system was then modified using asymmetric PCR to obtain ssDNA that could be integrated in a sandwich type hybridization strategy. This new format, associated with chrono-amperometry detection provided a good sensitivity (0.85 ng μL^{-1}) and specificity of the analysis [39].

Multiplex LATE-PCR assay was proposed to generate ssDNA amplicons from *toxR* gene that were integrated in an electrochemical genosensor also using a sandwich hybridization format. The complex was detected by an anti-FITC ALP conjugate that converts the substrate into electroactive α -naphthol, detected by amperometric measurement [53]. Nucleic acids extracted from bacterial cultures and from spiked stools samples were used to validate the reported system. Two years later, the same research group has proposed a simplified version of this genosensor by developing a dry-reagent LATE PCR that just needs addition of DNA

1 template and water to reconstitute the amplification mix, thus reducing the complexity of the
2 assay [40].

3 Interestingly, since 2013 magnetic beads and NPs were often included in the development
4 of electrochemical genosensors for *Vibrio* detection. As an example, the use of streptavidin
5 magnetic beads and antibody conjugated gold NPs was reported for the analysis of asymmetric
6 PCR amplicons from *V. cholerae* *lolB* gene, in order to increase the surface area for
7 bioreactions, and to label the complex, respectively [74]. The new biosensor was successfully
8 applied to the analysis of spiked stool samples, using differential pulse anodic stripping
9 voltammetry (DPASV) for the electrochemical detection of the chemically dissolved gold NPs,
10 leading to an LOD of 10^3 CFU mL⁻¹ of *V. cholerae*. A new strategy, developed by the same
11 authors, used gold NPs loaded onto a latex microsphere carrier to amplify the signal, and thus
12 to improve the sensitivity of the assay [46]. An LOD of 2 CFU mL⁻¹ of *V. cholerae* in spiked
13 stool samples was recorded using DPASV. Sensitivity of this approach was lower than that of
14 other methods. Furthermore, an internal control was incorporated to this system to provide
15 instant validation of the results, thus providing maximum diagnosis reliability. The new
16 platform was validated with 38 reference bacterial strains, along with 145 clinical isolates, with
17 a total assay time from sampling to measurement of less than 6h comprising 3h of enrichment
18 step [47]. In another work, gold NPs adsorbed on layer-by-layer modified latex were also
19 integrated in a novel lyophilized reagent-based test. Measurements were performed following
20 the same procedure as described above using either PCR or LAMP products for preliminary
21 assays [44]. Interestingly, the lyophilized reagents were stable at room temperature for up to 1
22 month.

23 Two genosensors for the detection of *V. cholerae* O1 were proposed by the same research
24 team using short fragments of genomic DNA, without the need of PCR amplification before
25 analysis. These devices are both based on the use of nanostructured magnesium oxide for the
26 electrode fabrication, either modified by chitosan [54], or grafted carboxyl functionalized
27 MWCNT [55]. In the first method, DPV was applied to study the electrochemical response of
28 methylene blue (MB), used as a redox probe to characterize the DNA complex formation. An
29 LOD of 35.2 ng μ L⁻¹ of DNA was obtained, associated to a fast response time of 3 seconds
30 [54]. In the second one, electrochemical impedance spectroscopy (EIS) was conducted with
31 $[\text{Fe}(\text{CN})_6]^{3-/4-}$ as redox probe, allowing a similar LOD of 21.7 ng μ L⁻¹ of DNA in 25 seconds
32 [55]. Although these two genosensors showed interesting results with *V. cholerae* strains, their
33 specificity was not tested with genomic DNA from other bacteria. Only interferences were
34 assessed with glucose, cholesterol, ascorbic acid and sodium chloride.

1 Recently, a PCR-free electrochemical genosensor was adapted to the detection of *V.*
2 *parahaemolyticus*, based on SPE modified with gold nanoparticles, and MB as the redox probe
3 [57] (Fig. 8). The hybridization reaction between the capture probe and the ssDNA molecule
4 was recorded through DPV, with an LOD of 2.6 pM. The reported portable genosensor was
5 successfully applied on spiked cockle samples. Another study reported the development of a
6 PCR-free genosensor for *V. cholerae* detection using latex-gold NPs as DNA probe
7 immobilization support in order to improve the sensitivity of the assay [76]. The system was
8 based on a sandwich hybridization format in which the target was bound between an
9 immobilized capture probe and a signal probe. The hybridization event was monitored by DPV
10 using an electroactive anthraquinone intercalator. The device allowed to detect as low as $1.0 \times$
11 10^{21} M of complementary DNA and no cross reaction with *Citrobacter freundii* was observed.
12 However, the genosensor was not tested with real samples.

13 5. Conclusions

14 *Vibrio* detection still primarily relies on culture approach which take days to be completed.
15 The development of sensitive, specific and rapid tools to detect these bacteria in the
16 environment remains challenging. These last years, biosensors and strip tests have been
17 proposed to meet these criteria. Compared to culture method, these tools allow to significantly
18 reduce the assay time (from days to hours) and to detect the viable but non culturable
19 microorganisms.

20 Thus, this review aims to illustrate the current state of the art in biosensors and strip tests
21 development for *Vibrio* monitoring. We directly noticed that the large majority of the devices
22 designed for *Vibrio* detection was focused on the analysis of species pathogenic for human,
23 especially *V. cholerae*. Surprisingly, despite the important economic losses attributed to some
24 *Vibrio* in aquaculture, the use of biosensing platforms in this area is still in its infancy (Bonnin-
25 Jusserand et al., 2017). We chose to deal with whole cell and nucleic acid detection since tools
26 developed for human *Vibrio* can be transferred to the analysis of environmental *Vibrio*, notably
27 those responsible for diseases in aquaculture settings. Toxin detection, restricted to *V. cholerae*,
28 was not addressed (Cecchini et al., 2016). We showed that each of the described device has its
29 own advantages and disadvantages.

30 Although strip tests constitute fast, low cost and easy-to-use devices, results are mostly
31 qualitative or semi-quantitative (Sajid et al., 2015). Thus, strip tests are often used to perform
32 a preliminary screening which can be confirmed by fully quantitative methods, such as

1 biosensors. Compared to optical biosensors, the sensitivity and specificity of the
2 electrochemical detection is restricted (Velusamy et al., 2010). However, due to their low cost
3 and their miniaturization capacities, electrochemical devices are nowadays preferred by most
4 end users.

5 The sensitivity of the developed tools is a critical point for bacteria detection. Thus, pushing
6 sensitivity towards single cell limit of detection is essential to prevent infections and to ensure
7 environmental safety. Besides, the specificity of the developed devices constitutes another
8 important aspect. However, in most of the studies presented here, this parameter was evaluated
9 only on a limited number of targeted and non-targeted strains. Furthermore, the applicability of
10 the devices was rarely tested on naturally contaminated samples.

11 In the case of nucleic acid analysis, the extraction is a critical step. Although the development
12 of a microfluidic system integrating DNA extraction was recently reported [52], efforts have
13 still to be made to automate and incorporate the extraction step into biosensing platforms.
14 Another point that we have to emphasize is that the majority of the reviewed tools requires a
15 preliminary amplification of the target, increasing analysis time.

16 These last years, nanotechnologies have attracted a great interest for the development and
17 improvement of biosensors. We showed that the most recent devices designed for *Vibrio*
18 detection, included nanomaterials such as nanoparticles, nanotubes and nanowires. The large
19 specific surface area and the high electrical conductivity of these materials allow to enhance
20 the performances of biosensors with increased sensitivities and lowered detection limits
21 (Holzinger et al., 2104).

22 In conclusion, two levels could be considered to detect *Vibrio* in the environment using
23 biosensing platforms. In order to rapidly diagnose and prevent the contamination directly on
24 the point of care, the strip tests could constitute interesting screening systems. Then, biosensors
25 could ensure *Vibrio* monitoring by performing repeated measures. However, although some
26 biosensors allow analysis on the field, these devices are still mainly used in laboratory. In order
27 to bring biosensors from the lab to the field, the future trend is towards the development of
28 integrated systems incorporating all the analytical steps from sample preparation to data
29 collection.

Acknowledgements

Authors would like to acknowledge the University of Perpignan Via Domitia, France, for its financial support through Doctoral School 305.

Conflicts of interest : none

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Figure and table captions :

Fig. 1 : General principle of biosensor devices.

Fig. 2 : Lateral flow strip test principle. **(A)** The sample is loaded on the sample pad ; **(B)** The antibodies conjugated to gold nanoparticles bind to the analyte ; **(C)** The analyte recognition leads to a response on the test line and the proper migration of the sample through the membrane is validated by a response on the control line.

Fig. 3 : Schematic representation of the detection principle. **(a)** Labeling particle and capture particle. **(b)** Detection process using the SLP LOC. The sample was added with LPs and CPs in the sample chamber, and moved to the detection chamber using a magnet. Detection was performed with TMB as the substrate of HRP. Reproduced from Ref. [27], with permission from Elsevier.

Fig. 4 : Schematic illustration of SERS-based aptasensor for *V. parahaemolyticus* detection using SiO₂@Au core/shell NPs as the substrate. Reproduced from Ref. [25], with permission from Elsevier.

Fig. 5 : The schematic diagram for the preparation of multi-functionalized graphene oxide and the ECL immunosensor. Reproduced from Ref. [69], with permission from Elsevier.

Fig. 6 : (a) A digital image of the integrated rotary microdevice (b) Schematic illustration of the integrated rotary microdevice for the DNA extraction, the LAMP reaction, and the lateral flow strip detection. (c-i) Schematic design of the solid phase DNA extraction unit and the fluorescence images of the FAM-labelled DNA adsorbed glass microbeads, (c-ii) Schematic image of the LAMP amplification of target DNA (c-iii) Schematics of a lateral flow strip which consists of a buffer loading pad, a conjugate pad that contains streptavidin coated gold NPs, a detection zone where an anti-Digoxigenin, an anti-Texas red, a biotin are immobilized in the test line 1, the test line 2 and the control line, respectively, and an absorbent pad. Reproduced from Ref. [52], with permission from Elsevier.

Fig. 7 : Schematic illustration of the chemiluminescent DNA-based assay for *Vibrio* spp. detection [51].

Fig. 8 : Schematic drawing of electrochemical DNA biosensor based on polylactide-stabilized gold nanoparticles modified electrode. Reproduced from Ref. [75], with permission from Elsevier.

Table 1 : Characteristics of the strip tests developed for whole *Vibrio* cell detection. - : not defined.

Table 2 : Characteristics of the optical biosensors designed for whole *Vibrio* cell detection. - : not defined.

Table 3 : Characteristics of the electrochemical biosensors developed for whole *Vibrio* cell detection. - : not defined.

Table 4 : Characteristics of the strip tests developed for the detection of nucleic acids extracted from *Vibrio* bacteria.

Table 5 : Characteristics of the optical biosensors designed for the detection of nucleic acids extracted from *Vibrio* bacteria. - : not defined.

Table 6 : Characteristics of the electrochemical biosensors developed for the detection of nucleic acids extracted from *Vibrio* bacteria. - : not defined.

Table 1

Target	Principle	Bioreceptor	Reference method	Sensitivity	Specificity	Evaluation of real samples	Reference
<i>V. cholerae</i> O1/O139	LFA (Crystal VC [®] RDT)	Antibody	Culture of frozen stool or stool on filter paper	94.2%-100%	84-100%	Frozen stools and rapid culture (6h) of stools collected on filter paper	[58]
<i>V. cholerae</i> O1/O139	LFA (Crystal VC [®] RDT)	Antibody	Culture on rectal swabs (multiplex PCR when RDT + / culture -)	93-96%	92-98%	Rectal swabs with 4h enrichment in APW	[36]
<i>V. cholerae</i> O1/O139	LFA (Crystal VC [®] RDT)	Antibody	PCR on rectal swabs	97%	71-76%	Fresh, bulk stools	[31]
<i>V. cholerae</i> O1	LFA (Crystal VC [®] RDT)	Antibody	Culture of rectal swabs and bulk stools	93-97%	77-97%	Rectal swabs with 4h enrichment in APW and fresh and bulk stools	[59]
<i>V. cholerae</i> O1/O139	LFA (Crystal VC [®] RDT)	Antibody	PCR on wet filters inoculated with stool samples and wet filter paper surnageants	86.1%	100%	Stool samples with 4-6h enrichment in APW	[37]
<i>V. cholerae</i> O1/O139	LFA (Crystal VC [®] RDT)	Antibody	Culture of water samples	89%	100%	Spiked water samples with 6-24h enrichment in APW	[60]
<i>V. cholerae</i> O1	LFA (Crystal VC [®] RDT)	Antibody	Culture of watery stools	65.6-75%	91.8-98.4%	Watery stool samples with/without 6h enrichment in APW	[35]
<i>V. cholerae</i> O1	LFA (Crystal VC [®] RDT)	Antibody	Culture of rectal swabs	93%	49%	Fresh, bulk stools	[61]
<i>V. cholerae</i> O1	LFA (Crystal VC [®] RDT)	Antibody	Culture of bulk stools	93-94%	67-76%	Fresh, bulk stools	[30]
<i>V. cholerae</i> O1/O139	LFA (Crystal VC [®] RDT)	Antibody	Culture of bulk stools	92%	73%	Fresh, bulk stools	[32]

<i>V. cholerae</i> O1/O139	LFA (Crystal VC® RDT)	Antibody	Culture and PCR on stool samples	88.2-92.9%	60.4-88.6%	Stools	[34]
<i>V. cholerae</i> O1 serotype Ogawa	LFA	Antibody	Culture on diarrhea feces	10 ⁴ CFU mL ⁻¹	100%	Diarrhea patients feces	[62]
<i>V. cholerae</i> O139	LFA	Antibody	-	10 ⁶ CFU mL ⁻¹	No cross reaction	Rectal swab specimens and specimens of enriched APW	[21]
<i>V. cholerae</i> O139	LFA	Antibody	-	10 ⁴ CFU mL ⁻¹	No cross reaction	Blood clams, mussels, oysters and shrimp spiked samples	[22]
<i>V. cholerae</i> O1/O139	LFA (Crystal VC® RDT)	Antibody	Culture and RT-PCR on stool samples	-	-	Watery spiked stool samples	[33]
<i>V. parahaemolyticus</i>	LFA	Antibody	Culture on shrimp hepatopancreas and fecal human samples	1.2 x 10 ³ CFU mL ⁻¹	No cross reaction	Shrimp and diarrheal stool samples	[26]
<i>V. anguillarum</i>	LFA	Antibody	-	10 ² CFU mL ⁻¹	No cross reaction	-	[23]

LFA : Lateral Flow Assay ; RDT : Rapid Diagnostic Test ; RT-PCR : Reverse Transcriptase PCR ; APW : Alkaline Peptone Water

Table 2

Target	Detection technique	Bioreceptor	LOD	Specificity	Evaluation of real samples (% recoveries)	Reference
<i>V. cholerae</i> O1	SPR	Antibody	10 ⁵ cells mL ⁻¹	No cross reaction	-	[63]
<i>V. cholerae</i> O1	SPR	Antibody	43 cells mL ⁻¹	No cross reaction	-	[64]
<i>V. alginolyticus</i>	Fluorescence	Antibody	2.8 x 10 ² CFU mL ⁻¹	No cross reaction	Spiked shrimp samples (89.4-94.0%) Fishpond water (95.9-102.3%)	[28]
<i>V. parahaemolyticus</i>	Colorimetry	Antibody	100 cells mL ⁻¹	No cross reaction	Spiked oyster samples (-)	[27]
<i>V. parahaemolyticus</i>	Fluorescence	Aptamer	35 CFU mL ⁻¹	No cross reaction	Spiked shrimp samples (-)	[29]
<i>V. parahaemolyticus</i>	Colorimetry	Aptamer	10 CFU mL ⁻¹	No cross reaction	Spiked water (92.0-102.0%)	[24]
<i>V. parahaemolyticus</i>	SERS	Aptamer	10 CFU mL ⁻¹	No cross reaction	Spiked shrimp samples (91.0-97.8%) Seawater (99.4-101.0%) Groundwater (98.0-100.6%)	[25]

SERS : Surface Enhanced Raman Spectroscopy ; SPR : Surface Plasmon Resonance

Table 3

Target	Detection technique	Bioreceptor	LOD	Specificity	Evaluation of real samples (LOD)	Reference
<i>V. cholerae</i> O1	Amperometry	Antibody	10 ⁵ CFU mL ⁻¹	No cross reaction	-	[65]
<i>V. cholerae</i> O1	Amperometry	Antibody	-	No cross reaction	Spiked seawater and groundwater (8 CFU cells mL ⁻¹) Spiked tap water and sewer water (80 CFU cells mL ⁻¹)	[66]
<i>Vibrio</i> spp.	Amperometry	Antibody	4.0 x 10 ² cells mL ⁻¹	No cross reaction	-	[67]
<i>V. cholerae</i> O1	Impedance	Antibody	10 ² CFU mL ⁻¹	No cross reaction	-	[68]
<i>V. parahaemolyticus</i>	Electrochemi-luminescence	Antibody	5 CFU mL ⁻¹	No cross reaction	Spiked seawater (5 CFU mL ⁻¹) and seafood (5 CFU g ⁻¹)	[69]

Table 4

Target (gene)	Principle	Bioreceptor	LOD	Specificity	Evaluation of real samples (LOD)	Reference
<i>V. cholerae</i> (<i>lolB</i>)	PCR + LFA	Antibody	5 ng of amplicons	No cross reaction	Spiked stool samples	[70]
<i>V. cholerae</i> (<i>ctxA</i>)	LATE-PCR + LFA	DNA probe	1 pg of genomic DNA 10 CFU mL ⁻¹	No cross reaction	Spiked stool samples	[45]
<i>V. alginolyticus</i> (<i>rpoX</i>)	LAMP + LFA	DNA probe	1.8 x 10 ² CFU mL ⁻¹	No cross reaction	Spiked whiteleg shrimp samples (2.0 x 10 ³ CFU g ⁻¹)	[43]
<i>V. parahaemolyticus</i> (<i>tlh</i>)	LAMP + LFA	DNA probe	120 CFU mL ⁻¹	No cross reaction	Spiked shrimp samples (1.8 x 10 ³ CFU g ⁻¹)	[48]
<i>V. harveyi</i> (<i>vvhP2</i>)	LAMP + LFA	DNA probe	1.1 x 10 ² CFU mL ⁻¹	No cross reaction	Spiked shrimp samples (1.8 x 10 ³ CFU g ⁻¹)	[49]
<i>V. vulnificus</i> (<i>rpoS</i>)	LAMP + LFA	DNA probe	1.5 x 10 ³ CFU mL ⁻¹	No cross reaction	Spiked oyster samples (1.2 x 10 ⁴ CFU g ⁻¹)	[42]
<i>V. parahaemolyticus</i> (<i>tlh</i>)	CPA + vertical flow	Antibody	1.8 CFU mL ⁻¹	No cross reaction	Spiked oyster samples (18 CFU g ⁻¹)	[41]
<i>V. parahaemolyticus</i> (<i>toxR</i>)	MCDA + LFA	Antibody	10 fg of DNA 4.2 x 10 ² CFU mL ⁻¹	No cross reaction	Spiked oyster homogenates (4.2 x 10 ² CFU g ⁻¹)	[71]
<i>V. parahaemolyticus</i> (<i>toxR</i>)	Microfluidic system incorporating LAMP + LFA	Antibody	10 ⁴ CFU mL ⁻¹	No cross reaction	Spiked milk samples (5.0 x 10 ³ CFU/5μL)	[52]

LFA : Lateral Flow Assay ; LATE-PCR : Linear After The Exponential PCR ; LAMP : Loop mediated isothermal AMplification ; CPA : Cross Priming Amplification ; MCDA : Multiple Cross Displacement Amplification

Table 5

Target (gene)	PCR amplification	Detection technique	Bioreceptor	LOD	Specificity	Evaluation of real samples (LOD)	Reference
<i>V. vulnificus</i> , <i>V. cholerae</i> , <i>V. parahaemolyticus</i> (<i>vvh</i> , <i>ctx</i> , <i>tlh</i>)	Yes	Colorimetry	DNA probe	-	No cross reaction	Oyster tissue homogenates (10 ² CFU g ⁻¹)	[72]
<i>V. cholerae</i> (nd)	No	Photoluminescence	DNA probe	3.133 ng µL ⁻¹	-	-	[50]
<i>V. cholerae</i> (<i>ompW</i>)	No	Fluorescence	DNA probe	2.34 ng mL ⁻¹	No cross reaction	-	[56]
<i>Vibrio</i> spp. (<i>16S rRNA</i>)	No	Chemiluminescence	DNA probe	5 ng µL ⁻¹	No cross reaction	Phytoplankton and spiked seawater (nd)	[51]

Table 6

Target (gene)	PCR amplification	Detection technique	Bioreceptor	LOD	Specificity	Evaluation of real samples (LOD)	Reference
<i>V. cholerae</i> (<i>lolB</i>)	Yes	Intermittent Pulse Amperometry	DNA probe	10 CFU mL ⁻¹	No cross reaction	-	[38]
<i>V. cholerae</i> (<i>lolB</i>)	Yes	Chrono amperometry	DNA probe	0.85 ng µL ⁻¹	No cross reaction	-	[39]
<i>V. cholerae</i> (<i>toxR</i>)	Yes	Amperometry	DNA probe	8-61 pM	No cross reaction	Spiked stools (-)	[53]
<i>V. cholerae</i> (<i>toxR</i>)	Yes	Amperometry	DNA probe	10 CFU mL ⁻¹	No cross reaction	Spiked stools (-)	[40]
<i>V. cholerae</i> (<i>lolB</i>)	Yes	DPASV	DNA probe	5 pg µL ⁻¹	No cross reaction	Spiked stools (10 ³ CFU mL ⁻¹)	[74]
<i>V. cholerae</i> (<i>lolB</i>)	Yes	DPASV	DNA probe	-	No cross reaction	Spiked stools (2 CFU mL ⁻¹)	[46]
<i>V. cholerae</i> (<i>lolB</i>)	Yes	DPASV	DNA probe	0.5 ng mL ⁻¹ 10 CFU mL ⁻¹	No cross reaction	Spiked stools (-)	[47]
<i>V. cholerae</i> (<i>lolB</i>)	Yes	DPASV	DNA probe	1 fM	No cross reaction	-	[44]
<i>V. cholerae</i> O1 (nd)	No	DPV	DNA probe	35.2 ng µL ⁻¹	-	-	[54]
<i>V. cholerae</i> O1 (nd)	No	EIS	DNA probe	21.7 ng µL ⁻¹	-	Human blood/serum (-)	[55]
<i>V. parahaemolyticus</i> (nd)	No	DPV	DNA probe	2.6 pM	No cross reaction	Spiked cockle samples (-)	[57]

<i>V. cholerae</i> (nd)	No	DPV	DNA probe	1.0 x 10 ⁻²¹ M of complementary DNA	No cross reaction	-	[76]
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DPASV : Differential Pulse Anodic Stripping Voltammetry ; DPV : Differential Pulse Voltammetry ; EIS : Electrochemical Impedance Spectroscopy

Figure 1

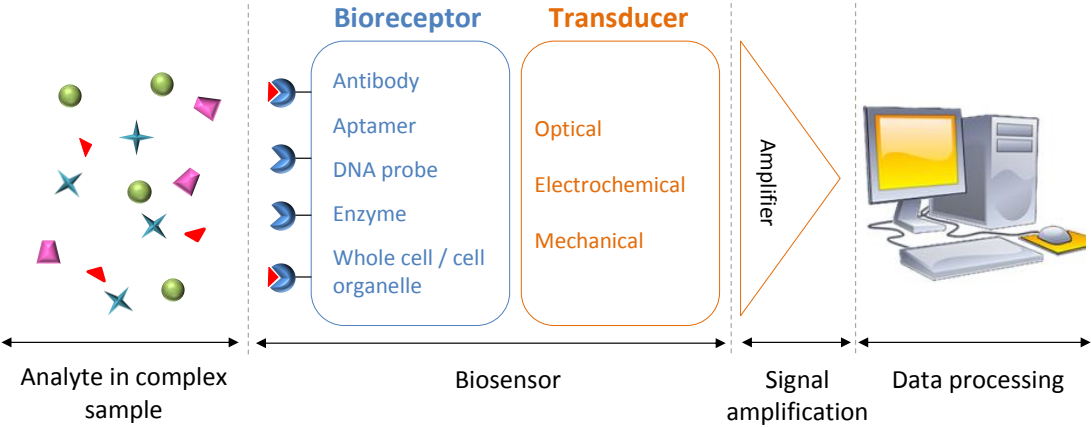


Figure 2

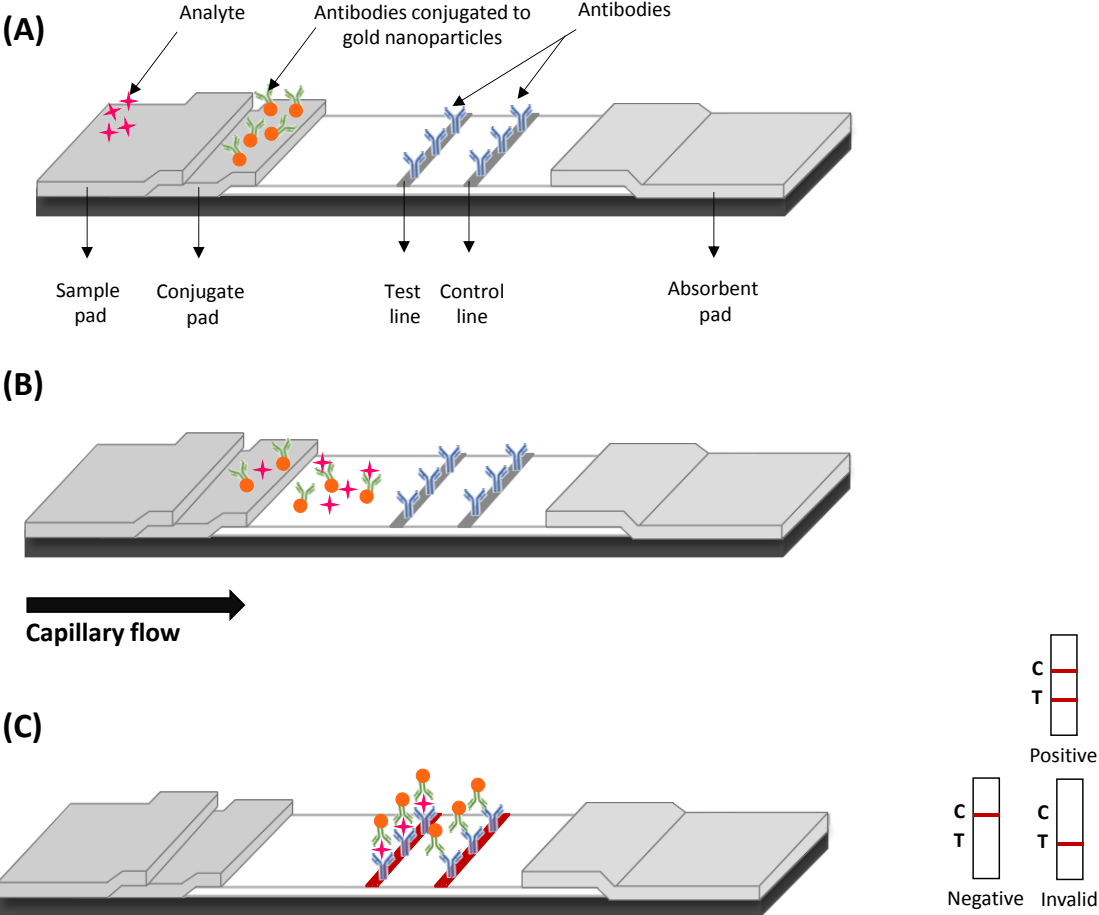


Figure 3

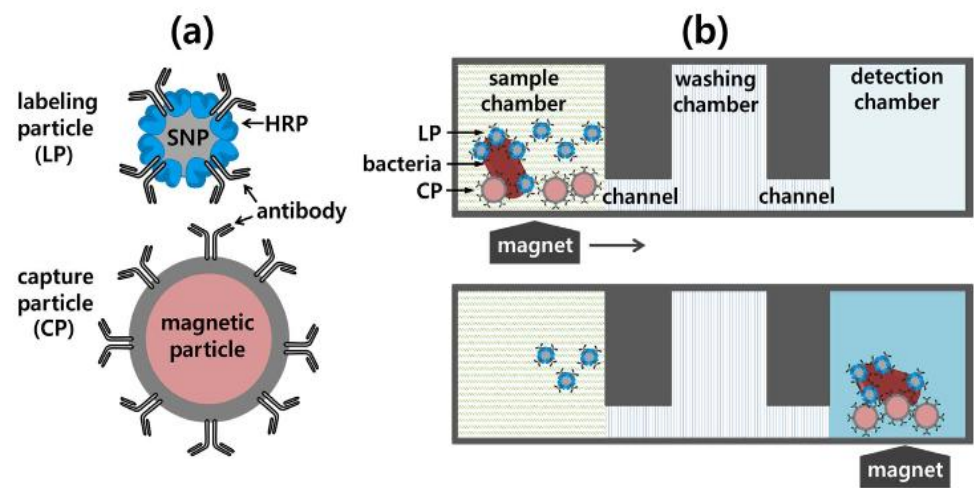


Figure 4

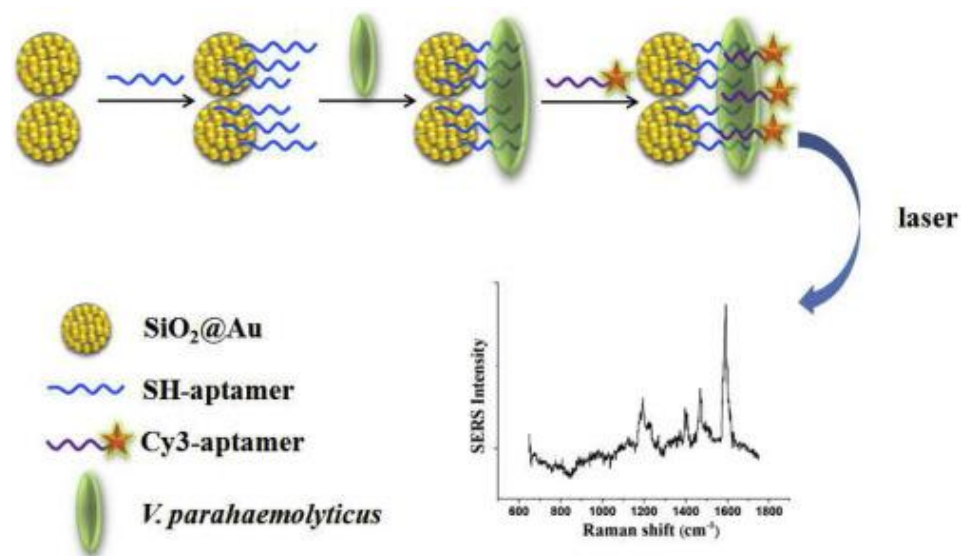


Figure 5

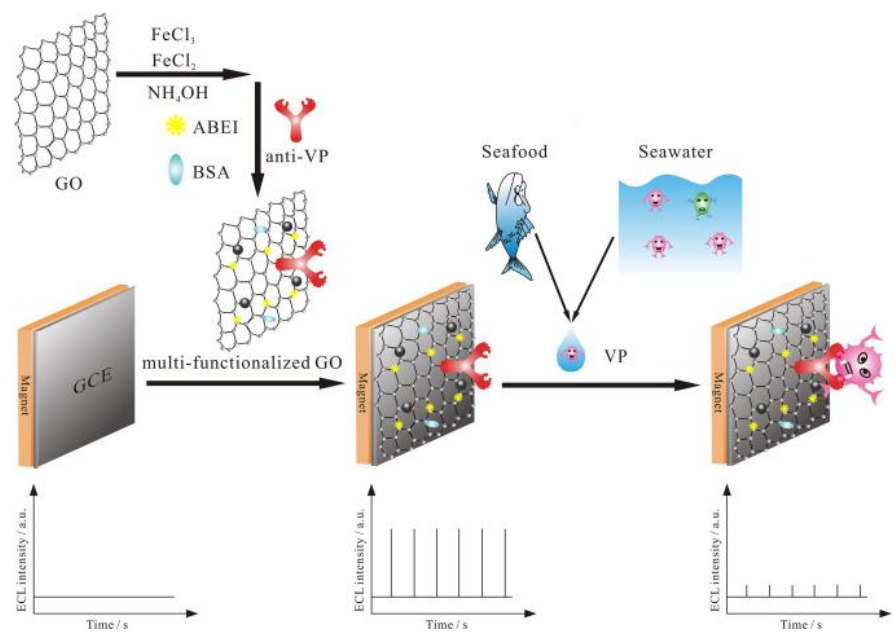


Figure 6

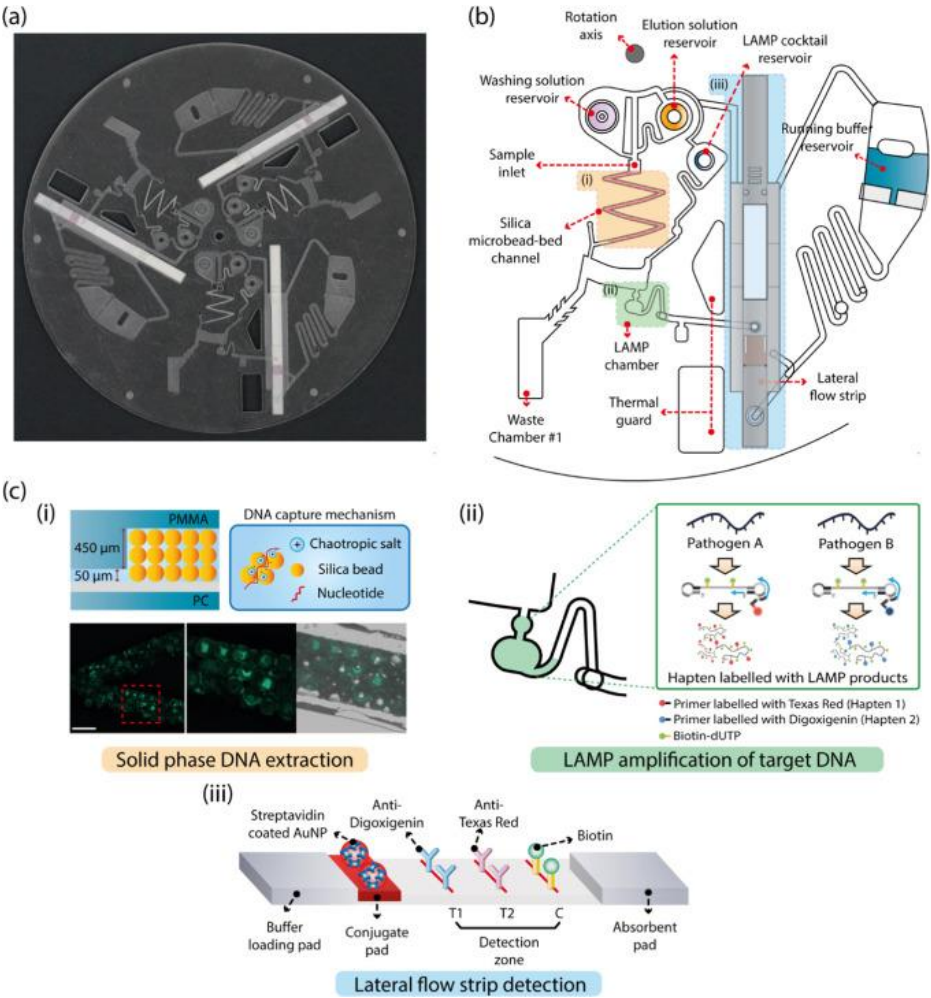


Figure 7

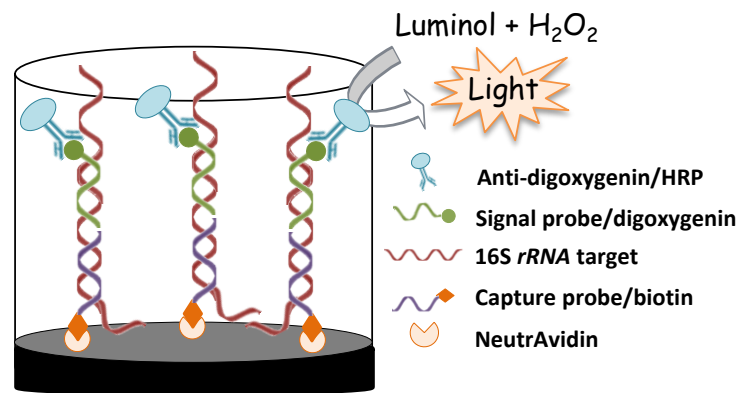


Figure 8

