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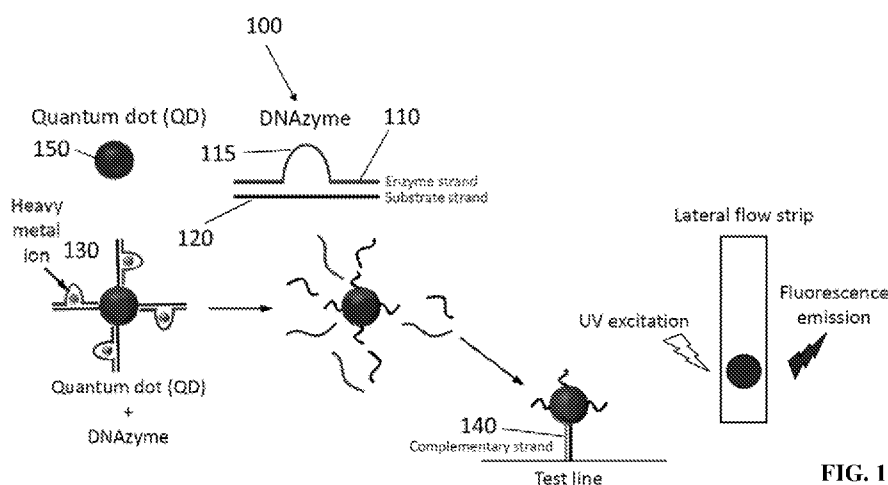


FIG. 1

(57) Abstract: A kit for detecting heavy metals and bacteria in an analyte is provided. The kit includes at least one heavy metal lateral flow test strip comprising a deoxyribozyme detection molecule. The deoxyribozyme detection molecule has an enzyme strand having a central catalytic core for binding to a heavy metal, and substrate strand, the substrate strand being conjugated with a biomolecule that binds to a quantum dot fluorescing indicator conjugated with a complementary biomolecule. The lateral flow test strip includes a test line having a complementary sequence to bind with the substrate strand to indicate that a heavy metal is present in the analyte; it also includes a control line for indicating that a test has been correctly performed. The kit further includes a bacteria test strip that includes an antibody that binds with at least one bacteria in the analyte.



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SYSTEM AND METHOD FOR DETECTING HEAVY METALS AND BACTERIA IN AN ANALYTE

Field of the Invention:

[0001] The present invention relates to a system for detecting and/or quantifying the presence of heavy metals and bacteria in an analyte. In particular, the present system is a lateral flow assay system incorporating specific deoxyribozymes and quantum dots for detecting one or more corresponding heavy metal(s) and/or an antibody-antigen specific recognition mechanism for detecting bacteria in an analyte such as water. A related method for detecting is also provided.

Background:

[0002] The following references are incorporated herein by reference in their entirety:

- [1] A.R. Flegel, D.R. Smith, Measurements of environmental lead contamination and human exposure, *Rev. Environ. Contam. Toxicol.* 143 (1995) 1-45.
- [2] Y. Pu, F. Cai, D. Wang, J.-X. Wang, and J.-F. Chen, “Colloidal Synthesis of Semiconductor Quantum Dots toward Large-Scale Production: A Review”, *Ind. Eng. Chem. Res.* 57, 1790-1802 (2018).
- [3] D. S. Kim, Y. T. Kim, S. B. Hong, J. Kim, N. S. Heo, M.-K. Lee, S. J. Lee, B. I. Kim, I. S. Kim, Y. S. Huh, and B. G. Choi, “Development of Lateral Flow Assay Based on Size-Controlled Gold Nanoparticles for Detection of Hepatitis B Surface Antigen”, *Sensors* 16, 2154 (2016).
- [4] W.C.W. Chan, D.J. Maxwell, et al., Luminescent quantum dots for multiplexed biological detection and imaging, *Current Opinion in Biotechnology* 13 (2002) 40-46.
- [5] US20050250141A1 - Diagnostic assays including multiplexed lateral flow immunoassays with quantum dots
- [6] US20060257958A1 - Magnetically-assisted test strip cartridge and method for using same
- [7] US20080032420A1 - Surface Enhanced Raman Scattering and Multiplexed Diagnostic Assays

[0003] Contamination by heavy metal ions and bacteria poses a serious threat to human health and the environment (Flegel and Smith, 1995). Heavy metals and bacteria poisoning has been related to several diseases associated with environmental pollution. Because of

health concerns and legal restrictions, it is critical to have probes that can provide rapid on-site evaluation of heavy metal and bacteria contents.

[0004] Most lateral flow assay systems in the market now use gold nanoparticles as a colorimetric signal indicator (Kim et al., 2016). Gold nanoparticles give a reddish purple color under white light. It is a convenient signal indicator as no excitation source is needed. However, the color signal becomes arbitrary when it is weak.

[0005] Currently, the most common and reliable way to examine the heavy metal content in water depends on the sophisticated equipment in the testing laboratories, such as atomic absorption spectroscopy (AAS), inductively coupled plasma optical emission spectroscopy (ICP-OES), and inductively coupled plasma mass spectroscopy (ICP-MS). In the case of bacteria detection in water sample, sophisticated pretreatments such as cell lysis and DNA purification are needed. Although these methodologies offer accurate and sensitive detection, the turnaround time is long and the cost is high.

[0006] Thus, there is a need in the art for a water test that can be performed in the field or in consumer homes that provides an immediate indication of the presence of heavy metals and bacteria in a water supply.

Summary of the Invention:

[0007] Accordingly, the present invention incorporates the highly efficient fluorescence properties of quantum dots (QD) and the highly sensitive but selective reactions of deoxyribozymes (DNAzymes) for heavy metal detection and antibodies for bacteria detection in a lateral flow assay system. A QD probe is chosen among different fluorescent probes as a labelling molecule in the present invention because the QD is not only rapid and highly sensitive but also suitable for quantitative analysis by conventional analytical systems such as spectrophotometer or the like.

[0008] In a first aspect, the lateral flow assay system of the present invention comprises a lateral flow strip as a test platform and for carrying or receiving at least one labeling molecule, at least one conjugating molecule, at least one detecting molecule and analyte. The interaction and/or reaction among the labeling molecule, the conjugating molecule, the detecting molecule and the target contaminant(s) in the analyte substantially takes place in the lateral flow strip of the present system. The labeling molecule includes quantum dots conjugated to a biomolecule; the detecting molecule includes one or more of deoxyribozymes (DNAzymes) and antibody specific for the target contaminant(s).

[0009] In another aspect, the present invention comprises a kit for detecting heavy metals and

bacteria in an analyte, that includes at least one heavy metal lateral flow test strip. The test strip has a deoxyribozyme detection molecule with an enzyme strand having a central catalytic core for binding to a heavy metal and a substrate strand. The substrate strand is conjugated with a biomolecule that binds to a quantum dot fluorescing indicator which is conjugated with the complementary biomolecule. The heavy metal detection test strip includes a test line with a complementary sequence to bind to the substrate strand to indicate that a heavy metal is present in the analyte. The lateral flow test strip further includes a control line for indicating that a test has been correctly performed. The kit includes a bacteria test strip that has an antibody that binds with at least one bacteria in the analyte. In an exemplary embodiment, the analyte is water.

[0010] The lateral flow strip according to one embodiment of the present invention comprises a signal amplifier for enhancing sensitivity of the labeling molecules.

[0011] The labeling molecule according to certain embodiments are Group II-VI semiconductors including cadmium selenide, cadmium sulfide, cadmium telluride, zinc selenide, zinc telluride, and/or alloys thereof.

[0012] In one embodiment, the semiconductor quantum dots of the present invention have an average particle size in a range of 5-20 nm.

[0013] In another embodiment, the conjugating molecules are biomolecules including streptavidin and biotin.

[0014] In certain embodiments, the lateral flow strip of the present system further comprises at least a detection pad and an absorbent pad.

[0015] In some embodiments, the lateral flow strip additionally comprises a sample pad and a conjugate pad.

[0016] The detectable heavy metals in water by the present system include but not limited to lead, chromium and cadmium.

[0017] Each of the DNAzymes used in the present invention comprises at least one substrate strand and one enzyme strand which are specific for detecting one or more heavy metals. In each specific pair of the substrate and enzyme strand, they are partially or fully complementary to each other in terms of the nucleotide sequence thereof, and they bind in a way that a central catalytic core or domain is flanked on the enzyme strand and a single ribonucleotide is resulted on the substrate strand such that the substrate strand is cleavable at the ribonucleotide site when the DNAzyme is exposed to the respective heavy metal ions.

[0018] Preferably, the at least one enzyme strand has a nucleotide sequence selected from any one of SEQ ID NOs: 1 to 3.

[0019] The at least one substrate strand has a nucleotide sequence selected from any one of SEQ ID Nos: 4 to 6, wherein the 5' or 3' end thereof is conjugated with at least one biomolecule. More specifically, the 5' or 3' end of the nucleotide sequence of the at least one substrate strand is biotinated.

[0020] In an exemplary embodiment, the lateral flow strip of the present invention further comprises at least one test line and one control line.

[0021] Preferably, the at least one test line comprises an oligonucleotide with a nucleotide sequence selected from any one of SEQ ID NOs: 7 to 9, wherein the 5' end of the nucleotide sequence is unmodified or modified by an amino modifier.

[0022] The at least one control line of the lateral flow strip comprises an oligonucleotide with a nucleotide sequence of SEQ ID NO: 10, wherein the 3' end thereof is conjugated with at least one biomolecule. More specifically, the 3' end of the nucleotide sequence of the oligonucleotide of the control line is biotinated.

[0023] The present system according to an embodiment has a detection limit for heavy metal lower than 50 ppb. In another embodiment, the detection limit for heavy metal is lower than 10 ppb. In other embodiment, the detection limit for heavy metal is lower than 3 ppb. In particular, the detection limit for chromium is approximately 50 ppb, that for lead is approximately 10 ppb and that for cadmium is approximately 3 ppb.

[0024] The antibody specifically bound to the antigen of the bacteria to be targeted is surface-conjugated with at least one biomolecule. In one embodiment, the at least one biomolecule which is conjugated to the antibody is biotin. In other embodiment, the quantum dots which interact with the signal amplifier are also conjugated with a complementary biomolecule, e.g., streptavidin.

[0025] In one embodiment, the signal amplifier comprises tyramide, horseradish peroxidase (HRP) and hydrogen peroxide.

[0026] In other embodiment, HRP is conjugated with streptavidin.

[0027] In another embodiment, tyramide is conjugated with biotin in order to pair with an HRP-streptavidin conjugate.

[0028] The target bacteria detectable in water by the present system include but not limited to *Escherichia coli* or *Staphylococcus aureus*.

[0029] The present system according to an embodiment has a detection limit for bacteria as low as 1,000 CFU/ μ L in water. In another embodiment, the detection limit for bacteria is about 2,000 CFU/ μ L in water.

[0030] In a second aspect, the present invention provides a method for detecting the presence

and/or quantifying the amount of heavy metals and/or bacteria in an analyte comprising using the lateral flow assay system of the present invention.

[0031] The present method includes preparing a test line and control line for the lateral flow strip; adding an analyte into a small vial which contains a dry form of labelling and detecting molecules followed by mixing to result into a sample mixture, or adding the analyte to the test line directly followed by drying; incubating one end of the lateral flow strip into the sample mixture such that the sample mixture flows across the whole length of the strip by capillary action, or adding the detection molecule to the test line where the analyte is added and dried followed by adding the labelling molecule; collecting the lateral flow strip after certain period of time followed by observing the presence and quantifying the intensity of the fluorescent signal from the strip under UV excitation or by strip reader. If the target contaminant(s) with a concentration above the corresponding detection limit are present in the analyte, a bright fluorescence spot will be observed on the test line under UV radiation. Fluorescence spot should be observed on the control line if the assay is correctly performed.

[0032] In an exemplary embodiment, signal amplifier is added after the detection molecule and before the labelling molecule are added in the case where the analyte is added directly to the test line of the lateral flow strip.

Brief Description of the Drawings:

[0033] Embodiments of the present invention are described in more detail hereinafter with reference to the drawings, in which:

[0034] FIG. 1 shows a schematic diagram of heavy metal detection in a water sample using DNazymes;

[0035] FIG. 2 shows a schematic diagram of a lateral flow assay system for bacteria detection in water sample;

[0036] FIG. 3 shows a design of the double-stranded DNzyme conjugated with biotin for lead detection; asterisk represents the ribonucleotide cleavage site;

[0037] FIG. 4 shows a design of the double-stranded DNzyme conjugated with biotin for chromium detection; asterisk represents the ribonucleotide cleavage site;

[0038] FIG. 5 shows a design of the double-stranded DNzyme conjugated with biotin for cadmium detection; asterisk represents the ribonucleotide cleavage site;

[0039] FIG. 6 shows a design of the test and control lines on the lateral flow strip for lead detection;

[0040] FIG. 7 shows a design of the test and control lines on the lateral flow strip for chromium or cadmium detection;

[0041] FIG. 8 shows the lateral flow assay results, where fluorescence signals at test lines represent the presence of lead ions of different concentrations in water samples;

[0042] FIG. 9A depicts a lateral flow strip for heavy metal detection; FIG. 9B shows a design of lateral flow strip for bacteria detection;

[0043] FIG. 10 shows the lateral flow assay result in terms of a fluorescence intensity curve along lateral flow direction with the presence of *Escherichia coli* at different concentrations;

[0044] FIG. 11 the lateral flow assay result in terms of a fluorescence intensity curve along lateral flow direction with the presence of *Staphylococcus aureus* at different concentrations.

Detailed Description:

[0045] The present invention provides a test kit of lateral assay flow strips that may be used in the field or in a residential setting to quickly and accurately determine the safety of a water supply. There are at least two main target health-threatening contaminants in water that are detectable by the kit, that is, heavy metals and bacteria. A lateral flow assay system is provided that incorporates specific detection mechanisms such as deoxyribozymes (DNAzymes) for detection of heavy metals. In one embodiment, an antibody-antigen specific recognition molecule in combination with tyramide signal amplification is used for the detection of bacteria.

1. Detection of Heavy Metals

[0046] The DNAzymes used in the present invention are engineered strands of DNA each of which is configured to target a specific heavy metal. An example of DNAzyme-based detection of a heavy metal is depicted in FIG. 1. Each DNAzyme 100 includes a separate enzyme strand 110 and a substrate strand 120. The two strands are complementary and bind in a way that flanks a central catalytic core or domain 115 on the enzyme strand and a single ribonucleotide on the substrate strand. Once the DNAzyme is exposed to a target heavy metal ion 130, the substrate strand 120 is cleaved at the ribonucleotide site. The cleaved substrate strand is then captured by the complementary strand 140 at the test line.

[0047] To show the presence of heavy metal or bacteria, robust, highly accurate, and easy-to-read indicator molecules are coupled with the DNAzymes. To this end, quantum dots are selected as the indicators. Quantum dots are semiconductor nanocrystals which can offer

fluorescence emission over the entire spectrum. Quantum dots have diameters sufficiently small so that quantum confinement effects are present causing widening of the bandgap. Therefore, by choosing a suitable material and size, it is possible to obtain a fluorescence emission suitable for field detection. Compared to organic dyes, quantum dots have much brighter fluorescence and higher photostability. These properties can increase the sensitivity and reliability of the lateral flow assay of the present invention. In particular, the emission wavelength of quantum-dot nanocrystals can be continuously tuned and a single light source can be used for simultaneous excitation of all different-sized dots. High-quality dots are also highly stable against photobleaching and have narrow, symmetric emission spectra. These novel optical properties render quantum dots ideal fluorophores for ultrasensitive, multicolor, and multiplexing applications in molecular biotechnology and bioengineering.

[0048] Semiconductor quantum dots are initially functionalized and conjugated with biomolecules such as streptavidin, biotin, etc. For example, in FIG. 6 the quantum dot is conjugated with streptavidin. One end of the DNAzyme substrate strand is conjugated with the complementary biomolecule (e.g., either streptavidin or biotin), as depicted in FIGS. 3 and 4, which can bind with strong affinity to the biomolecule conjugated at the surface of quantum dots. The cleaved substrate strand, (after exposure to the target metal, described above) together with the quantum dot, is then captured by the complementary strand (test line) anchored at the lateral flow strip, as seen in FIG. 1.

[0049] To demonstrate that a water quality test has been successfully performed, the test strip includes a control line. The control line is a single DNA strand that is conjugated with a biomolecule, for example, biotin (when the quantum dot is conjugated with streptavidin). Thus, the biotin-conjugated DNA strand will bind with strong affinity to the streptavidin-conjugated quantum dot, proving that the quantum dots have been successfully flowing through a test strip and that the test has been correctly performed.

[0050] The present system includes a lateral flow strip as a test platform which is configured to carry the essential chemical reagents for labeling and detecting the corresponding health-threatening contaminant(s) in water. In the example of detecting the presence of heavy metals, the lateral flow strip can include at least a detection pad and absorbent pad.

[0051] The quantum dots used in the present invention may be one or more II-VI semiconductor compounds such as cadmium selenide, cadmium sulfide, cadmium telluride, zinc selenide, zinc telluride, and/or mixtures thereof. The quantum dots have an average particle size in a range of 5-20 nm.

[0052] The heavy metals detected by the lateral flow strip include but are not limited to lead,

chromium and cadmium. Other metals may be selected for detection depending upon the water supply being tested.

[0053] For heavy metal detection, each of the DNazymes comprises at least a substrate strand and an enzyme strand specific to one or more heavy metal.

[0054] For example, the DNzyme specific for lead detection comprises an enzyme strand with a nucleotide sequence of 5'-ACA GAC ATC ATC TCT GAA GTA GCG CCG CCG TAT AGT GAG-3' (SEQ ID NO: 1), and a substrate strand with a nucleotide sequence of 5'-CTC ACT AT rA GGA AGA GAT GAT GTC TGT-3' (SEQ ID NO: 4), wherein a biotin-TEG is conjugated at the 5' end of the nucleotide sequence of the substrate strand which can be represented by 5'-/5BiotinTEG/-CTC ACT AT rA GGA AGA GAT GAT GTC TGT-3'.

[0055] In another example, the DNzyme specific for chromium detection comprises an enzyme strand with a nucleotide sequence of 5'-TT TCG CCA TAG GTC AAA GGT GGG TGC GAG TTT TTA CTC GTT ATA GTG ACT CGT GAC-3' (SEQ ID NO: 2), and a substrate strand with a nucleotide sequence of 5'-GTC ACG AGT CAC TAT rA GG AAG ATG GCG AAA-3' (SEQ ID NO: 5), wherein a biotin-TEG is conjugated at the 3' end of the nucleotide sequence of the substrate strand which can be represented by 5'-GTC ACG AGT CAC TAT rA GG AAG ATG GCG AAA-/3BiotinTEG/-3'.

[0056] In other example, the DNazymes specific for cadmium detection comprises an enzyme strand with a nucleotide sequence of 5'-TT TCG CCA TCT TCC TTC GAT AGT TAA AAT AGT GAC TCG TGA C-3' (SEQ ID NO: 3), and a substrate strand with a sequence of 5'-GTC ACG AGT CAC TAT rA GG AAG ATG GCG AAA-3' (SEQ ID NO: 6) wherein a biotin-TEG is conjugated at the 3' end of the nucleotide sequence of the substrate strand which can be represented by 5'-GTC ACG AGT CAC TAT rA GG AAG ATG GCG AAA-/3BiotinTEG/-3'.

[0057] The lateral flow strip of the present system for detecting heavy metals further comprises a test line having a specific oligonucleotide.

[0058] For example, the oligonucleotide specific for lead detection has a nucleotide sequence of 5'-GT ATA GTG AG-3' (SEQ ID NO: 7), wherein AmMC6 is conjugated at the 5' end thereof which can be represented by 5'-/5AmMC6/-GT ATA GTG AG-3'.

[0059] In another example, the oligonucleotide specific for chromium detection has a nucleotide sequence of 5'-TTT CGC CAT CTT CC-3' (SEQ ID NO: 8).

[0060] In another example, the oligonucleotide specific for cadmium detection has a nucleotide sequence of 5'-TTT CGC CAT CTT CC-3' (SEQ ID NO: 9).

[0061] The lateral flow strip of the present invention further comprises a control line having

an oligonucleotide with a nucleotide sequence of 5'-TAG TCG ACA TGC TCC-3' (SEQ ID NO: 10), wherein a biotin-TEG is conjugated at the 3' end of the nucleotide sequence which is represented by 5'-TAG TCG ACA TGC TCC-/3BiotinTEG/-3'.

[0062] FIG. 9A depicts an example of a lateral flow strip 400 for testing heavy metals according to an embodiment. The strip 400 includes an absorbent pad 410, and a detection pad 420. Positioned within the detection pad 420 is a control line 430 and a test line 440. The control line 430 indicates that the test has been correctly performed while the test line 440 indicates the presence of the target heavy metal.

[0063] In use, a lateral flow assay strip for heavy metal works in a two-step process. The water sample (analyte) is added into a small vial which contains the dry form of necessary reaction chemicals. After facilitating slight mixing, a lateral flow strip with a prepared test line and a control line is put into the vial which contains the sample to be tested. One end of the flow strip is then soaked with the test sample and the sample will flow across the whole length of the strip by capillary action. After the test sample has flowed across the length of the strip, the strip is taken out and observed under UV excitation or read by a strip reader. If heavy metal ions with concentration above certain range are present in the water sample, a bright fluorescence spot is observed on the test line. A fluorescence spot is observed on the control line if the test is correctly performed. An example of fluorescence indicating the presence of heavy metal (and a spot indicating that the test has been correctly performed) is depicted in FIG. 8.

[0064] The lateral flow assay system of present invention has a detection limit for the presence of heavy metal ions at a concentration lower than 50 ppb. More specifically, the detection limit specific for the presence of lead ions can be lower than 10 ppb; the detection limit for the presence of chromium ions can be lower than 50 ppb; and the detection limit for the presence of cadmium ions can be lower than 3 ppb in water.

2. Detection of Bacteria

[0065] The lateral flow assay system of the present invention for detecting bacteria in water includes an antibody specifically bound to a surface antigen of the bacteria which is conjugated with a biomolecule such as streptavidin or biotin.

[0066] In the lateral flow assay for bacteria, the detection is based on selective recognition between a bacterial surface antigen and a certain antibody. Antibodies are conjugated with biotin while quantum dots are conjugated with streptavidin. An indication of the presence of bacteria is based on the fluorescence of quantum dots under an ultraviolet light source, with

optional signal amplification based on tyramide signal amplification (TSA). TSA reaction occurs between tyramide and protein, mediated by horseradish peroxidase (HRP) and hydrogen peroxide, resulting in high-density tyramide labelling of protein in situ. In this case, TSA reagents are composed by a HRP-streptavidin conjugate, hydrogen peroxide, tyramide-biotin conjugate and reaction buffer.

[0067] After bacteria in the water sample are absorbed on the test line of a test strip, a specific antibody-biotin conjugate and HRP-streptavidin conjugate will be captured one by one, through antibody-antigen and streptavidin-biotin recognition, respectively. High-density tyramide-biotin conjugate and quantum dot-streptavidin conjugate will further label on antibody one by one, through TSA reaction and streptavidin-biotin recognition. In the absence of bacteria, no quantum dot conjugated with streptavidin will be captured and detected.

[0068] In one example, the antibody may be a rabbit polyclonal antibody.

[0069] The bacteria detectable in water by the present invention may include, but is not limited to, *Escherichia coli* and *Staphylococcus aureus*.

[0070] For *Escherichia coli*, the present system can detect at a concentration lower than 1000 CFU/ μ L in water.

[0071] For *Staphylococcus aureus*, the present system can detect at a concentration lower than 2000 CFU/ μ L in water.

[0072] FIG. 9B depicts an exemplary lateral flow strip 300 for bacteria detection. The flow is from right to left in FIG. 9B. The lateral flow strip 300 includes a sample pad 310 for absorbing a water sample. Through capillary action, the water proceeds to the detection pad 315. Detection pad 315 includes the conjugate pad 320, test line 330 (for indicating whether bacteria is present), and bacteria-blocking line 340. Excess water is absorbed by absorbent pad 350.

[0073] The detection pad of the lateral flow strip may be made of nitrocellulose. The absorbent pad 350 of the lateral flow strip can be made of cotton pulp. Any one or both of the sample pad 310 and conjugate pad 320 of the lateral flow strip 300 can be made of fiberglass.

[0074] The bacteria-blocking line 340 is positioned in front of the test line and may include a hydrophilic polymer such as polyvinyl alcohol (PVA), and bovine serum albumin (BSA).

[0075] The lateral flow assay for bacteria is performed as follows. First, a water sample is introduced on the test line position 330 and left to dry naturally. Second, an antibody-biotin conjugate flows across the lateral flow strip, which selectively captures a surface antigen of a bacteria. Tyramide signal amplification (TSA) reagents are then optionally applied and

activated on the test line 330. Lastly, a conjugated quantum dot flows across the lateral flow strip. After a certain period of time, the strip is observed under UV radiation or read by a strip reader. If bacteria with a concentration above a certain range are present in the water sample, a bright fluorescence signal can be observed on the test line under UV radiation. Similarly, a fluorescent peak at the test line position can be read out using a strip reader.

[0076] Note that the above quantum-dot-based lateral flow assay is only one example of bacteria test that can be included in the kits of the present invention. Other bacteria tests may be incorporated in the kits of the present invention. Further, plural test strips may be integrated into a single detection element. For example, this may take the form of a single test strip with an array of test and control lines indicating the presence or absence of multiple metals and bacteria. Such single strips may be particularly useful for consumer applications, such as testing home water supplies.

[0077] It should be apparent to those skilled in the art that many modifications besides those already described are possible without departing from the inventive concepts herein. The inventive subject matter, therefore, is not to be restricted except in the spirit of the disclosure. Moreover, in interpreting the disclosure, all terms should be interpreted in the broadest possible manner consistent with the context. In particular, the terms “includes”, “including”, “comprises” and “comprising” should be interpreted as referring to elements, components, or steps in a non-exclusive manner, indicating that the referenced elements, components, or steps may be present, or utilized, or combined with other elements, components, or steps that are not expressly referenced.

Claims:

1. A kit for detecting heavy metals and bacteria in an analyte comprising:
at least one heavy metal lateral flow test strip comprising a deoxyribozyme detection molecule, the deoxyribozyme detection molecule including an enzyme strand having a central catalytic core for binding to a heavy metal, and substrate strand, the substrate strand being conjugated with a biomolecule that binds to a quantum dot fluorescing indicator conjugated with a complementary biomolecule, the lateral flow test strip including a test line having a complementary sequence to bind with the substrate strand to indicate that a heavy metal is present in the analyte, the lateral flow test strip further comprising a control line for indicating that a test has been correctly performed;
a bacteria test strip, the bacteria test strip including an antibody that binds with at least one bacteria in the analyte.
2. The kit according to claim 1, wherein said lateral flow strip includes a signal amplifier for enhancing sensitivity of the indicator.
3. The kit according to claim 1, wherein the quantum dot comprises one or more of Group II-VI semiconductors, and/or mixtures thereof.
4. The kit according to claim 3, wherein the quantum dot comprises cadmium selenide, cadmium sulfide, cadmium telluride, zinc selenide, zinc telluride, and/or alloys thereof.
5. The kit according to claim 1, wherein the quantum dot has an average particle size in a range of 5-20 nm.
6. The kit according to claim 1, wherein the biomolecule is selected from streptavidin or biotin.
7. The kit according to claim 1, wherein the lateral flow strip further comprises at least a detection pad and an absorbent pad.

8. The kit according to claim 1, wherein the lateral flow strip additionally comprises a sample pad and a conjugate pad.
9. The kit according to claim 1, wherein the heavy metal is selected from one or more of lead, chromium, or cadmium.
10. The kit according to claim 1, wherein the substrate strand includes a single ribonucleotide cleavable at the ribonucleotide site when the deoxyribozyme is exposed to a target heavy metal.
11. The kit according to claim 1, wherein the enzyme strand has a nucleotide sequence selected from any one of SEQ ID NOs: 1 to 3.
12. The kit according to claim 1, wherein the substrate strand has a nucleotide sequence selected from any one of SEQ ID NOs: 4 to 6.
13. The kit according to claim 1, wherein the substrate strand is conjugated with at least one biomolecule at the 5' or 3' end of the nucleotide sequence thereof.
14. The kit according to claim 1, wherein the test line comprises an oligonucleotide with a nucleotide sequence selected from any one of SEQ ID NOs: 7 to 9,
15. The kit according to claim 13, wherein the 5' end of the nucleotide sequence of the oligonucleotide is modified by an amino modifier.
16. The kit according to claim 1, wherein the control line comprises an oligonucleotide with a nucleotide sequence of SEQ ID NO: 10.
17. The kit according to claim 13, wherein the 3' end of the nucleotide sequence of the oligonucleotide is conjugated with at least one biomolecule.
18. The kit according to claim 1, wherein the antibody is conjugated with at least one biomolecule.

19. The kit according to claim 18, wherein the at least one biomolecule which is conjugated to the antibody is biotin.
20. The kit according to claim 2, wherein said signal amplifier comprises tyramide, horseradish peroxidase and hydrogen peroxide.
21. The kit according to claim 20, wherein tyramide is conjugated with biotin in order to pair with an HRP-streptavidin conjugate.
22. The kit according to claim 1, wherein the bacteria detectable comprise *Escherichia coli* and *Staphylococcus aureus*.
23. The kit according to claim 1, in which the heavy metal detection limit is lower than 50 ppb.
24. The kit according to claim 1, in which the detection limit for bacteria is 1,000 CFU/ μ L.
25. The kit according to claim 1, wherein the heavy metal lateral flow test strip and the bacteria strip are integrated with one another into a single strip.
26. The kit according to claim 18, wherein the bacteria test strip includes biomolecule-conjugated quantum dots for binding to the biomolecule-conjugated antibody.
27. A method for detecting the presence and/or quantifying the amount of heavy metals and/or bacteria in an analyte comprising using the kit according to any one of claims 1 to 26.
28. The method according to claim 27, further comprising:
 - preparing a test line and a control line for the lateral flow strip;
 - adding an analyte into a small vial which contains a dry form of the conjugating and detecting molecules followed by mixing to result in a sample mixture, or adding the analyte to the test line directly followed by drying;

incubating one end of the lateral flow strip into the sample mixture such that the sample mixture flows across the whole length of the strip by capillary action, or adding the detection molecule to the test line where the analyte is added and dried followed by adding the labelling molecule;

collecting the lateral flow strip after certain period of time followed by observing the presence and quantifying the intensity of the fluorescent signal from the strip under UV excitation or by strip reader.

29. The method according to claim 28, further comprising adding signal amplifier after the detection molecule and before the labelling molecule are added to the test line when the analyte is added directly to the test line of the lateral flow strip.

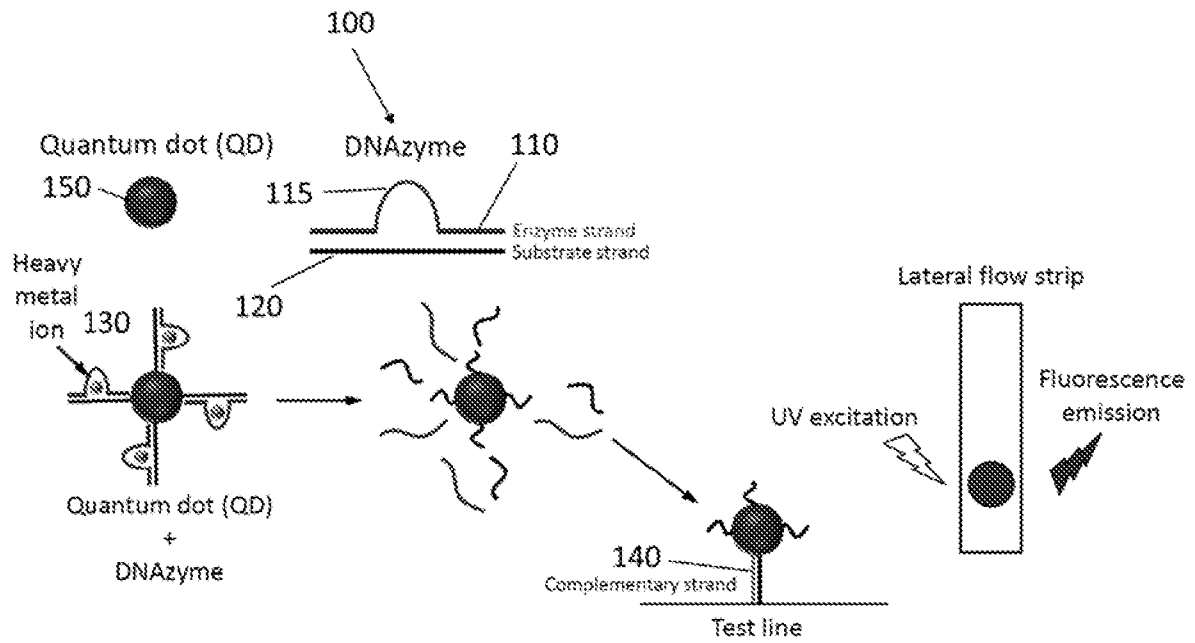


FIG. 1

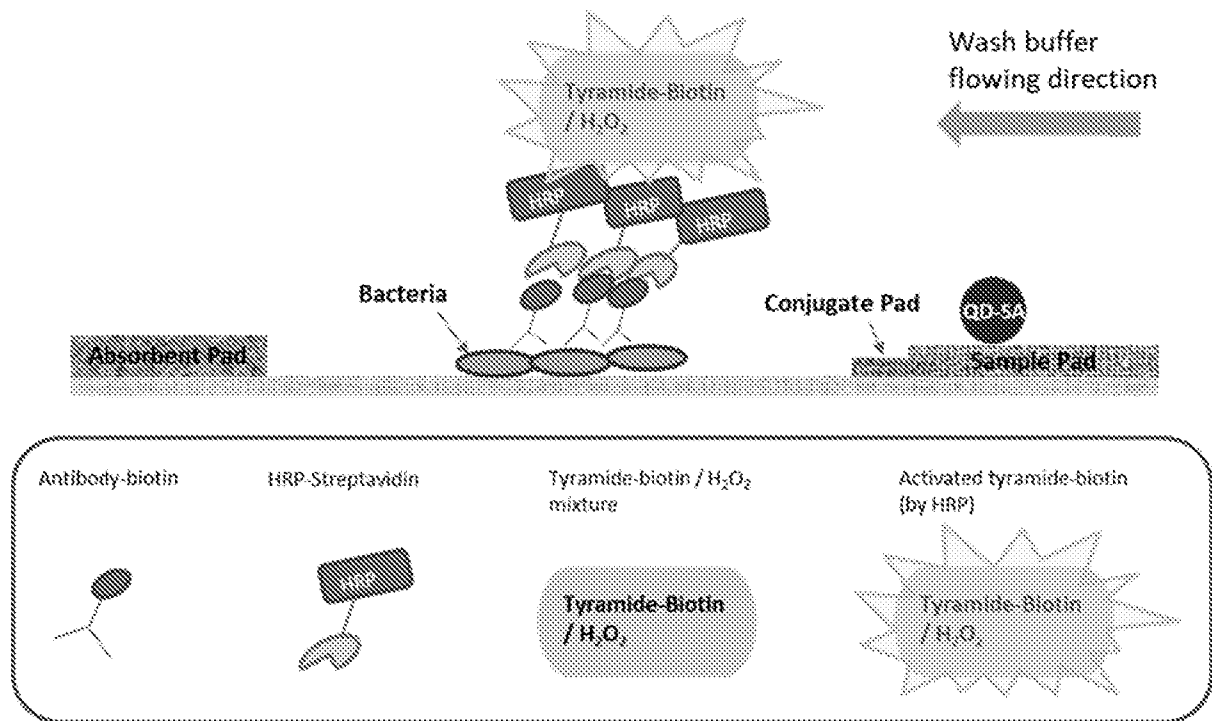


FIG. 2

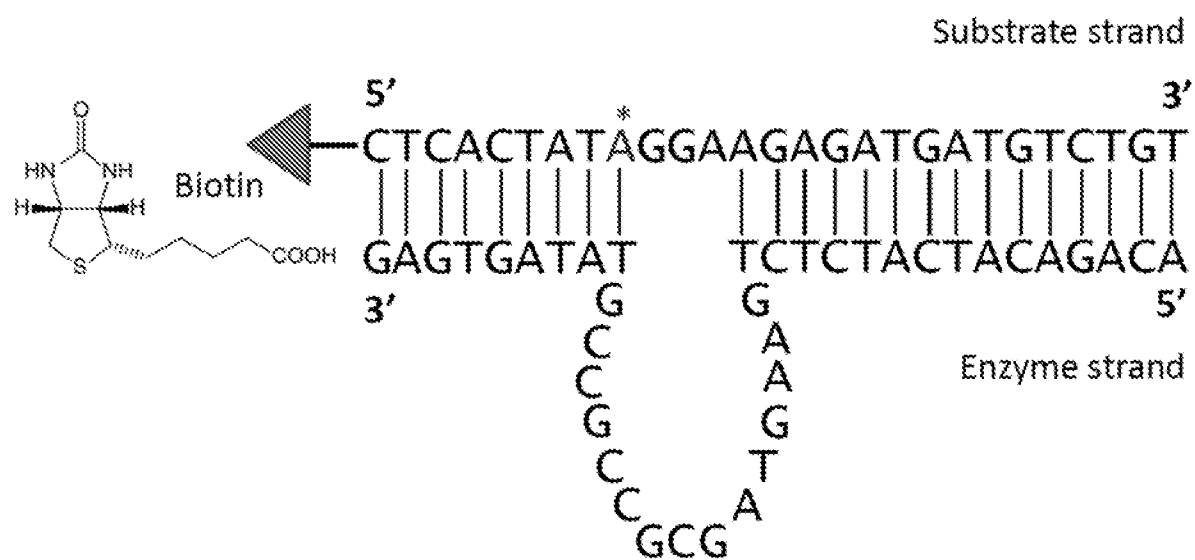


FIG. 3

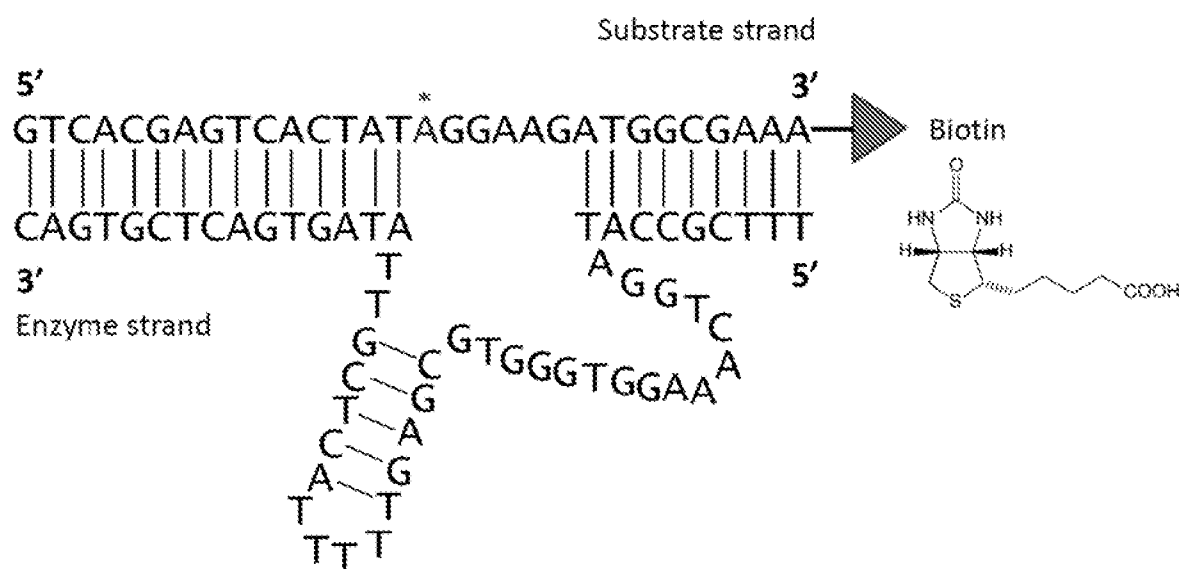


FIG. 4

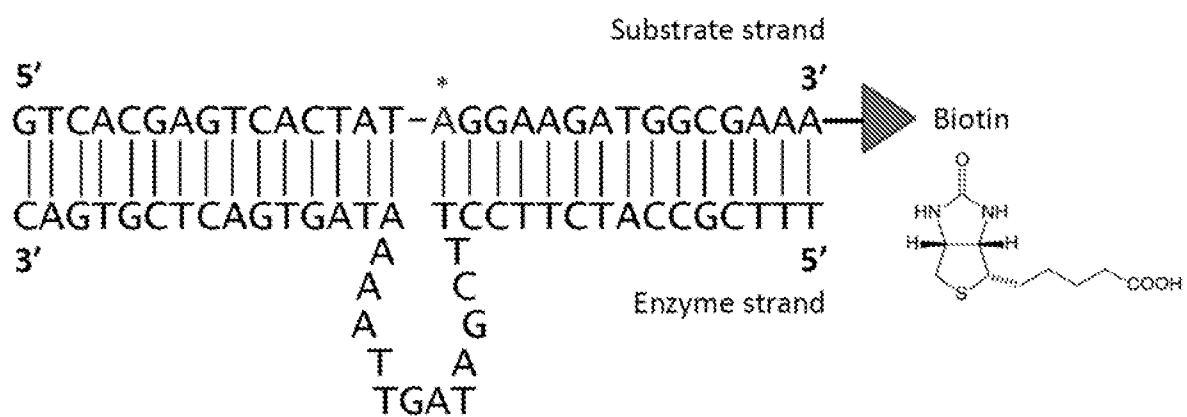


FIG. 5

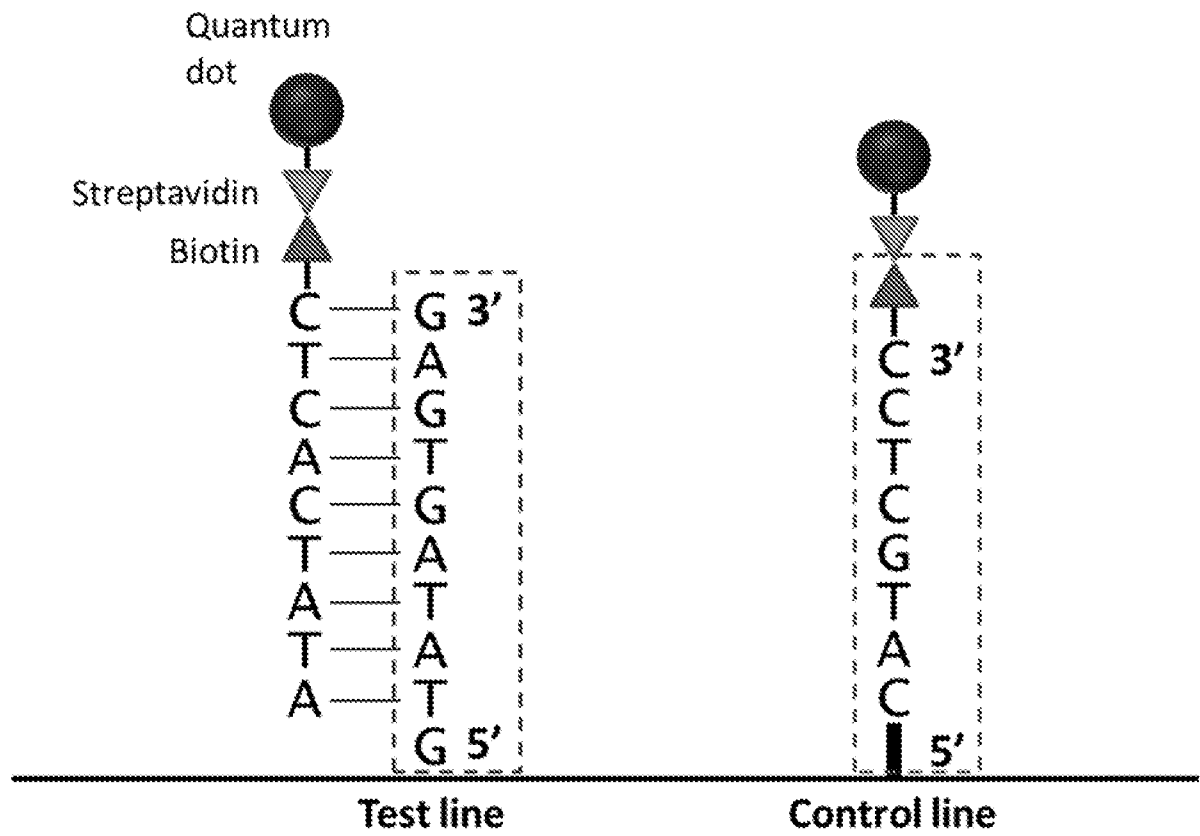


FIG. 6

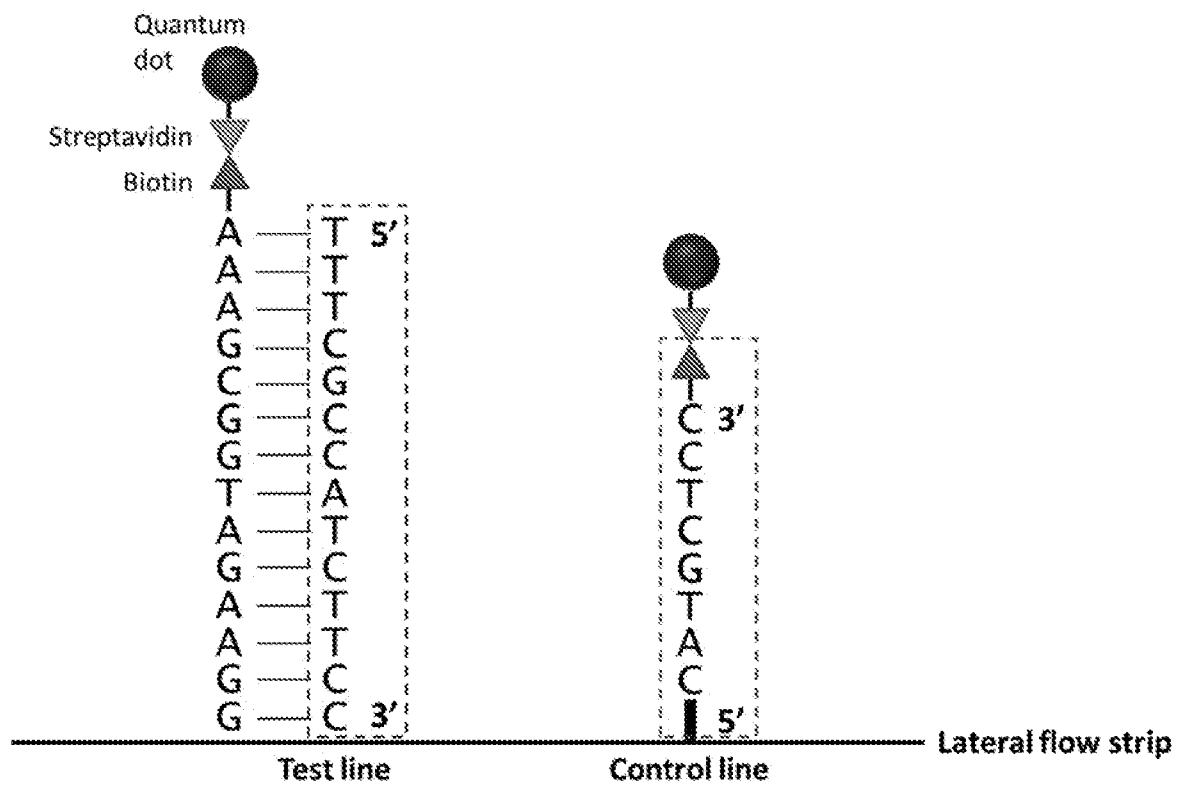


FIG. 7

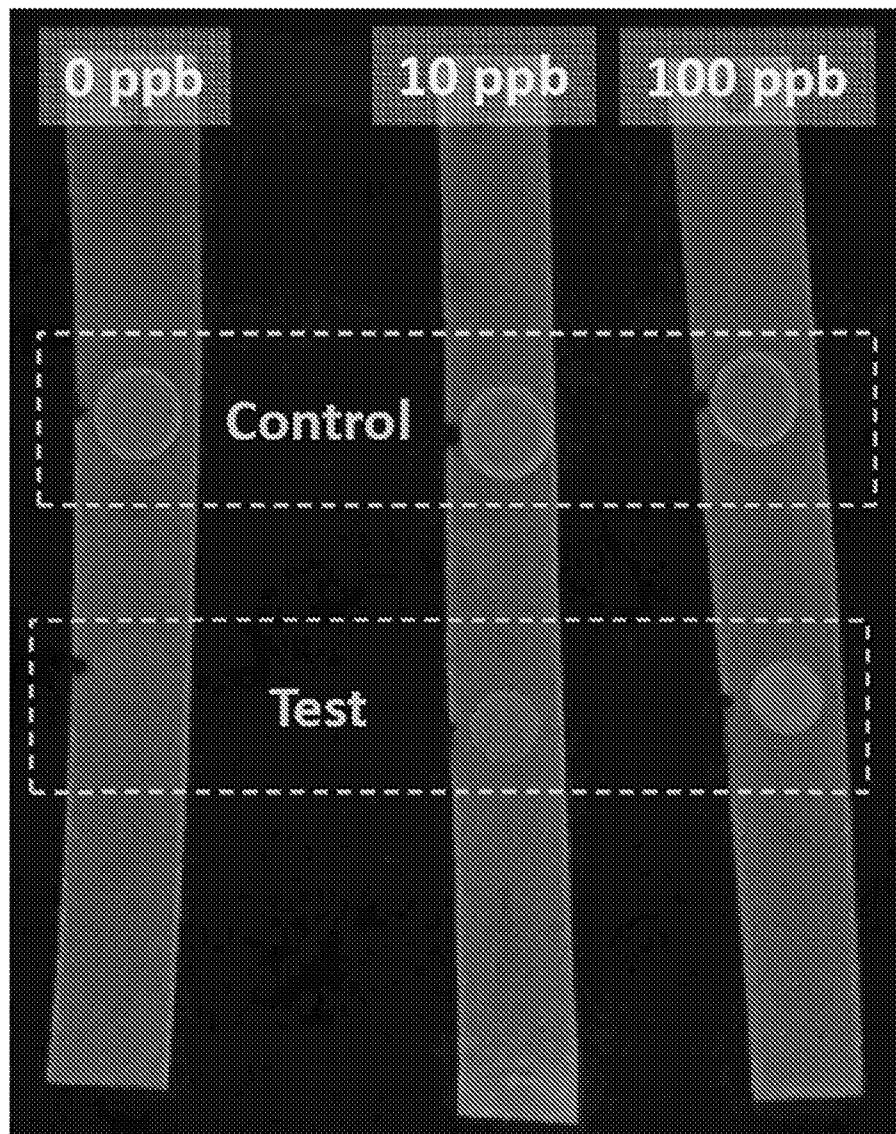


FIG. 8

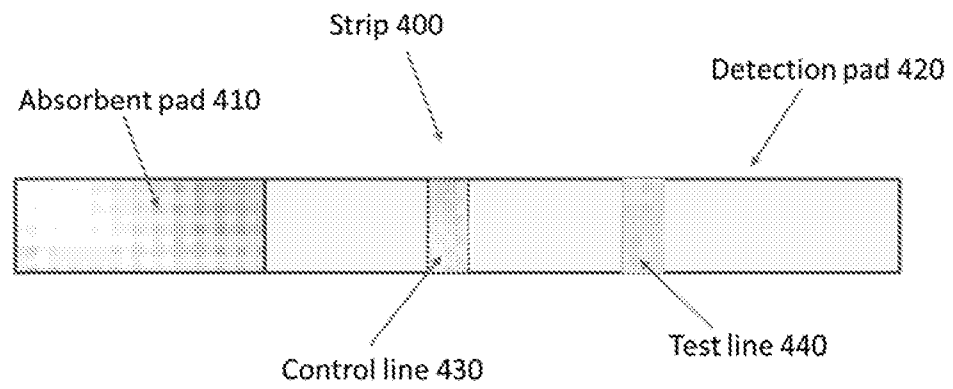


FIG. 9A

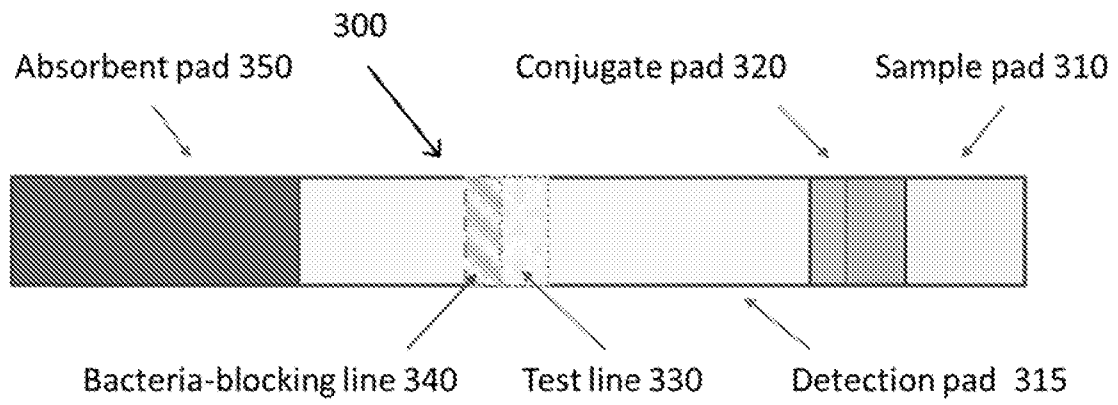


FIG. 9B

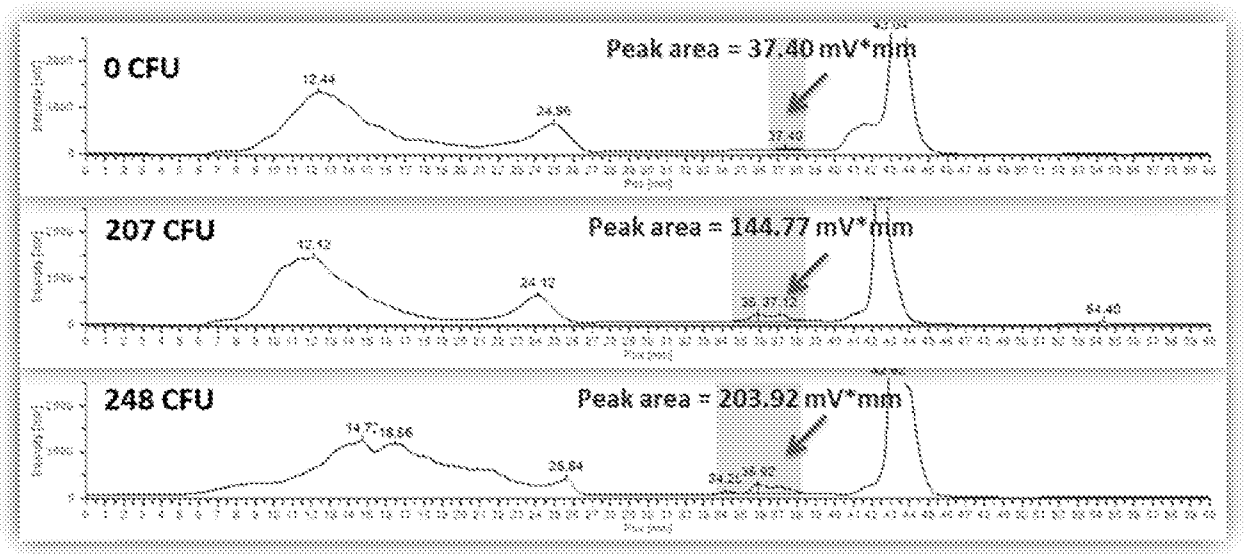


FIG. 10

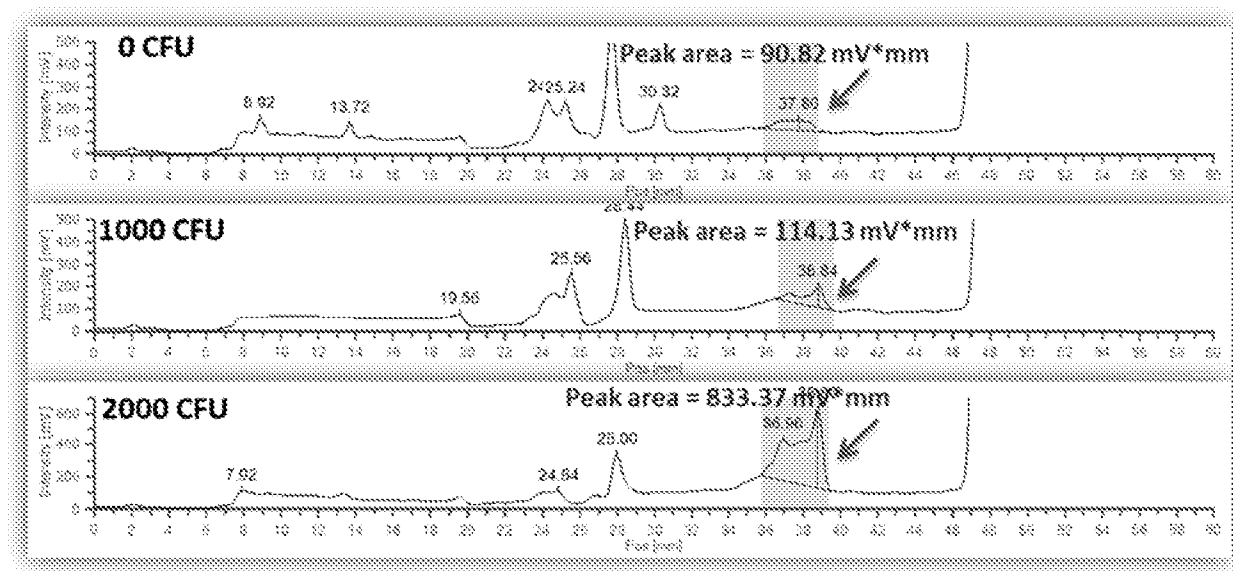


FIG. 11

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2019/076886

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/543(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N33, C12Q1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

VEN;CNABS;ISI;CNKI: heavy metal, lateral flow, deoxyribozyme, Immunochromatography, DNAzyme, lead ion

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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A	Zhiyuan Fang, et al. "Lateral flow nucleic acid biosensor for Gu2+ detection in aqueous solution with high sensitivity and selectivity." <i>Chem. Commun.</i> , No. 46, 31 December 2010 (2010-12-31), pages 9043-9045	1-29
A	Wang Dou, et al. "A simple lateral flow biosensor for the rapid detection of copper(II) ions based on click chemistry." <i>RSC ADVANCES</i> , Vol. 92, No. 5, 31 December 2015 (2015-12-31), pages 75722-75727	1-29
A	Wang Yulong, et al. "Signal-Amplified Lateral Flow Test Strip for Visual Detection of Cu2+" <i>PLOS ONE</i> , Vol. 12, No. 1, 10 January 2017 (2017-01-10), pages 1-13	1-29
A	US 2015031014 A1 (UNIV ILLINOIS FOUND) 29 January 2015 (2015-01-29) the whole document	1-29



Further documents are listed in the continuation of Box C.



See patent family annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

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"&" document member of the same patent family

Date of the actual completion of the international search

30 September 2019

Date of mailing of the international search report

18 October 2019

Name and mailing address of the ISA/CN

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Facsimile No. (86-10)62019451

Telephone No. 86-(010)-62085241

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2019/076886

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CN2019/076886

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US	2012315621	A1	13 December 2012	EP	2576806	A4	11 December 2013
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				EP	2576806	A1	10 April 2013
				WO	2011150186	A1	01 December 2011
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				US	2013065224	A1	14 March 2013
				EP	2576806	B1	08 March 2017
				CA	2800257	C	05 March 2019
				CN	103025885	B	20 January 2016
				CN	103025885	A	03 April 2013
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JP	2003052373	A	25 February 2003	None			
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				IL	236363	D0	26 February 2015
				JP	2015523864	A	20 August 2015
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PCT/CN2019/076886

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