

Method and Sensor for Detecting *Vibrio* Bacteria

Field of Invention

The present invention relates generally to the field of biotechnology. In particular, the invention relates to methods of detecting the presence of *Vibrio spp.* in a sample.

Background

Vibrio is a genus of bacteria that is pathogenic and can cause mortality to various fish, shrimps, or other aquaculture organisms. Detection of *Vibrio* in aquaculture water and aquaculture organisms is important to prevent disease breakout. Currently *Vibrio* detection relies on “standard” bacteria culture and plate count (requires at least 1-2 days in lab), or Polymerase Chain Reaction (PCR) that typically requires pre-enrichment culture (18-20 h) followed by at least 2 h of assay time, and sophisticated lab equipment. Developing rapid, on-site, and culture-free *Vibrio* detection method is in demand. Specifically, the sensor should be able to detect *Vibrio spp.* in fish tank water within range 900-50,000 CFU/ ml, to early monitor *Vibrio spp.* presence when infection to fish is potentially high when the *Vibrio spp.* reaches 50,000 CFU/ml. It should be able to detect *Vibrio* from fish tissue samples in order to identify *Vibrio* and *non-Vibrio* infection.

It would be desirable to overcome or ameliorate at least one of the above-described problems, or at least to provide a useful alternative.

Summary

Disclosed herein is a method of detecting the presence of *Vibrio spp.* in a sample, the method comprising:

- a) contacting the sample with a substrate to capture *Vibrio spp.* onto the substrate; and
- b) contacting the substrate with an anti-*Vibrio* aptamer or antibody nanoparticle conjugate, wherein specific binding of the anti-*Vibrio* aptamer or antibody nanoparticle conjugate to the *Vibrio spp.* on the substrate indicates the presence of *Vibrio spp.* in the sample; wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCAG-3'

(SEQ ID NO: 1) or a derivative thereof.

Disclosed herein is a method of detecting the presence of *Vibrio spp.* in a liquid sample, the method comprising:

- a) flowing the liquid sample through a porous substrate to capture *Vibrio spp.* on the porous substrate; and
- b) contacting the porous substrate with an anti-*Vibrio* aptamer or antibody nanoparticle conjugate, wherein specific binding of the anti-*Vibrio* aptamer or antibody nanoparticle conjugate to the *Vibrio spp.* on the porous substrate indicates the presence of *Vibrio spp.* in the sample;

wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCAG-3'

(SEQ ID NO: 1) or a derivative thereof.

Disclosed herein is a method of detecting the presence of *Vibrio spp.* in a solid/semi-solid sample, the method comprising:

- a) contacting the sample with a swab to capture *Vibrio spp.* onto the surface of the swab; and
- b) contacting the swab with an anti-*Vibrio* aptamer or antibody nanoparticle conjugate, wherein specific binding of the anti-*Vibrio* aptamer or antibody nanoparticle conjugate to the *Vibrio spp.* on the swab indicates the presence of *Vibrio spp.* in the sample;

wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCAG-3'

(SEQ ID NO: 1) or a derivative thereof.

Disclosed herein is a kit or sensor for performing a method as defined herein.

Disclosed herein is an aptamer comprising a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCAG-3'

(SEQ ID NO: 1) or a derivative thereof.

Disclosed herein is a composition for capturing *Vibrio spp.* from a sample, the composition comprising a substrate and an anti-*Vibrio* aptamer and/or antibody that has been immobilised on the substrate, wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCAG-3'
(SEQ ID NO: 1) or a derivative thereof.

Brief Description of Drawings

Embodiments of the present invention are hereafter described, by way of non-limiting example only, with reference to the accompanying drawings in which:

Figure 1. Fluorescent depletion experiment screening of the affinity for eight anti-*Vibrio* DNA aptamers and two anti-*Vibrio* antibodies (average of at least 3 tests with good bacteria growth).

Figure 2. Conjugation of VHa aptamer to 13 nm gold nanoparticle (AuNP). Stability test of the VHa-AuNP in water, 0.9% NaCl and 3.5% NaCl.

Figure 3. Conjugation of Ab1 antibody to 13 nm gold nanoparticles (AuNPs). Stability test of the Ab1-AuNPs in water, 0.9% NaCl and 3.5% NaCl.

Figure 4. (A) The “Filter and Stain” process. (B) Selective staining of *Vibrio* relative to non-*Vibrio* bacteria, using VHa-AuNPs, at a bacteria concentration of 10^7 CFU/ml for each bacteria.

Figure 5. Detection of *V. harveyi* in a mixture of *V. harveyi* (at various concentrations) and *S. aureus* (at a concentration of 10^7 CFU/ml).

Figure 6. (A) Selectivity of Ab1-AuNPs to *Vibrio* relative to non-*Vibrio* bacteria at a concentration of 10^7 CFU/ml for each bacterium. (B) Detection of *V. parahaemolyticus* at various concentration by Ab1-AuNPs.

Figure 7. (A). Schematic of “Filter and Stain” method for water analysis with gold deposition. (B). Detector reading of filters after VHa-AuNPs staining and gold deposition (12.5 mM HAuCl₄ with 3% H₂O₂). Insert: image of filters with various *Vibrio* concentration after VHa-AuNPs staining and gold deposition.

Figure 8. (A). Schematic of “Swab and Stain” method for biological tissue analysis with nanoparticles staining and gold deposition. (B) and (C) Two sets of swabs images (top) and their corresponding image analysis (bottom). The swabs were dipped in fish kidney homogenates spiked with various bacteria.

Detailed Description

The present specification teaches a method of detecting the presence of *Vibrio spp.* in a sample. The specification teaches a method of detecting the presence of *Vibrio spp.* in a sample, the method comprising: a) contacting the sample with a substrate to capture *Vibrio spp.* onto the substrate; and b) contacting the substrate with an anti-*Vibrio* aptamer or antibody, wherein specific binding of the anti-*Vibrio* aptamer or antibody to the *Vibrio spp.* on the substrate indicates the presence of *Vibrio spp.* in the sample.

In one embodiment, there is provided a method of detecting the presence of *Vibrio spp.* in a sample, the method comprising b) contacting a substrate with an anti-*Vibrio* aptamer or antibody, wherein specific binding of the anti-*Vibrio* aptamer or antibody to the *Vibrio spp.* on the substrate indicates the presence of *Vibrio spp.* in the sample, wherein the substrate has been contacted with a sample to capture *Vibrio spp.* prior to step b).

Disclosed herein is a method of detecting the presence of *Vibrio spp.* in a sample, the method comprising:

- a) contacting the sample with a substrate to capture *Vibrio spp.* onto the substrate; and
- b) contacting the substrate with an anti-*Vibrio* aptamer or antibody nanoparticle conjugate, wherein binding of the anti-*Vibrio* aptamer or antibody nanoparticle conjugate to the *Vibrio spp.* on the substrate indicates the presence of *Vibrio spp.* in the sample;

wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-AGCCGGGGTGGTCATTAGCAGCAGCACAACAGGCAGCACAACAGGCAG-3'
(SEQ ID NO: 1) or a derivative thereof.

In one embodiment, the method comprises detecting anti-*Vibrio* aptamer or antibody that is present on the substrate, wherein an increased level of anti-*Vibrio* aptamer or antibody that is present on the substrate as compared to a reference indicates the presence of *Vibrio spp.* in the sample.

The method may also comprise detecting anti-*Vibrio* aptamer or antibody nanoparticle conjugate that is present on the substrate, wherein an increased level of anti-*Vibrio* aptamer or antibody nanoparticle conjugate that is present on the substrate as compared to a reference indicates the presence of *Vibrio spp.* in the sample.

As used herein, the term “increase” or “increased” may refer to a statistically significant and measurable increase in the level of the anti-*Vibrio* aptamer or antibody, or anti-*Vibrio* aptamer or antibody nanoparticle conjugate, as compared to a reference. The increase in level may be an increase of 1.1 times, 1.2 times, 1.3 times, 1.4 times, 1.5 times, 1.6 times, 1.7 times, 1.8 times, 1.9 times, 2 times, 3 times, 4 times, 5 times, 6 times, 7 times, 8 times, 9 times, 10 times, 11 times, 12 times, 13 times, 14 times, 15 times, 16 times, 17 times, 18 times, 19 times, 20 times, 21 times, 22 times, 23 fold, 24 times, 25 times, 26 times, 27 times, 28 times, 29 times, 30 times, 31 times, 32 times, 33 times, 34 times, 35 times, 36 times, 37 times, 38 times, 39 times, 40 times, 41 times, 42 times, 43 times, 44 times, 45 times, 46 times, 47 times, 48 times, 49 times, 50 times, 51 times, 52 times, 53 times, 54 times, 55 times, 56 times, 57 times, 58 times, 59 times, 60 times, 61 times, 62 times, 63 times, 64 times, 65 times, 66 times, 67 times, 68 times, 69 times, 70 times, 71 times, 72 times, 73 times, 74 times, 75 times, 76 times, 77 times, 78 times, 79 times, 80 times, 81 times, 82 times, 83 times, 84 times, 85 times, 86 times, 87 times, 88 times, 89 times, 90 times, 91 times, 92 times, 93 times, 94 times, 95 times, 96 times, 97 times, 98 times, 99 times or 100 times or anywhere in between as compared to a reference.

The reference may be a substrate that has been contacted with a sample that does not contain *Vibrio spp.*

In one embodiment, a lack of a statistically significant and measurable increase or no

change in the level as compared to a reference indicates the absence of *Vibrio spp.* in the sample.

Vibrio spp.

The term “*Vibrio spp.*” may refer to any Gram-negative bacterium that falls under the *Vibrio spp.* genus, or any serovar thereof. This genus of bacteria is responsible for diseases in a large number of animal species, including in fish, shellfish and humans. Embodiments of this disclosure contain aptamers and/or antibodies that bind to at least one of the *Vibrio* species selected from the group consisting of: *Vibrio aerogenes*, *V. aestivus*, *V. aestuarianus*, *V. agarivorans*, *V. albensis*, *V. alfacensis*, *V. alginolyticus*, *V. anguillarum*, *V. areninigrae*, *V. artabrorum*, *V. atlanticus*, *V. atypicus*, *V. azureus*, *V. brasiliensis*, *V. bubulus*, *V. calviensis*, *V. campbellii*, *V. casei*, *V. chagasii*, *V. cholerae*, *V. cincinnatiensis*, *V. coralliilyticus*, *V. crassostreae*, *V. cyclitrophicus*, *V. diabolicus*, *V. diazotrophicus*, *V. ezurae*, *V. fluvialis*, *V. fortis*, *V. furnissii*, *V. gallicus*, *V. gazogenes*, *V. gigantis*, *V. haliotocoli*, *V. harveyi*, *V. hepatarius*, *V. hippocampi*, *V. hispanicus*, *V. ichthyenteri*, *V. indicus*, *V. kanaloae*, *V. lentus*, *V. litoralis*, *V. logei*, *V. mediterranei*, *V. metschnikovii*, *V. mimicus*, *V. mytili*, *V. natriegens*, *V. navarrensis*, *V. neonates*, *V. neptunius*, *V. nereis*, *V. nigripulchritudo*, *V. ordalii*, *V. orientalis*, *V. pacinii*, *V. parahaemolyticus*, *V. pectenica*, *V. penaeicida*, *V. pomeroyi*, *V. ponticus*, *V. proteolyticus*, *V. rotiferianus*, *V. ruber*, *V. rumoiensis*, *V. salmonicida*, *V. scophthalmi*, *V. splendidus*, *V. superstes*, *V. tapetis*, *V. tasmaniensis*, *V. tubiashii*, *V. vulnificus*, *V. wodanis*, and *V. xuii*.

In some embodiments, the bacterium is selected from the group consisting of *Vibrio parahaemolyticus*, *Vibrio vulnificus*, *Vibrio harveyi* and *Vibrio alginolyticus*. In some embodiments, the methods herein detect living *Vibrio* bacteria. In some embodiments *Vibrio spp.* is present in the sample along with at least one other species of microorganism from a different genus, e.g., another non-*Vibrio* bacterial species.

Substrate

The term “substrate” refers any solid support suitable for capturing *Vibrio spp.* A substrate may be made from woven or non-woven fabric of any suitable material. Suitable materials include but are not limited to nylon, nitrocellulose, regenerated cellulose, activated agarose,

diazotized cellulose, latex, polyvinylidene fluoride (PVDF), plastic, polystyrene, glass, or a combination thereof. A substrate may have a further coating of a different material, e.g., a polymer coating. A substrate may further comprise a moiety that binds to *Vibrio spp.* Non-limiting examples of such a moiety include a peptide, an aptamer, an antibody or antigen-binding fragment thereof, a lectin, or a combination thereof. A substrate may be in the form of microscope slides, membranes, microtiter plates, beads, microparticles, nanoparticles, fibres, filters, swabs, probes, dipsticks, etc.

The substrate may be porous or non-porous. As defined herein, the term “porous” means a substrate that permits the flow and drainage of a fluid through its body. The term “non-porous” means a substrate that does not permit flow and/or drainage of a fluid through its body. A porous substrate will have an interconnected network of channels and/or open spaces running from its surface through the volume of its body, i.e., a network of pores. A porous substrate may thus allow solid matter with dimensions larger than the dimensions of the pores to be filtered out from a liquid.

Sample

In some embodiments, the sample is a liquid sample and wherein the substrate is a porous substrate that is capable of filtering *Vibrio spp.* from the sample.

The liquid sample may be an environmental sample. An environmental sample may include, but is not limited to, a microbial suspension; water from naturally-occurring or artificial water systems and sources (e.g., naturally-sourced freshwater, drinking water, seawater, reservoir water, wetlands, ponds, etc.); water from an aquatic life support system (e.g., aquarium, mariculture enclosure, aquaculture enclosure, aquaponics tank, etc.); a biological fluid or other biological product (e.g., amniotic fluid, bile, blood, cerebrospinal fluid, cerumen, exudate, pus, fecal matter, tears, gastric fluid, interstitial fluid, intracellular fluid, cavity fluid, lymphatic fluid, ocular fluid, milk, mucus, rumen content, saliva, sebum, semen, sweat, urine, vaginal secretion, vomit, etc.); and sewage (e.g., sanitary, commercial, industrial, agricultural wastewater and surface runoff, aquaculture wastewater, etc.).

In some embodiments the liquid sample is an environmental water sample. In some embodiments the environmental water sample is taken from a mariculture farm, an

aquaculture farm or an aquatic life support system. In some embodiments the liquid sample is a water sample taken from an aquaculture fish farm.

In some embodiments, the method comprises flowing the liquid sample through the porous substrate to filter *Vibrio spp.* from the sample.

In some embodiments, the porous substrate has a pore size that is smaller than the size of a *Vibrio* bacterium, for instance, a pore size of about 0.22 μm .

As used herein, “flowing” refers to moving the liquid sample continuously as a stream through the porous substrate. In some embodiments, the sample (and/or a liquid solution) is passed through the porous substrate. The sample contacts the porous substrate at one surface, permeates the porous substrate and exits the porous substrate via an opposite surface.

The passing of the sample in a liquid form through a porous substrate serves to trap or retain bacterial cells on the surface of the substrate. In some embodiments, the sample is filtered through the porous substrate. This step allows for the separation of bacterial cells from the other contents in the sample, which may reduce background noise in subsequent analyses.

In some embodiments, the method further comprises a step prior to step (a) of flowing the sample through a pre-filter. In some embodiments, the pre-filter has a pore size of about 5 μm , or more than 5 μm . In other embodiments, the pre-filter comprises Nylon, polyvinylidene difluoride (PVDF) or another hydrophilic material that does not adsorb or interact with bacteria cells. The pre-filter is intended to remove larger particulates, if necessary. The larger particulates may interfere with the quantification by blocking the filter which traps the bacteria cells. The use of a pre-filter is an optional step, and may be considered when the skilled person encounters difficulties in passing the sample through the porous substrate, which may indicate the presence of larger particulates.

In some embodiments, the sample is a solid or semi-solid sample and wherein the substrate is a swab that is capable of capturing *Vibrio spp.* onto its surface. A solid sample may first be converted to a semi-solid form before analysing the sample with the present method.

The solid or semi-solid sample may be an environmental sample. An environmental sample may include, but is not limited to, soil (including, e.g., minerals, organic matter, gases, liquids, organisms, etc.); sediment (e.g., agricultural, aquacultural, marine, etc.); living or dead organic matter; and sewage (e.g., sanitary, commercial, industrial, agricultural wastewater and surface runoff, aquaculture wastewater, etc.). An environmental sample may also be an inanimate object, such as an inanimate object that has been in contact with water or an aqueous solution or an aqueous suspension that contains or is able to support the growth of *Vibrio spp.* The inanimate object may have been enclosing a body of water or aqueous solution or suspension, or the object may have been immersed in, or partially submerged in, or floating on a body of water or aqueous solution or suspension. The object may have been in contact with the water or aqueous solution or suspension for any length of time.

The solid or semi-solid sample may be a biological tissue sample. The tissue sample may be obtained from a human or animal. The sample may be obtained from a vertebrate or an invertebrate, e.g., a fish, mollusk, crustacean, echinoderm, bird or mammal, a wild animal, a farm animal, a sport animal, or a pet. The sample may be obtained from a living or dead subject. The sample may be obtained fresh from a subject or may have undergone some form of pre-processing, storage, or transport. A biological tissue sample may be taken from e.g., connective, muscle, nervous, epithelial, skeletal, cartilage, gastrointestinal, reproductive, vascular tissue, etc. A biological tissue sample may comprise a biological fluid or other biological secretions or excretions from a subject. A biological tissue sample may also comprise native microbiota present in a tissue. A biological tissue sample may be obtained from a food ingredient or a food product suitable for animal and/or human consumption, which includes but is not limited to raw animals or animal parts, pre-processed food, and partially or fully processed food.

In some embodiments the solid or semi-solid sample is a biological tissue sample. In some embodiments the biological tissue sample is taken from an animal in a mariculture farm, an aquaculture farm or an aquatic life support system. In some embodiments the solid or semi-solid sample is a biological tissue sample taken from fish in an aquaculture farm.

As used herein "swab" and "sampling swab" are used interchangeably to refer to a woven or non-woven fabric of any suitable material that is used to collect a solid or semi-solid sample. Non-limiting examples of suitable materials include natural fibres (e.g., cotton), semi-synthetic fibres (e.g., regenerated cellulose) and synthetic polymers (e.g., polyester). The material may be in the form of a foam, mesh, fibres, flocked fibres, etc. In some embodiments a cotton swab was used to obtain the solid or semi-solid sample.

Aptamer

The term "aptamer" refers to a nucleic acid molecule that naturally folds into specific and stable secondary structures that enable it to bind with high specificity and high affinity to a selected target molecule (e.g. on *Vibrio spp.*), such a target molecule being a three dimensional chemical structure other than a polynucleotide that binds to the aptamer through a mechanism which is independent of Watson/Crick base pairing or triple helix formation. The target molecule of an aptamer may be a cell wall component of *Vibro spp.*, including but not limited to a cell wall lipopolysaccharide or a cell wall protein.

The term "nucleic acid" refers to single-stranded or double-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) and any chemical modifications thereof.

The term "sequence identity" as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis over a window of comparison. Thus, a "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G and I) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity.

In one embodiment, the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-

AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCAG-3'

(SEQ ID NO: 1) or a derivative thereof.

In one embodiment, the aptamer consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-

AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCAG-3'

(SEQ ID NO: 1) or a derivative thereof.

In one embodiment, the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-

TCAGTCGCTTCGCCGTCTCCTTCAGCCGGGGTGGTCAGTAGGAGCAGCACAA

AGAGGGAGACCCCAGAGGG-3' (SEQ ID NO: 2) or a derivative thereof.

The aptamer may have a compact sequence with fewer non-essential nucleotides that may interfere with aptamer folding and binding to a target. The compact sequence may be beneficial for generic binding to multiple *Vibrio* species.

The aptamer may be 48nt to 70nt, 48nt to 69nt, 48nt to 68nt, 48nt to 67nt, 48nt to 66nt, 48nt to 65nt, 48nt to 64nt, 48nt to 63nt, 48nt to 62nt, 48nt to 61nt, 48nt to 60nt, 48nt to 59nt, 48nt to 58nt, 48nt to 57nt, 48nt to 56nt, 48nt to 55nt, 48nt to 54nt, 48nt to 53nt, 48nt to 52nt, 48nt to 51nt, 48nt to 50nt, 48nt to 49nt. The aptamer may be 48nt, 49nt, 50nt, 51nt, 52nt, 53nt, 54nt, 55nt, 56nt, 57nt, 58nt, 59nt, 60nt, 61nt, 62nt, 63nt, 64nt, 65nt, 66nt, 67nt, 67nt, 68nt, 69nt or 70nt.

A derivative of a nucleic acid sequence of this disclosure may be chemically modified. The chemical modification may confer nuclease resistance to the nucleic acid or aptamer. Chemical modifications may include backbone modifications, methylations, unusual base-pairing combinations such as the isobases isocytidine and isoguanidine, and the like. Modifications may also include 3' and 5' modifications, such as capping. Other modifications may include substitution of one or more of the naturally occurring nucleotides with an analogue, internucleotide modifications such as, for example, those with uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.) and those with charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), those with intercalators (e.g., acridine, psoralen, etc.), those

containing chelators (e.g., metals, radioactive metals, boron, oxidative metals, etc.), those containing alkylators, and those with modified linkages (e.g., alpha-anomeric nucleic acids, etc.). Further, any of the hydroxyl groups ordinarily present on the sugar of a nucleotide may be replaced by a phosphonate group or a phosphate group; protected by standard protecting groups; or activated to prepare additional linkages to additional nucleotides or to a solid support. The 5' and 3' terminal OH groups may be phosphorylated or substituted with amines, organic capping group moieties of from about 1 to about 20 carbon atoms, or other hydrophilic or hydrophobic biological or synthetic polymers.

A derivative of a nucleic acid sequence of this disclosure may also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including 2'-O-methyl-, 2'-O-allyl, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs, alpha-anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogues and abasic nucleoside analogs such as methyl riboside. As noted above, one or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include embodiments wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), (O)NR₂ ("amidate"), P(O)R, P(O)OR', CO or CH₂ ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical.

A nucleic acid or aptamer may be chemically modified to introduce a moiety for conjugation to a substrate or surface. In one embodiment, the aptamer is modified to have a thiol moiety and a nine-carbon alkyl linker at the 5' terminus for conjugating to a gold nanoparticle.

Antibody

The term "antibody", as used herein, refers to a protein, or polypeptide sequence derived from an immunoglobulin molecule which specifically binds with an antigen. Antibodies may be polyclonal or monoclonal, multiple or single chain, or intact immunoglobulins, and may be derived from natural sources or from recombinant sources.

The term "antibody fragment" may refer to at least one portion of an antibody that retains the ability to specifically interact with an epitope of an antigen. Examples of antibody fragments include, but are not limited to, Fab, Fab', F(ab')₂, Fv fragments, scFv antibody fragments, disulfide-linked Fv (sdFv), a Fd fragment consisting of the VH and CH1 domains, linear antibodies, single domain antibodies such as sdAb (either VL or VH), camelid VHH domains, multi-specific antibodies formed from antibody fragments such as a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region, and an isolated CDR or other epitope-binding fragments of an antibody. An antigen binding fragment can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv.

The term "antigen" as used herein, refers to a molecule capable of being recognized by an antibody. An antigen can be, for example, a peptide or a modified form thereof. An antigen can comprise one or more epitopes. The antigen for an anti-*Vibrio* antibody may be a cell wall component of *Vibrio spp.*, including but not limited to a cell wall lipopolysaccharide or a cell wall protein.

In some embodiments the substrate is conjugated with an anti-*Vibrio* aptamer and/or antibody to capture *Vibrio spp.* from the sample.

Conjugation may involve a reactive amino acid side-chain in an antibody (e.g., cysteine, lysine, serine and tyrosine), or a functional group in an antibody glycan (e.g., amine, aldehyde), or a functional group in a nucleic acid (e.g., purine or pyrimidine functionality or alcohol). As summarised *inter alia* in G. T. Hermanson, "Bioconjugate Techniques", Elsevier, 3rd Ed. 2013, incorporated by reference, a large number of reactive functional groups have become available over the years for chemoselective targeting of one of these functional groups, such as maleimide, haloacetamide, activated ester, activated carbonate, sulfonyl halide, activated thiol derivative, alkene, alkyne, allenamide and more, each of which requiring its own specific conditions for conjugation (pH, concentration, stoichiometry, light, etc.).

Alternatively, conjugation may be a two-step process involving the addition of a unique reactive group to the aptamer/antibody or the substrate, followed by the coupling of the

aptamer/antibody to the substrate. Non-limiting examples of a reactive group that may be added to the aptamer/antibody or the substrate include a (strained) alkyne, (strained) alkene, norbornene, tetrazine, azide, phosphine, nitrile oxide, nitron, nitrile imine, diazo compound, carbonyl compound, (O-alkyl)hydroxylamine, hydrazine, and any of the functional groups mentioned in the previous paragraph. The reactive group may be present in a carrier molecule that is added to the aptamer/antibody, such as a protein, a peptide and the like. The addition of the reactive group may be achieved by either chemical or molecular biology approach.

Conjugation may also occur via non-covalent interactions between complementary protein-ligand pairs on the aptamer/antibody and the substrate, such as biotin-streptavidin pairing, IgG-protein A pairing, etc. which are known in the art. In some embodiments, a two-step process is used to conjugate an anti-*Vibrio* antibody to a cotton swab, involving the step-wise silanization and functionalization of the swab with Protein G, followed by the addition and binding of the anti-*Vibrio* antibody to Protein G.

Label

As used herein, the term “label” for detecting an aptamer or antibody that is bound to *Vibrio* spp. refers to molecules that are either directly or indirectly involved in the production of a detectable signal to indicate the presence of the aptamer or antibody. Any label or indicating means can be linked to or incorporated in an aptamer or antibody molecule, and those atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well known in clinical diagnostic chemistry.

The label can be a fluorescent agent or a fluorochrome (dye) that is conjugated to an aptamer or antibody to form a “fluorescent” aptamer or “fluorescent” antibody that is a useful immunofluorescent tracer. Suitable fluorescent labeling agents are fluorochromes such as fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC), 5-dimethylamine-1-naphthalenesulfonyl chloride (DANSC), tetramethylrhodamine isothiocyanate (TRITC), lissamine, rhodamine 8200 sulphonyl chloride (RB 200 SC) and the like.

In some embodiments, the indicating group is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, or the like. In such cases where the principle indicating group is an enzyme such as HRP or glucose oxidase, additional reagents are required to indicate the

presence of the aptamer or antibody. Such additional reagents for HRP include hydrogen peroxide and an oxidation dye precursor such as diaminobenzidine. An additional reagent useful with glucose oxidase is 2,2'-azino-di-(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS), as an example.

Radioactive elements are also useful labeling agents and are used illustratively herein. An exemplary radiolabeling agent is a radioactive element that produces gamma ray emissions. Elements which themselves emit gamma rays, such as ^{124}I , ^{125}I , ^{128}I , ^{132}I and ^{51}Cr represent one class of gamma ray emission-producing radioactive element indicating groups. Another group of useful labeling means are those elements such as ^{11}C , ^{18}F , ^{15}O and ^{13}N which themselves emit positrons. Also useful is a beta emitter, such as ^{111}In or ^3H .

Detection of an aptamer or antibody that is bound to *Vibrio spp.* can be performed by several methods. For example, the aptamer/antibody (or primary aptamer/antibody) may be prepared with a label such as biotin, an enzyme, a fluorescent marker, or radioactivity, and may be detected directly using this label. Alternatively, a labeled "secondary aptamer/antibody" or "reporter aptamer/antibody" which recognizes the primary antibody may be added, forming a complex comprised of antigen-aptamer/antibody-antibody. Again, appropriate reporter reagents are then added to detect the labeled aptamer/antibody. Any number of additional aptamers or antibodies may be added as desired. These aptamers or antibodies may also be labeled with a marker, including, but not limited to an enzyme, fluorescent marker, or radioactivity.

As used herein, the term "reporter reagent" is used in reference to compounds which are capable of detecting the presence of an antibody. For example, a reporter reagent may be a colorimetric substance which is attached to an enzymatic substrate. Upon binding of aptamer/antibody, the enzyme acts on its substrate and causes the production of a color. Other reporter reagents include, but are not limited to fluorogenic and radioactive compounds or molecules.

Nanoparticle

In some embodiments, the anti-*Vibrio* aptamer or antibody is conjugated to a nanoparticle. The term “an anti-*Vibrio* aptamer or antibody nanoparticle conjugate” refers to an anti-*Vibrio* aptamer or antibody that is conjugated to a nanoparticle.

“Nanoparticle” refers to a solid object with external dimensions in the nanoscale. For example, a nanoparticle may be a particle between 1 nm and 990 nm in size. The term includes nanorods, nanospheres, nanocones, nanopyramids, nanoprisms and other geometric shapes. A nanoparticle may be irregular in shape. In some embodiments, the nanoparticle is less than about 100 nm in diameter. Because of their nano-size, properties which are different from the physical bulk properties are manifested.

In some embodiments, the nanoparticle is a plasmonic and/or fluorescent nanoparticle.

“Plasmonic nanoparticle” refers to particles whose electron density can couple with electromagnetic radiation of wavelengths that are far larger than the size of the particle due to the nature of the dielectric-metal interface between the medium and the particles. What differentiates these particles from normal surface plasmons is that plasmonic nanoparticles also exhibit interesting scattering, absorbance, and coupling properties based on their geometries and relative positions. Plasmons are the oscillations of free electrons that are the consequence of the formation of a dipole in the material due to electromagnetic waves. The electrons migrate in the material to restore its initial state; however, the light waves oscillate, leading to a constant shift in the dipole that forces the electrons to oscillate at the same frequency as the light. This coupling only occurs when the frequency of the light is equal to or less than the plasma frequency and is greatest at the plasma frequency that is therefore called the resonant frequency. The scattering and absorbance cross-sections describe the intensity of a given frequency to be scattered or absorbed. Examples of plasmonic nanoparticle include, but are not limited to, gold nanoparticles and silver nanoparticles.

“Fluorescent nanoparticle” refers to particles which are able to emit light (fluoresce) after excitation with electromagnetic radiation of a certain wavelength. In most cases, the emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation. A fluorescent nanoparticle may cease to glow nearly immediately when the radiation source stops.

In some embodiments, the plasmonic and/or fluorescent nanoparticle is selected from metallic nanoparticles, II-VI binary, ternary and quaternary semiconductor nanocrystals, IV-VI semiconductor nanocrystals, III-V semiconductor nanocrystals, I-V semiconductor nanocrystals, I-III-V semiconductor nanocrystals, group IV elemental semiconductor nanocrystals, Cu^+ and Mn^{2+} doped semiconductor nanocrystals, and their related core/shell structures thereof. In some embodiments, the plasmonic and/or fluorescent nanoparticle is selected from gold nanoparticles, silver nanoparticles or CdSe/CdS core/shell nanorods. In other embodiments, the plasmonic and/or fluorescent nanoparticle is a semiconductor nanoparticle. In other embodiments, the plasmonic and/or fluorescent nanoparticle is a semiconductor nanorod. An example of a semiconductor nanorod is CdSe/CdS core/shell nanorods. Examples of II-VI binary, ternary and quaternary semiconductor nanocrystals and related core/shell structures are CdSe, CdZnS, CdZnSeS, CdSe/ZnS; examples of IV-VI semiconductor nanocrystals and related core/shell structures are PbSe, PbSe/PbS; examples of III-V semiconductor nanocrystals and related core/shell structures are InP, InAs, InP/ZnS; example of I-V semiconductor nanocrystals and related core/shell structures is Ag_2S ; examples of I-III-V semiconductor nanocrystals and related core/shell structures are AgInS_2 , $\text{CuInS}_2/\text{ZnS}$; examples of elemental group IV semiconductor nanocrystals are Carbon dots and Si nanocrystals; examples of Cu^+ and Mn^{2+} doped semiconductor nanocrystals are Cu^+ doped ZnSe/ZnS, and Mn^{2+} doped ZnS. Other examples of such nanocrystals are CdSe/ZnS, CdTe, CdZnS/ZnS, CdZnSeS, $\text{CuInS}_2/\text{ZnS}$, $\text{CuInSe}_2/\text{ZnS}$, $\text{CuInZnS}/\text{ZnS}$, PbS, PbSe/PbS, InP/ZnS, Ag_2S , Ag_2Se and $\text{AgInS}_2/\text{ZnS}$. Another example of a plasmonic nanoparticle is a platinum nanoparticle.

The skilled person would understand that, for example, Group 14 (IUPAC notation) is the same as Group IV. For avoidance of doubt, group 14 (or group IV) includes elements such as carbon, silicon, germanium, tin and lead. As another example, II-VI semiconductor compounds are compounds composed of a metal from either group 2 or 12 of the periodic table (the alkaline earth metals and group 12 elements, formerly called groups IIA and IIB) and a nonmetal from group 16 (the chalcogens, formerly called group VI).

In some embodiments, the nanoparticles have a high plasmonic or fluorescence output. For example, semiconductor nanorods may be used instead of semiconductor nanodots.

In some embodiments, the nanoparticle is a metallic nanoparticle. In some embodiments, the metallic nanoparticle is a gold nanoparticle.

In some embodiments, the nanoparticle is at a concentration of about 2 nM to about 30 nM. In other embodiments, when the nanoparticle is a gold nanoparticle, the nanoparticle is at a concentration of about 5 nM to about 30 nM, or about 8 nM to about 20 nM. In other embodiments, when the nanoparticle is a semiconductor nanorod, the nanoparticle is at a concentration of about 2 nM to about 15 nM, or about 3 nM to about 10 nM.

In some embodiments the nanoparticles are formulated with a surfactant to reduce non-specific binding to the substrate when they are added in step (b) of the methods herein. The surfactant may be an ionic or a non-ionic surfactant, and should be compatible with the aptamer or antibody conjugated to the nanoparticle. In some embodiments, an aptamer nanoparticle conjugate is formulated with a non-ionic surfactant, such as Tween-20. In some embodiments, an antibody nanoparticle conjugate is formulated with a cationic surfactant, such as cetyltrimethylammonium bromide (CTAB) or cetyltrimethylammonium chloride (CTAC).

In some embodiments, the nanoparticle is detected by addition of a metallic precursor to improve visual detection of the nanoparticle. The metallic precursor may be a metallic salt that can be deposited on the nanoparticle. The metallic salt may be in solution prior to contact with the nanoparticle. The metallic salt may be deposited on the nanoparticle in the presence of a reducing agent.

In one embodiment, the metallic salt is a gold salt. The gold salt may be Au^{3+} (e.g. HAuCl_4). The reducing agent may be H_2O_2 . In one embodiment, about 12.5 mM Au^{3+} and about 0.3% to 3% H_2O_2 are added in a 1:5 volume ratio.

In some embodiments the method comprises washing the substrate to remove unbound anti-*Vibrio* aptamer or antibody nanoparticle conjugate after contacting the substrate with the anti-*Vibrio* aptamer or antibody nanoparticle conjugate.

Detection

The method as defined herein may involve detecting bound nanoparticles by eye or with a detector. The method may involve detecting a colorimetric and/or fluorescence output emitted from a nanoparticle that is bound to *Vibrio spp.* captured by the substrate. The colorimetric output can be due to light absorption, light transmission, and/or reflection.

Quantification

In one embodiment, there is provided a method of quantifying *Vibrio spp.* bacteria in a sample. The method may be capable of quantifying the total number of cells (e.g. the total number of live cells).

Disclosed herein is a method of detecting the presence of *Vibrio spp.* in a liquid sample, the method comprising:

- a) passing the liquid sample through a porous substrate to capture *Vibrio spp.* on the porous substrate; and
- b) contacting the porous substrate with an anti-*Vibrio* aptamer or antibody, wherein specific binding of the anti-*Vibrio* aptamer or antibody to the *Vibrio spp.* on the porous substrate indicates the presence of *Vibrio spp.* in the sample;

wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCAG-3'
(SEQ ID NO: 1) or a derivative thereof.

Disclosed herein is a method of detecting the presence of *Vibrio spp.* in a liquid sample, the method comprising:

- a) passing the liquid sample through a porous substrate to capture *Vibrio spp.* on the porous substrate; and
- b) contacting the porous substrate with an anti-*Vibrio* aptamer or antibody nanoparticle conjugate, wherein specific binding of the anti-*Vibrio* aptamer or antibody nanoparticle conjugate to the *Vibrio spp.* on the porous substrate indicates the presence of *Vibrio spp.* in the sample;

wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCAG-3'

(SEQ ID NO: 1) or a derivative thereof.

Disclosed herein is a method of detecting the presence of *Vibrio spp.* in a solid/semi-solid sample, the method comprising:

- a) contacting the sample with a swab to capture *Vibrio spp.* onto the surface of the swab; and
- b) contacting the swab with an anti-*Vibrio* aptamer or antibody, wherein specific binding of the anti-*Vibrio* aptamer or antibody to the *Vibrio spp.* on the swab indicates the presence of *Vibrio spp.* in the sample;

wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCAG-3'

(SEQ ID NO: 1) or a derivative thereof.

Disclosed herein is a method of detecting the presence of *Vibrio spp.* in a solid/semi-solid sample, the method comprising:

- a) contacting the sample with a swab to capture *Vibrio spp.* onto the surface of the swab; and
- b) contacting the swab with an anti-*Vibrio* aptamer or antibody nanoparticle conjugate, wherein specific binding of the anti-*Vibrio* aptamer or antibody nanoparticle conjugate to the *Vibrio spp.* on the swab indicates the presence of *Vibrio spp.* in the sample;

wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCAG-3'

(SEQ ID NO: 1) or a derivative thereof.

Disclosed herein is a kit or sensor for performing a method as defined herein. The kit or sensor may comprise a substrate (e.g., a porous substrate or a swab) and an anti-*Vibrio* aptamer or antibody nanoparticle conjugate. In preferred embodiments the kit or sensor is portable so that a method defined herein may be performed in a remote locale instead of in a central facility.

Disclosed herein is an aptamer comprising or consisting a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-AGCCGGGGTGGTCATTAGCAGCAGCACAACAGGCAGCACAACAGGCAG-3'
(SEQ ID NO: 1) or a derivative thereof.

Disclosed herein is a composition for capturing *Vibrio spp.* from a sample, the composition comprising a substrate and an anti-*Vibrio* aptamer and/or antibody that has been conjugated to the substrate, wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-AGCCGGGGTGGTCATTAGCAGCAGCACAACAGGCAGCACAACAGGCAG-3'
(SEQ ID NO: 1) or a derivative thereof.

In some embodiments the substrate is a porous substrate or a swab.

By "about" is meant a quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that varies by as much 15, 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 % to a reference quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length.

As used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative (or).

As used in this application, the singular form "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "an agent" includes a plurality of agents, including mixtures thereof.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or

admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in the field of endeavour to which this specification relates.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications which fall within the spirit and scope. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Unless otherwise defined, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs.

Certain embodiments of the invention will now be described with reference to the following examples which are intended for the purpose of illustration only and are not intended to limit the scope of the generality hereinbefore described.

EXAMPLES

1. Selection of affinity probes specific to *Vibrio* spp.

Two types of affinity probes are involved in this TD, namely (1) aptamer and (2) antibody. In order to find aptamer and antibody candidates that are generic to *Vibrio* spp., “fluorescent depletion” experiments were performed for a list of aptamers reported in literatures for four *Vibrio* species that are prominent pathogens in fish farms, i.e. *Vibrio alginolyticus* (VA), *Vibrio vulnificus* (VV), *Vibrio harveyi* (VH), *Vibrio parahaemolyticus* (VP). The process of the fluorescent depletion experiment is: in 1.5 ml Eppendorf tubes, 50 μ L of 1 μ M FAM-labelled aptamers was mixed with 400 μ L of 10^8 CFU/ml bacteria cells and 50 μ L of 10x PBS with 10 mM MgCl_2 . After 30 min incubation in a thermomixer at room temperature with moderate shaking, the solution is centrifuged at 14,000 rpm for 30 min. After that, 100 μ L of the supernatant was transferred into 96 well plate (black) and measured its fluorescence intensity (F_{sample}) at suitable excitation wavelength (e.g. 470 nm for AF488 and 470 nm for

FAM, etc). The initial fluorescence intensity of the fluorophore labelled antibody or aptamer without bacteria was recorded as F_{control} . The % fluorescence intensity decrease (F_{decrease}) due to affinity probe binding to bacteria cells is calculated as:

$$\% F_{\text{decrease}} = [(F_{\text{control}} - F_{\text{sample}}) / F_{\text{control}}] \times 100\%$$

The same experiments were performed with two non-Vibrio bacteria of *E. coli* (EC) and *S. aureus* (SA) to generate negative controls.

Similar experiments were performed for two anti-Vibrio antibodies (Ab1 from SPD scientific and Ab2 from ARC), by replacing the aptamers with 0.02 mg/mL of antibody. **Figure 1** shows the results. Among the 8 anti-Vibrio DNA aptamers screened, the 48 nt aptamer (5'-AGCCGG GGTGGTCATTAGCAGCAGCACAACAGGCAGCACAACAGGCAG-3') (SEQ ID NO: 1), named "VHa", shows generic selectivity to all Vibrio species, relative to the two negative controls. The anti-Vibrio antibody from SPD (Ab1) has higher affinity and better selectivity than Ab2. In the following assay development, VHa aptamer and Ab1 were used to prepare affinity probe conjugated nanoparticles for staining and affinity-probe modified swabs for bacteria capture.

2. Preparation and formulation of affinity probes conjugated nanostains (*Vibrio* nanostains)

***Vibrio* Nanostains preparation:**

Nanostains for *Vibrio* detection are prepared from spherical gold nanoparticles (AuNPs) with diameter around 13 nm, synthesized from HAuCl₄ reduction by sodium citrate. The *Vibrio* spp. specific affinity probes (i.e. VHa aptamer or Ab1 antibody), selected through in our screening test (session 1) are conjugated on the AuNPs.

For VHa aptamer and AuNPs conjugation, thiol labelled VHa aptamer was used. A protocol was developed to conjugate the VHa aptamer with desired surface coverage, inter aptamer spacing and retained stability in salt or sea water. The as-prepared VHa-AuNPs conjugates are characterized for their hydrodynamic size of 23.3 ± 2.1 nm (diameter). The stability test for the VHa-AuNP (**Figure 2**) further confirmed the successful conjugation of the aptamer

which make the particle stable (no aggregation) in salt water with NaCl as high as 3.5%, which is equivalent to sea water.

For Ab1-AuNPs conjugation, the AuNPs was first coated with molecules that can capture the IgG antibody through the Fc region. For example, we use protein G or protein A that specifically binds to the Fc region of IgG so that the Fab region (epitope binding region) of antibody is orientated towards the outside part of AuNPs and remain available for further binding to target analyte.

The as-prepared Ab1-AuNPs hydrodynamic size is 43.8 ± 0.3 nm. As shown in Figure 3, the Ab1-AuNPs are also stable (no aggregation) in high salt solution (3.5% NaCl), which is close to the salt condition of sea water.

Formulation of *Vibrio* nanostains:

The nanostains (both VHa-AuNPs and Ab1-AuNPs) were formulated with surfactants to reduce non-specific attachment of nanostains on blank filter membranes (without bacteria). VHa-AuNPs were formulated with non-ionic surfactant, such as Tween-20 at optimized concentration of 6 μ L/ml. Tween-20 is not suitable for Ab1-AuNPs because Tween-20 induces non-specific attachment of Ab1-AuNPs on blank membrane. Therefore, Ab1-AuNPs were formulated with positive charged surfactant such as CTAB or CTAC at 6 mg/mL. Negatively charged surfactants (e.g. SDS) are not used because they are known to denature proteins which may affect the binding ability of these probes to *Vibrio* cells.

3. Detection protocol for *Vibrio* spp. in water samples (“Filter and Stain”)

The *Vibrio* nanostains (prepared in Example 2) were used to detect *Vibrio* spp. from water samples in the “Filter and Stain” protocol. This protocol (**Figure 4A**) consists of the following steps: (1) Flow 1 mL water sample through a filter membrane with 0.22 μ m pore-size, (2) Flow 0.2 mL of nanostain solution (VHa-AuNPs or Ab1-AuNPs) and incubate for 10 min, (3) wash with 1 ml of washing solution (water formulated with at least 1% Tween 20 to remove non-specific binding of AuNPs to non-*Vibrio* bacteria), (4) blow dry with air till dry, (5) measure color intensity (sample reading, R_{sample}) using detector. Dirty water with large particulates can be pre-filtered by using a 5 μ m pore-size filter to remove solid particulates larger than 5 μ m while passing through bacteria cells. Blank experiment was performed with

no bacteria cells. The staining reading is recorded as R_{blank} . The detector reading difference (R_{diff}) is calculated:

$$R_{\text{diff}} = R_{\text{blank}} - R_{\text{sample}}$$

The selectivity of the staining to *Vibrio* spp. is firstly demonstrated for four *Vibrio* representative (*V. parahaemolyticus* (VP), *V. vulnificus* (VV), *V. harveyi* (VH), *V. alginolyticus* (VA)) relative to the negative controls of *E. coli* (EC) and *S. aureus* (SA) (all at concentration of 10^7 CFU/ml) (**Figure 4B**), with stronger staining (larger R_{diff} value) to all *Vibrio* species relative to negative control bacteria.

Next, using *V. harveyi* (VH) as a representative, the selective staining to *Vibrio* in mixtures of *Vibrio* (VH) and non-*Vibrio* (SA) bacteria is demonstrated (**Figure 5**). VH of various concentrations is spiked into *S. aureus* of very high concentration (10^7 CFU/ml) to mimic the real situation that total bacteria count in fish pond water can range from $10^5 - 10^7$ CFU/ml. The results showed that *Vibrio* can be detected from non-*Vibrio* bacteria mixtures and the signal increases with the increase of *Vibrio* concentration. The LOD is 10^5 CFU/ml.

Similarly, the Ab1-AuNPs nanostain was used to detect *Vibrio* species, relative to negative controls of *E. coli* (EC) and *S. aureus* (SA) (all at concentration of 10^7 CFU/ml) (**Figure 6A**). Stronger staining (larger R_{diff} value) is observed to all *Vibrio* species relative to negative control bacteria, confirming the selectivity. The LOD of Ab1-AuNPs is at 10^6 CFU/ml (**Figure 6B**).

4. Gold deposition step for improving limit of detection (LOD) of *Vibrio* spp. in “Filter and Stain”

In order to enhance the detection sensitivity, a post staining signal amplification step is introduced (**Figure 7A**). It is a reduction reaction of Au^{3+} by H_2O_2 . The reaction solution is a mixture HAuCl_4 (12.5 mM) and H_2O_2 (0.3 to 3%) in 1:5 volume ratio. This solution, 0.2 mL, is added to the AuNP-stained filters. After 5 min of incubation, the solution is washed away with 1% Tween 20 aqueous solution. The reading is recorded as R_{Au} . With this additional step, the limit of detection (LOD) for *Vibrio* is improved to 100 cfu/ml (**Figure 7B**)

5. Detection protocol for *Vibrio* spp. in biological tissue samples (“Swab and Stain”) with gold deposition step

Biological tissue samples, such as fish kidneys, are typically solid/ semi-solid samples that contain high number of particulates in their homogenates. The “Filter & Stain” protocol is not applicable, largely due to the membrane jammed by the tissue particulates. To detect bacteria *Vibrio* from biological tissues, a “Swab and Stain” protocol is developed that is suitable for bacteria detection from biological solid/semi solid tissue samples homogenized in liquid medium (**Figure 6A**). In this method, the cotton swabs were firstly functionalized with one type of affinity probe (affinity probe 1), e.g. Ab1 to specifically capture *Vibrio* spp. cells when dipped into biological tissue homogenates. After that the swabs are washed with 0.9% - 1.5% NaCl to remove tissue particulates stuck on the swabs. Then, 100 μ L of the VHa-AuNPs (affinity probe 2 conjugated to AuNPs) is added to the swabs that may have captured the *Vibrio* spp. cells (positive samples). A sandwiched complex will form and cause the swabs being stained if *Vibrio* presents in the sample. The swabs are then washed with 1% Tween-20 aqueous solution to remove nonspecific adsorption of VHa-AuNPs on the swabs. Following this, gold deposition step is applied by adding 100 μ L of HAuCl₄ (12.5 mM) and H₂O₂ (3%) in 1:5 volume ratio mixture onto the swabs, for 15 min to enhance the staining intensity. The total assay time from dipping the swabs into tissue homogenates into results is 30 min. While not much stain is observed on swabs dipped in homogenates without spiked bacteria and homogenates spiked with negative controls, i.e. *S. aureus* and *E. coli* at 10⁷ CFU/ml, darker stain is observed on swabs from homogenates spiked with *Vibrio* bacteria at various concentration (10² – 10⁷ CFU/ml), even at 100 CFU/ml of *Vibrio* (**Figure 8B and 8C**). Corresponding image analysis shows that swabs dipped in homogenates spiked with *Vibrio* typically have brightness value below 150, which is lower than the brightness value from controls (no bacteria spiked) or homogenates spiked with *S. aureus* and *E. coli*. These results show that the “Swab and Stain” method can selectively detect *Vibrio* spp. as low as 100 CFU/ml and differentiate *Vibrio* from non-*Vibrio* bacteria collected from biological tissue samples.

CLAIMS

1. A method of detecting the presence of *Vibrio spp.* in a sample, the method comprising:

- a) contacting the sample with a substrate to capture *Vibrio spp.* onto the substrate; and
- b) contacting the substrate with an anti-*Vibrio* aptamer or antibody nanoparticle conjugate, wherein specific binding of the anti-*Vibrio* aptamer or antibody nanoparticle conjugate to the *Vibrio spp.* on the substrate indicates the presence of *Vibrio spp.* in the sample;

wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-

AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCA
G-3' (SEQ ID NO: 1) or a derivative thereof.

2. The method of claim 1, wherein the method comprises detecting anti-*Vibrio* aptamer or antibody nanoparticle conjugate that is present on the substrate, wherein an increased level of anti-*Vibrio* aptamer or antibody nanoparticle conjugate that is present on the substrate as compared to a reference indicates the presence of *Vibrio spp.* in the sample.
3. The method of claim 1 or 2, wherein the sample is a liquid sample (such as a water sample) and wherein the substrate is a porous substrate that is capable of filtering *Vibrio spp.* from the sample.
4. The method of claim 3, wherein step a) comprises flowing the liquid sample through the porous substrate to capture *Vibrio spp.* from the sample.
5. The method of any one of claims 1 to 4, wherein the sample is a solid/semi-solid sample (such as a biological tissue sample) and wherein the substrate is a swab that is capable of capturing *Vibrio spp.* onto its surface.

6. The method of any one of claims 1 to 5, wherein the substrate is conjugated with an anti-*Vibrio* aptamer and/or antibody to capture *Vibrio spp.* from the sample.
7. The method of any one of claims 1 to 6, wherein the nanoparticle is a plasmonic and/or fluorescent nanoparticle.
8. The method of claim 7, wherein the nanoparticle is a metallic nanoparticle.
9. The method of claim 7 or 8, wherein the nanoparticle is detected by addition of a metallic precursor to improve visual detection of the nanoparticle.
10. The method of any one of claims 1 to 9, wherein the method comprises washing the substrate to remove unbound anti-*Vibrio* aptamer or antibody nanoparticle conjugate after contacting the substrate with the anti-*Vibrio* aptamer or antibody nanoparticle conjugate.
11. A method of detecting the presence of *Vibrio spp.* in a liquid sample, the method comprising:
 - a) flowing the liquid sample through a porous substrate to capture *Vibrio spp.* on the porous substrate; and
 - b) contacting the porous substrate with an anti-*Vibrio* aptamer or antibody nanoparticle conjugate, wherein specific binding of the anti-*Vibrio* aptamer or antibody nanoparticle conjugate to the *Vibrio spp.* on the porous substrate indicates the presence of *Vibrio spp.* in the sample;
wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:
5'-
AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCA
G-3' (SEQ ID NO: 1) or a derivative thereof.
12. A method of detecting the presence of *Vibrio spp.* in a solid/semi-solid sample, the method comprising:
 - a) contacting the sample with a swab to capture *Vibrio spp.* onto the surface of the swab; and

b) contacting the swab with an anti-*Vibrio* aptamer or antibody nanoparticle conjugate, wherein specific binding of the anti-*Vibrio* aptamer or antibody nanoparticle conjugate to the *Vibrio spp.* on the swab indicates the presence of *Vibrio spp.* in the sample;

wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-

AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCA
G-3' (SEQ ID NO: 1) or a derivative thereof.

13. The method of claim 12, wherein the method comprises conjugating an anti-*Vibrio* antibody or aptamer to the swab to capture *Vibrio spp.* from the sample.

14. A kit or sensor for performing a method according to any one of claims 1 to 13.

15. An aptamer comprising or consisting of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-

AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCA
G-3' (SEQ ID NO: 1) or a derivative thereof.

16. A composition for capturing *Vibrio spp.* from a sample, the composition comprising a substrate and an anti-*Vibrio* aptamer and/or antibody that has been conjugated to the substrate, wherein the aptamer comprises or consists of a nucleic acid sequence having at least 90% sequence identity to a nucleic acid sequence of:

5'-

AGCCGGGGTGGTCATTAGCAGCAGCACAAACAGGCAGCACAAACAGGCA
G-3' (SEQ ID NO: 1) or a derivative thereof.

17. The composition of claim 16, wherein the substrate is a porous substrate or a swab.