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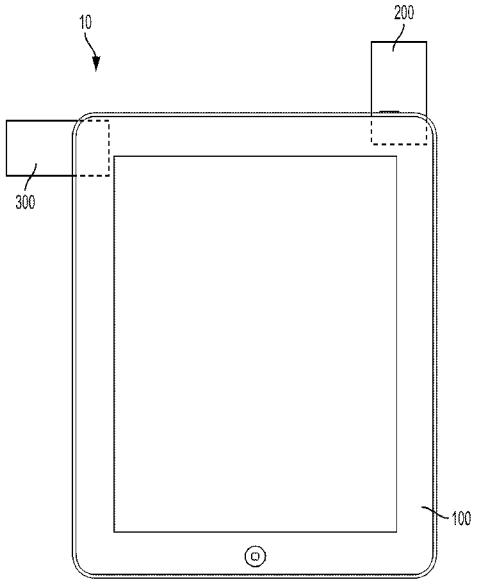

FIG. 1

(57) Abstract: A device for detecting the presence of a target in a sample including a first port configured to receive a multi-layered substrate having a sample inlet and a reagent inlet. The sample inlet is connected to a first microfluidic channel and the reagent inlet is connected to both the first microfluidic channel and a second microfluidic channel. The second microfluidic channel has a longer pathway than the first microfluidic channel. A first test strip and a second test strip are each connected to both the first microfluidic channel and the second microfluidic channel, while a third test strip is connected only to the first microfluidic channel. Each test strip includes a conjugate section, a detection section, and a collection section.

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DEVICE AND METHOD FOR POINT-OF-CARE DIAGNOSTICS AND ANTIBIOTIC RESISTANCE IDENTIFICATION, AND APPLICATIONS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to and the benefit of U.S. provisional patent application number 62/194,389, filed on July 20, 2015, the entire contents of which are hereby incorporated by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] The present disclosure is directed generally to a device for molecular diagnostics, and more particularly, to a portable device with a three-stage test immunoassay and minimum inhibitory concentration assay for point-of-care diagnosis.

2. Description of the Related Art

[0002] Currently, sepsis at different life stages, neonatal, childhood, or in adults, is a leading cause of death globally. Neonatal sepsis, in particular, presents vague signs and symptoms; therefore, current management of neonatal sepsis requires a high index of suspicion, even on the part of highly trained clinicians. For example, one presenting sign may only include an alteration in feeding behavior. Further, the use of antibiotics must be carefully calculated as the inappropriate use of antibiotics leads to the emergence of resistant strains of pathogens.

[0003] The sustained increase in antibiotic resistance (ABR) is a major concern worldwide that is affecting patient outcomes causing significant increases in morbidity and mortality. As per the 2015 World Health Organization (WHO) report on current practices in place to address ABR, many governments have initiatives, but there are major discontinuities

in action across all 6 WHO regions and many low-income countries do not have a response plan. A recent review has estimated 10 million deaths worldwide and economic loss of around $100 trillion due to drug-resistant infections. The Centers for Disease Control and Prevention (CDC) reports that almost 50% of antibiotics prescribed for people are not required and also not effective. Additionally, the CDC reports that each year in the U.S., at least 2 million people acquire bacterial infections resistant to one or more antibiotics and at least 23,000 people die each year as a result.

[0004] Traditional minimum inhibitory concentration (MIC) assays are performed by diffusion or dilution methods. Diffusion method involves a hydrophilic strip or disc infused with antibiotic that is placed in contact with the agar plate surface on which a microbe is cultured. The MIC is estimated based on a visual 'zone of inhibition' around the disc or strip. The analyses of results obtained by diffusion method are subjective and variable. In dilution method, a series of culture tubes or agar plates with nutrient media and serial dilution of an antibiotic are used to grow bacteria. The MIC is determined by visual inspection, by identifying the lowest concentration of antibiotics that inhibits bacterial growth. The guidelines for determining MIC by dilution-based methods have been published by the Clinical Laboratory and Standards Institute (CLSI) in the U.S.

[0005] The majority of quantitative ABR evaluation is done via automated systems that are not portable and rely on some variant of traditional microdilution testing. Conceptually, in these systems, the bacterial sample is split and exposed to an array of different antibiotics and doses. The plate or card is incubated for a period of time and then read to determine the MIC of each antibiotic that halts cell growth. The specifics of the assay and their read out format vary from manufacturer to manufacturer (see the Vitek II, *Brilliance*™ ESBL, MicroScan WalkAway, Phoenix, and Sensititre systems), but generally

require at least 16 to 24 hours for obtaining final susceptibility results depending on the organism.

[0006] Several microfluidic implementations of diffusion/dilution methods have been reported to reduce assay time and rely on applying plugs of fluids, concentration gradient generators, microparticles and dielectrophoresis. However, these approaches require multiple steps, technician training and other external equipment such as syringe pump, which are barriers to translating these devices to clinical applications and point-of-care diagnostics. There is a need for a point-of-care MIC assay technology that does not require external equipment, can be operated without extensive user training, and can measure the MIC of various antibiotics with required specificity/sensitivity in a cost-effective manner.

[0007] Technological advancements, especially in the medical field, seldom reach resource-limited populations. For example, current medical diagnostic equipment can be costly, bulky, and require sophisticated training to operate and maintain. Therefore, there is a need in the art for a point-of-care molecular diagnostic device that can identify infectious disease pathogens and antibiotic resistance quickly and with little skill required such to enable health workers around the globe appropriately refer and manage infections and sepsis.

[0008] Description of the Related Art Section Disclaimer: To the extent that specific patents/publications/products are discussed above in this Description of the Related Art Section or elsewhere in this Application, these discussions should not be taken as an admission that the discussed patents/publications/products are prior art for patent law purposes. For example, some or all of the discussed patents/publications/products may not be sufficiently early in time, may not reflect subject matter developed early enough in time and/or may not be sufficiently enabling so as to amount to prior art for patent law purposes. To the extent that specific patents/publications/products are discussed above in this Description of the Related Art Section and/or throughout the application, the

descriptions/disclosures of which are all hereby incorporated by reference into this document in their respective entirety(ies).

SUMMARY OF THE INVENTION

[0009] Embodiments of the present invention recognize that there are potential problems and/or disadvantages with the conventional devices for molecular diagnostics. Therefore, the need exists for a simple-to-use device which can identify pathogens and their resistance to antibiotics at the point-of-care. Various embodiments of the present invention may be advantageous in that they may solve or reduce one or more of the potential problems and/or disadvantages discussed herein.

[0010] The present disclosure is directed to an inventive configuration, structure, and resulting function of a device for detecting the presence of a target in a sample. The device comprises a first port configured to receive a multi-layered substrate having a sample inlet and a reagent inlet. The sample inlet is connected to a first microfluidic channel and the reagent inlet is connected to both the first microfluidic channel and a second microfluidic channel. The second microfluidic channel comprises a longer pathway than the first microfluidic channel. A first test strip and a second test strip are each connected to both the first microfluidic channel and the second microfluidic channel, while a third test strip is connected only to the first microfluidic channel. Each test strip comprises a conjugate section, a detection section, and a collection section.

[0011] According to an alternative embodiment, a method for detecting a target in a sample comprises the step of first providing a device having a first port configured to receive a multi-layered substrate having a sample inlet and a reagent inlet. The sample inlet is connected to a first microfluidic channel and the reagent inlet is connected to both the first microfluidic channel and a second microfluidic channel. The second microfluidic channel

comprises a longer pathway than the first microfluidic channel. A first test strip and a second test strip are each connected to both the first microfluidic channel and the second microfluidic channel, while a third test strip is connected only to the first microfluidic channel. Each test strip comprises a conjugate section, a detection section, and a collection section. Once the device is provided, the method further comprises the steps of labeling detection antibodies of the target with nanoparticles and depositing the detection antibodies at the conjugate section. Secondary antibodies of the target are also labeled with nanoparticles and deposited at the conjugate section. Next, the sample is inserted into the sample inlet and a reagent is inserted into the reagent inlet. At the next step, the sample flows across each test strip. Finally, detection antibodies and secondary antibodies are captured at the detection section.

[0012] According to another embodiment, a method for detecting a target in a sample comprises the step of first providing a device having a first port configured to receive a multi-layered substrate having a sample inlet and a reagent inlet. The sample inlet is connected to a first microfluidic channel and the reagent inlet is connected to both the first microfluidic channel and a second microfluidic channel. The second microfluidic channel comprises a longer pathway than the first microfluidic channel. A first test strip and a second test strip are each connected to both the first microfluidic channel and the second microfluidic channel, while a third test strip is connected only to the first microfluidic channel. Each test strip comprises a conjugate section, a detection section, and a collection section. The device comprises a second port configured to receive a MIC chip interface. The MIC chip interface has an open volume configured to receive a MIC chip therein. The MIC chip comprises one or more wells and the MIC chip interface comprises one or more magnets in alignment with the wells when the MIC chip is inserted into the open volume of the MIC chip interface. Once the device is provided, the method further comprises the steps of applying an antibiotic to a solid media in each well, inserting the MIC chip into the MIC chip interface, mixing a

sample with a biorecognition element to create a mixture, depositing the mixture into the MIC chip, and flowing the mixture across the wells.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] The present invention will be more fully understood and appreciated by reading the following Detailed Description in conjunction with the accompanying drawings. The accompanying drawings illustrate only typical embodiments of the disclosed subject matter and are therefore not to be considered limiting of its scope, for the disclosed subject matter may admit to other equally effective embodiments.

[0014] Reference is now made briefly to the accompanying drawings, in which:

[0015] FIG. 1 is a perspective view schematic representation of an exemplary embodiment of a device to detect the presence of a target in a sample;

[0016] FIG. 2 is a perspective view schematic representation of an exemplary embodiment of the fluid network of the device;

[0017] FIG. 3 is a structure diagram of an exemplary embodiment of the fluid network of the device;

[0018] FIG. 4 is a perspective view schematic representation of an exemplary embodiment of a test strip of the fluid network;

[0019] FIG. 5 is a perspective view schematic representation of an exemplary embodiment of the minimum inhibitory concentration (MIC) chip assembly of the device;

[0020] FIG. 6 is a perspective view schematic representation of an exemplary embodiment of a sample mixture inserted into the MIC chip;

[0021] FIG. 7 is a diagram of an exemplary embodiment of a well during the MIC assay;

[0022] FIG. 8 is a perspective view schematic representation of an exemplary embodiment of the MIC chip in the device; and

[0023] FIG. 9 is a flowchart illustrating the method for diagnosing neonatal sepsis and determining the appropriate treatment.

[0024] Where applicable, like reference characters designate identical or corresponding components and units throughout the several views, which are not to scale unless otherwise indicated. Moreover, the embodiments disclosed herein may include elements that appear in one or more of the several views of in combinations of the several views.

DETAILED DESCRIPTION

[0025] Referring now to the drawings, wherein like reference numerals refer to like parts throughout, there is seen in FIG. 1 a perspective view schematic representation of an exemplary embodiment of a device 100 to detect the presence of a target in a sample. The device 100 is part of a system 10 that can include a first port configured to receive a fluid network 200 and a second port configured to receive a minimum inhibitory concentration (MIC) chip assembly 300. In use, the system 10 can identify infectious disease pathogens and determine antibiotic resistance. Examples of targets identified by the system include, but are not limited to C-Reactive Protein (CRP), procalcitonin (PCT), and Endotoxin. In one embodiment, the device 100 is an electronic device, such as a smartphone, for example. As smartphones are becoming increasingly ubiquitous and user-friendly, they provide a compact platform that can transform health care.

[0026] Referring now to FIG. 2, there is shown a perspective view schematic representation of an exemplary embodiment of the fluid network 200 of the device 100. In the embodiment shown in FIG. 2, the fluid network 200 comprises a substrate having a

sample inlet 202 and a reagent inlet 204. The sample inlet 202 is configured to receive a sample, such as a drop of blood and the reagent inlet 204 is configured to receive a reagent, such as a buffer. The sample inlet 202 may additionally comprise a filtration membrane to maintain the sample before the flow of the sample is initiated.

[0027] The sample inlet 202 is connected to a first microfluidic channel 206. The first microfluidic channel 206 extends to a first test strip 208a, a second test strip 208b, and a third test strip 208c. The reagent inlet 204 is connected to the first microfluidic channel 206 and is also connected to a second microfluidic channel 210. The second microfluidic channel 210 connects only to the first test strip 208a and the second test strip 208b. In the depicted embodiment, the sample inlet 202 is disposed in the first microfluidic channel 206 between the reagent inlet 204 and the test strips 208a-c. When a sample is inserted into the sample inlet 202, the sample remains in a filtration membrane of the sample inlet 202 until contacted by the reagent. Thus, application of the reagent via the reagent inlet 204 initiates flow of the sample to the all three test strips 208a-c.

[0028] The application of a reagent via the reagent inlet 204 also initiates flow of the reagent into the second microfluidic channel 210, which comprises an enhancement solution membrane 212 therein. The enhancement solution membrane 212 contains an enhancement solution used to amplify the detection of the target in the sample as some targets are found in very low concentrations. For example, PCT and Endotoxin are found in very low concentrations (pg/ml – ng/ml) in blood. Thus, the enhancement solution would permit detection of low concentrations of PCT and Endotoxin that would otherwise be difficult or impossible to detect. In one embodiment, the enhancement solution is a silver enhancement solution.

[0029] The second microfluidic channel 210 comprises a longer pathway than the first microfluidic channel 206. For example, in the embodiment shown in FIG. 2, the second

microfluidic channel 210 is tortuous thereby creating a longer pathway than the first microfluidic channel 206. However, other pathway configurations are contemplated. When a reagent is applied via the reagent inlet 204, the reagent must travel the longer pathway in the second microfluidic channel 210 before it contacts the enhancement solution membrane 212. Once the reagent reaches the enhancement solution membrane 212, the enhancement solution is released and flows in the second microfluidic channel 210 to the first test strip 208a and the second test strip 208b.

[0030] As described above and shown in FIG. 2, the tortuous second microfluidic channel 210 comprises a longer pathway creating a time delay from the time the sample reaches the test strips 208a-c to the time the enhancement solution reaches the first test strip 208a and the second test strip 208b. The time delay created by the longer pathway of the second microfluidic channel 210 allows optimal exposure of the first test strip 208a and the second test strip 208b to the sample before the enhancement solution is introduced.

[0031] A structure diagram of an exemplary embodiment of the fluid network 200 is shown in FIG. 3. The embodiment of the fluid network 200 shown in FIG. 3 is composed of a substrate having a four-layer structure. As shown in FIG. 3, the first microfluidic channel 206 and the second microfluidic channel 210 extend through different layers of the substrate to allow for optimal flow in a compact structure. The substrate may be composed of a plastic composition or other like materials.

[0032] In the embodiments shown in FIGs. 2 – 3, there are three test strips 208a, 208b, 208c, which may detect Endotoxin, PCT, and CRP, respectively. The three-plex test configuration provides rapid detection and identification of pathogens known to cause or otherwise contribute to neonatal sepsis. As stated above, PCT and Endotoxin are found in very low concentrations in the blood. Thus, in the embodiments shown in FIGs. 2 – 3, test strips representing an Endotoxin test strip 208a and a PCT test strip 208b are connected to

both the second microfluidic channel 210 and the first microfluidic channel 206. This configuration allows the Endotoxin test strip 208a and the PCT test strip 208b to receive and be exposed to the sample before the enhancement solution arrives from the second microfluidic channel 210. However, as CRP is found in higher concentrations in the blood and thus does not require the enhancement solution, the CRP test strip 208c is only connected to the first microfluidic channel 206. Other configurations are contemplated for specific targets and combinations of targets.

[0033] Referring now to FIG. 4, there is shown a perspective view schematic representation of an exemplary embodiment of a test strip 208a-c of the fluid network 200. Each test strip may comprise a plurality of sections. In the embodiment shown in FIG. 4, the test strip 208a-c comprises three sections: a conjugate section 214, a detection section 216, and a collection section 218. In the depicted embodiment, the sections are contiguous, with the detection section 216 between the conjugate section 214 and the collection section 218. Such a configuration creates test strips 208a-c according to sandwich-type lateral flow principles.

[0034] The conjugate section 214 may store detection antibodies 220. In one embodiment, the detection antibodies are labeled with gold nanoparticles. The detection section 216 captures and immobilizes the detection antibodies 220 for the target molecules 222 only if the target molecules 222 are present. The detection section 216 captures detection antibodies 220 because it is composed of nitrocellulose or similar material that has a high protein-binding affinity. Captured detection antibodies 220 may include, but are not limited to, anti-CRP, anti-PCT, and anti-Endotoxin. The collection section 218 immobilizes secondary antibodies 224 with an affinity for the common species of the detection antibodies 220. The common species may include a mouse, rabbit, goat and the like. The secondary

antibodies 224 are similarly captured by the detection section 216 if the target molecules 222 are present.

[0035] As the lateral flow assay is conducted, the detection section 216 captures the detection antibodies 220 and the secondary antibodies 224 as a test line 226 and a control line 228, respectively. The test line 226 changes color when detection antibodies 220 are captured, indicating that target molecules are present in the sample. For example, the test line 226 may turn a reddish color when the detection antibodies 220 are captured at the detection section 216. The color change of the test line 226 may be more vibrant and conspicuous when a high concentration of target molecules are present in the sample. Similarly, when a low concentration of target molecules are present in the sample, the color change may be more subtle. Determining the concentration of target molecules in a sample at the point-of-care is critical as concentrations of target molecules are correlated to certain types of diseases. For example, CRP levels of 1 – 6 ug/ml can be an indication of heart disease risk, while CRP levels greater than 10 ug/ml indicate inflammation, either from infection or other inflammatory diseases.

[0036] Referring now to FIG. 5, there is shown a perspective view schematic representation of an exemplary embodiment of the minimum inhibitory concentration (MIC) chip assembly 300 of the device 100. The MIC chip assembly 300 comprises a MIC chip interface 302 having an open volume configured to receive a MIC chip 304 therein. In the embodiment shown in FIG. 5, the MIC chip interface 302 slidably receives the MIC chip 304. The MIC chip 304 comprises one or more wells 306 configured to receive a sample. The MIC chip interface 302 comprises one or more magnets 308 that align with the wells 306 when the MIC chip interface 302 receives the MIC chip 304. In the embodiment shown in FIG. 5, the wells 306 may be arranged into an array for optimal alignment with the magnets 308.

[0037] Referring now to FIG. 6, there is shown a perspective view schematic representation of an exemplary embodiment of a sample mixture inserted into the MIC chip. In the embodiment shown in FIG. 6, a sample 310 is mixed with a biorecognition element 312. The sample 310 can be a biological sample, such as a blood sample. In one embodiment, the biorecognition element 312 is a magnetic nanoparticle. Magnetic nanoparticles can be functionalized with capture ligands specific to bacterial organisms. Thus, when a sample is mixed with functionalized magnetic nanoparticles, the nanoparticles attach to the specified bacterial organisms in the sample. As shown in FIG. 6, the sample 310 and the biorecognition element 312 are combined to create a mixture 314, such as a blood magnetic nanoparticle mixture. The mixture 314 is then inserted into an inlet 316 on the MIC chip 304.

[0038] Referring now to FIG. 7, there is shown a diagram of an exemplary embodiment of a well during the MIC assay. In the embodiment shown in FIG. 7, each well is pre-functionalized with specific varying concentrations of an antibiotic within a solid media 318. For example, the solid media 318 may be doped agar, although numerous other like materials can be used. Once the MIC chip 304 is inserted into the MIC chip interface 302 and the wells 306 align with the magnets 308, the mixture 314 is flown through the MIC chip 304 such that it is evenly distributed to each of the wells 306. The magnets 308 attract the magnetic nanoparticles, thereby collecting bacterial organisms attached to the magnetic nanoparticles in the mixture 314 while the remainder of the mixture 314 flows through the wells 306.

[0039] In some embodiments, a culture medium with an indicator is inserted into the inlet 316 on the MIC chip 304. The indicator washes the remainder of the mixture 314 from the wells 306. It also introduces growth or culture medium, if necessary, and a chemical indicator. A chemical indicator can be any compound or solution that indicates whether an

organism is alive, dead, or metabolically, or otherwise, active. An example of a chemical indicator is phenol red, which will change the color of a solution in the presence of metabolically active organisms due to the changes in pH of the solution caused by the metabolic activity changes occurring in the organisms.

[0040] After the mixture 314 and culture medium with a chemical indicator has been added, antibiotic 320 begins to diffuse from the solid media 318. If the organisms captured in the solid media 318 are not resistant to the antibiotic 320, the organisms will not experience metabolic activity changes that trigger a change in pH of the solution in the well 306. However, if the organisms thrive despite the antibiotic 320, the organism experience metabolic activity changes that alter the pH of the solution in the well 306. As the pH of the solution decreases and becomes more acidic, the chemical indicator changes the color of the solution. Thus, the wells 306 comprising organisms which are resistant to the antibiotic 320, will have a different color, or other indication, than wells 306 comprising organisms which are not resistant to the antibiotic 320. Further, as each well 306 in the array comprises a different concentration of antibiotic 320, a minimum inhibitory concentration can be ascertained.

[0041] Referring now to FIG. 8, there is shown a perspective view schematic representation of an exemplary embodiment of the MIC chip assembly in the device. In the embodiment of the system 10 shown in FIG. 8, the MIC chip assembly 300 can be inserted into a second port on the device 100. For example, the MIC chip assembly may be inserted into a port on an electronic device, such as a smartphone. The integration of the MIC chip assembly 300 into the device 100 is critical for interpreting colorimetric results that may not be interpreted efficiently by eyesight. It is contemplated that the device 100 may comprise a digital camera, sensor, or other imaging mechanism that can capture the colorimetric result produced in the wells 306 and transmit data indicating the results to a processor in device

100. An imaging mechanism of the device 100 will allow the device 100 to interpret the minimum inhibitory concentration, which is the lowest concentration where no significant color change is shown.

[0042] Referring now to FIG. 9, there is shown a flowchart illustrating the method for diagnosing neonatal sepsis and determining the appropriate treatment. The flowchart shown in FIG. 9 is exemplary of one embodiment wherein the targets are C-Reactive Protein (CRP), procalcitonin (PCT) and Endotoxin. At the first step, the system 10 as shown in FIG. 1, detects the presence of a bacterial infection. At this step, lateral flow assays are conducted in the fluid network 200 to detect Endotoxin, CRP, and PCT. The CRP and PCT assays are analyzed first as CRP and PCT have been shown to have high sensitivity and specificity in this context. If neither CRP nor PCT is detected, supportive management is recommended. Next, the Endotoxin assay is analyzed to determine if the bacterial infection is due to gram-negative bacterium. If Endotoxin is detected, gram-positive antibodies are recommended for treatment. Finally, the system 10 will determine whether the gram-negative bacterium causing the infection is sensitive to first-line antibiotics. This step occurs using the MIC assay in the MIC chip assembly 300. Once antibiotic sensitivity is assessed, the proper antibiotics can be administered. This method can be conducted rapidly at the point-of-care using the system 10 shown in FIG. 1 and explained in detail above.

[0043] While embodiments of the present invention have been particularly shown and described with reference to certain exemplary embodiments, it will be understood by one skilled in the art that various changes in detail may be effected therein without departing from the spirit and scope of the invention as defined by claims that can be supported by the written description and drawings. Further, where exemplary embodiments are described with reference to a certain number of elements, it will be understood that the exemplary

embodiments can be practiced utilizing either less than or more than the certain number of elements.

CLAIMS

What is claimed is:

1. A device for detecting the presence of a target in a sample, the device comprising:

a first port configured to receive a multi-layered substrate having a sample inlet and a reagent inlet;

wherein the sample inlet is connected to a first microfluidic channel and the reagent inlet is connected to both the first microfluidic channel and a second microfluidic channel;

wherein the second microfluidic channel comprises a longer pathway than the first microfluidic channel;

a first test strip and a second test strip each connected to both the first microfluidic channel and the second microfluidic channel; and

a third test strip connected only to the first microfluidic channel;

each test strip comprising a conjugate section, a detection section, and a collection section.

2. The device of claim 1, further comprising an enhancement solution membrane disposed in the second microfluidic channel.

3. The device of claim 1, wherein the sample inlet is disposed in the first microfluidic channel between the reagent inlet and each test strip.

4. The device of claim 1, further comprising:

a second port configured to receive a MIC chip interface;

wherein the MIC chip interface has an open volume configured to receive a MIC chip therein, the MIC chip comprising one or more wells.

5. The device of claim 4, wherein the MIC chip interface further comprises one or more magnets.

6. The device of claim 4, wherein the wells are arranged in an array.

7. The device of claim 5, wherein the magnets are in alignment with the wells when the MIC chip is inserted into the open volume of the MIC chip interface.

8. A method for detecting the presence of a target in a sample, comprising the steps of:

providing a device having a first port configured to receive a multi-layered substrate having a sample inlet and a reagent inlet;

wherein the sample inlet is connected to a first microfluidic channel and the reagent inlet is connected to both the first microfluidic channel and a second microfluidic channel,

further wherein the second microfluidic channel comprises a longer pathway than the first microfluidic channel;

a first test strip and a second test strip each connected to both the first microfluidic channel and the second microfluidic channel; and

a third test strip connected only to the first microfluidic channel,

wherein each test strip comprises a conjugate section, a detection section, and a collection section;

labeling detection antibodies of the target with nanoparticles and depositing the detection antibodies at the conjugate section;

labeling secondary antibodies of the target with nanoparticles and depositing the secondary antibodies at the conjugate section;

inserting a sample into the sample inlet and a reagent into the reagent inlet;

flowing the sample across each test strip; and

capturing detection antibodies and secondary antibodies at the detection section.

9. The method of claim 8, further comprising the step of:

flowing an enhancement solution through the second microfluidic channel and across the first test strip and the second test strip.

10. The method of claim 8, further comprising the steps of:

generating a test line indicative of a concentration of the detection antibodies captured at the detection section; and

generating a control line indicative of a concentration of the secondary antibodies captured at the detection section.

11. A method for detecting the presence of a target in a sample, comprising the steps of:

providing a device having a first port configured to receive a multi-layered substrate having a sample inlet and a reagent inlet,

wherein the sample inlet is connected to a first microfluidic channel and the reagent inlet is connected to both the first microfluidic channel and a second microfluidic channel,

further wherein the second microfluidic channel comprises a longer pathway than the first microfluidic channel;

a first test strip and a second test strip each connected to both the first microfluidic channel and the second microfluidic channel; and

a third test strip connected only to the first microfluidic channel,

wherein each test strip comprising a conjugate section, a detection section, and a collection section; and

a second port configured to receive a MIC chip interface,

wherein the MIC chip interface has an open volume configured to receive a MIC chip therein, the MIC chip comprising one or more wells,

wherein the MIC chip interface comprises one or more magnets that align with the wells when the MIC chip is inserted into the open volume of the MIC chip interface;

applying an antibiotic to a solid media in each well;

inserting the MIC chip into the MIC chip interface;

mixing a sample with a biorecognition element to create a mixture;

depositing the mixture into the MIC chip; and

flowing the mixture across the wells.

12. The method of claim 11, further comprising the step of:

capturing the target in the wells,

wherein the target in the mixture is magnetically attracted to the magnets in the MIC chip interface.

13. The method of claim 12, further comprising the step of:

growing a culture of the target captured in the wells.

14. The method of claim 11, wherein each well comprises a different concentration of the antibiotic.

15. The method of claim 11, wherein the biorecognition element is a magnetic nanoparticle.

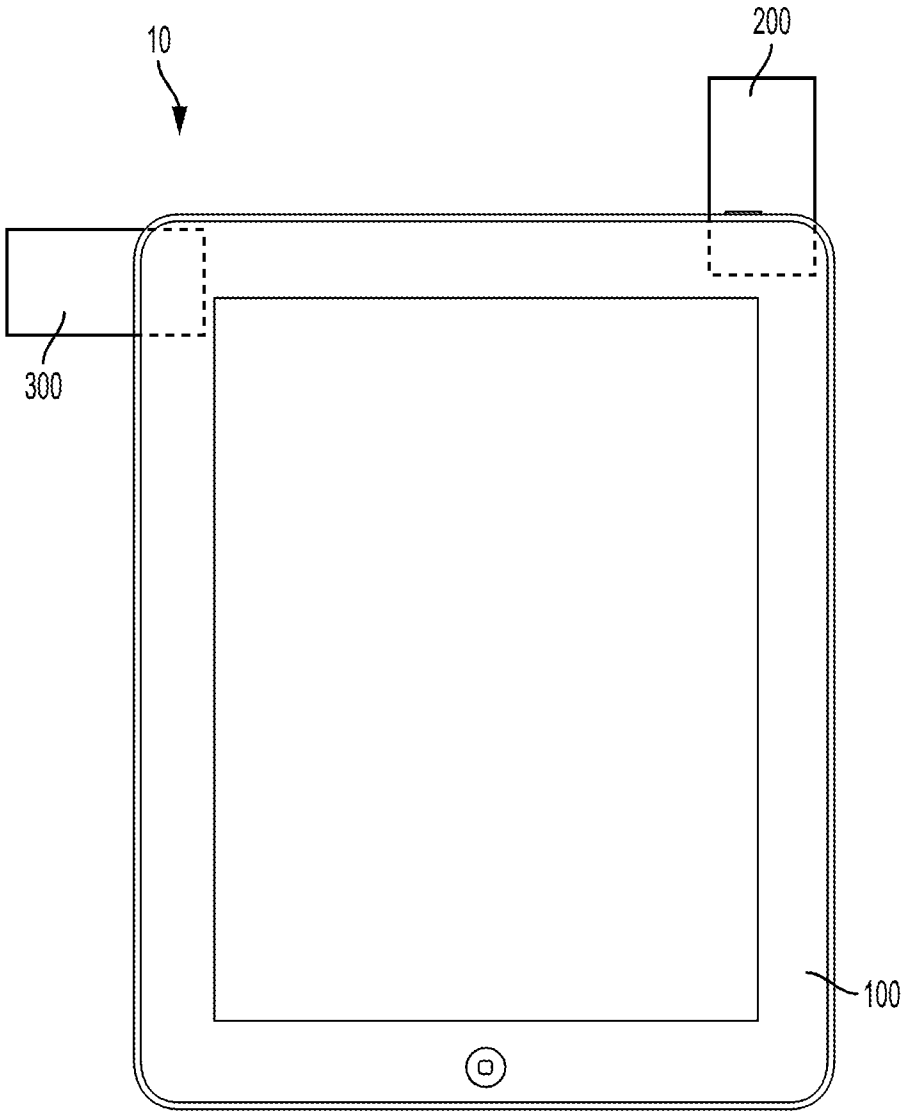
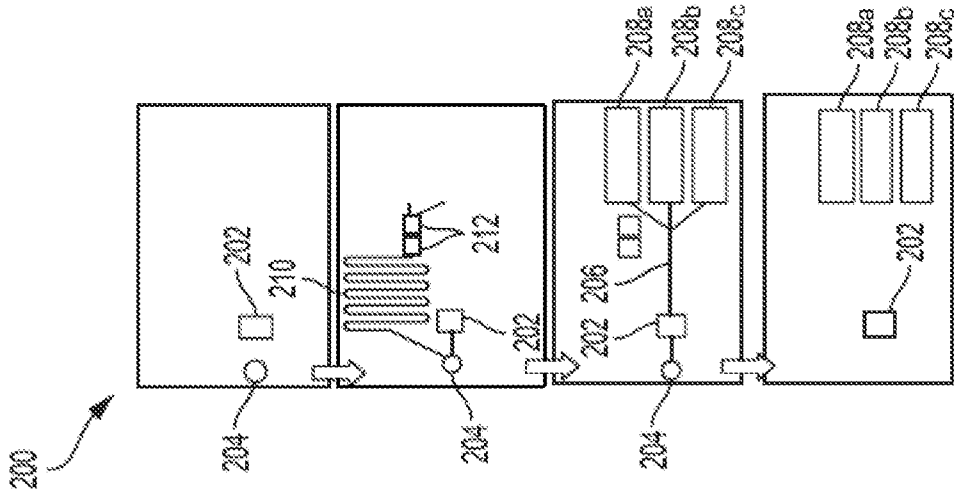


FIG. 1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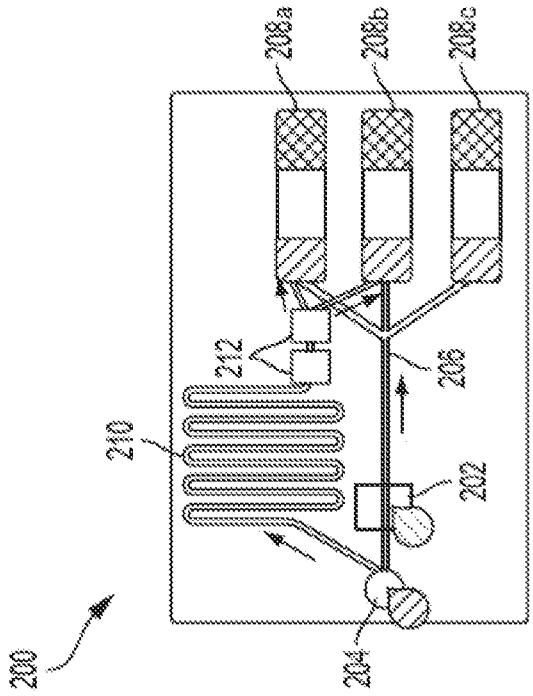
202
210
212
204
206
208a
208b
208c

FIG. 3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210
212
202
204
206
208a
208b
208c

FIG. 2

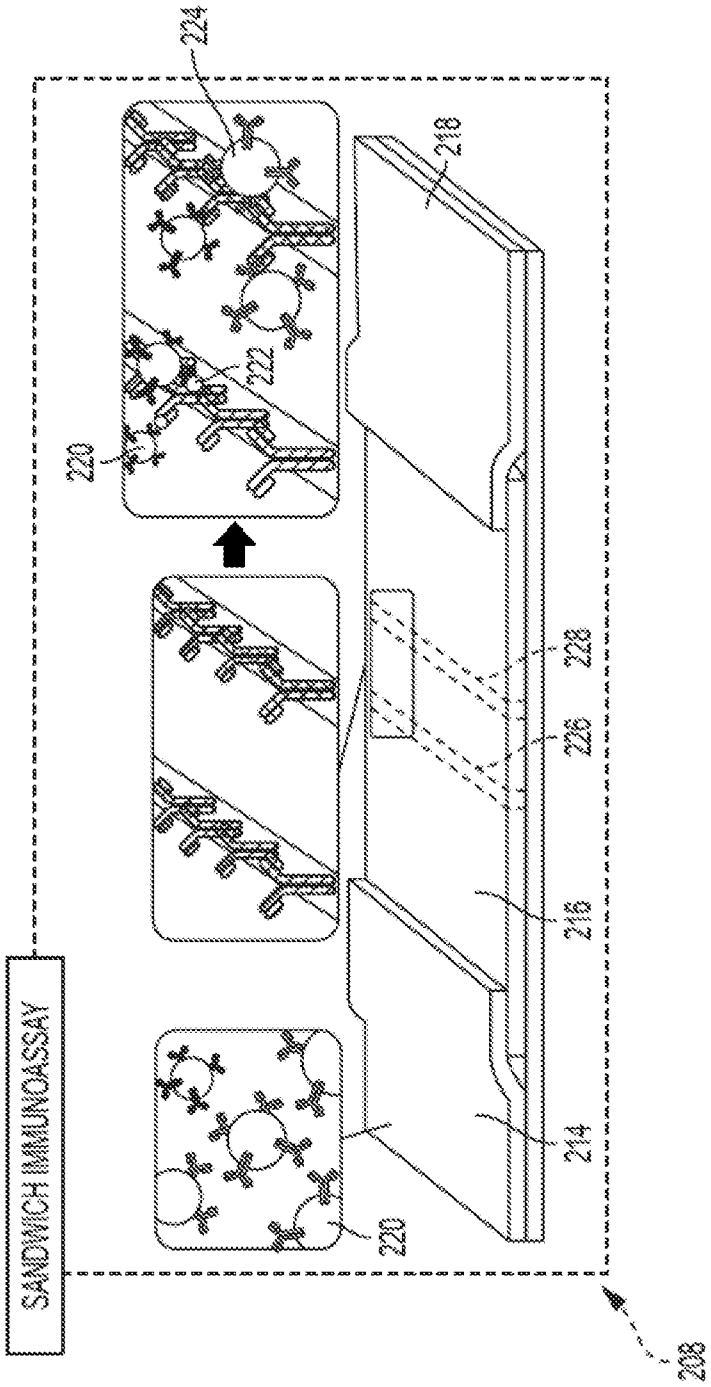


FIG. 4

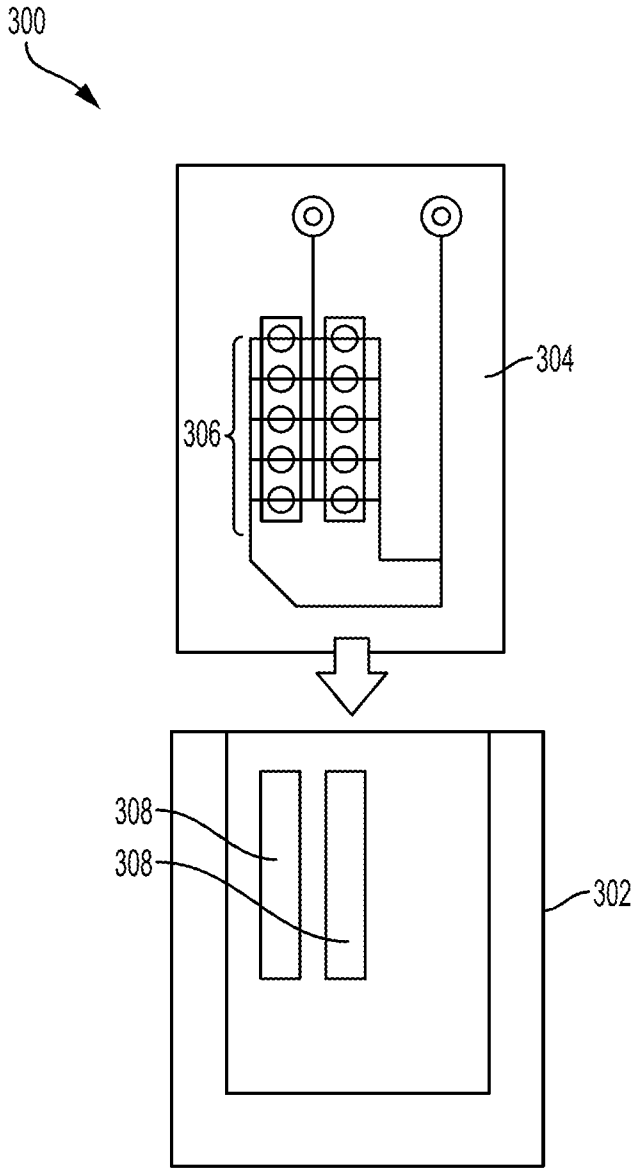


FIG. 5

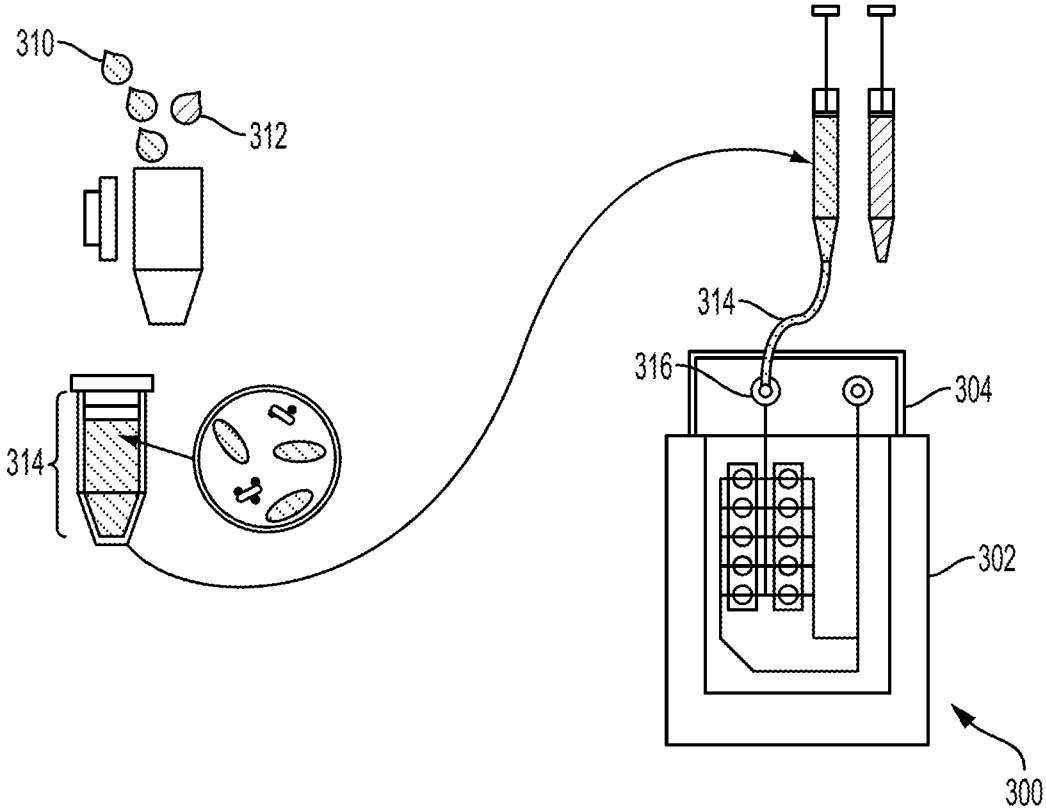
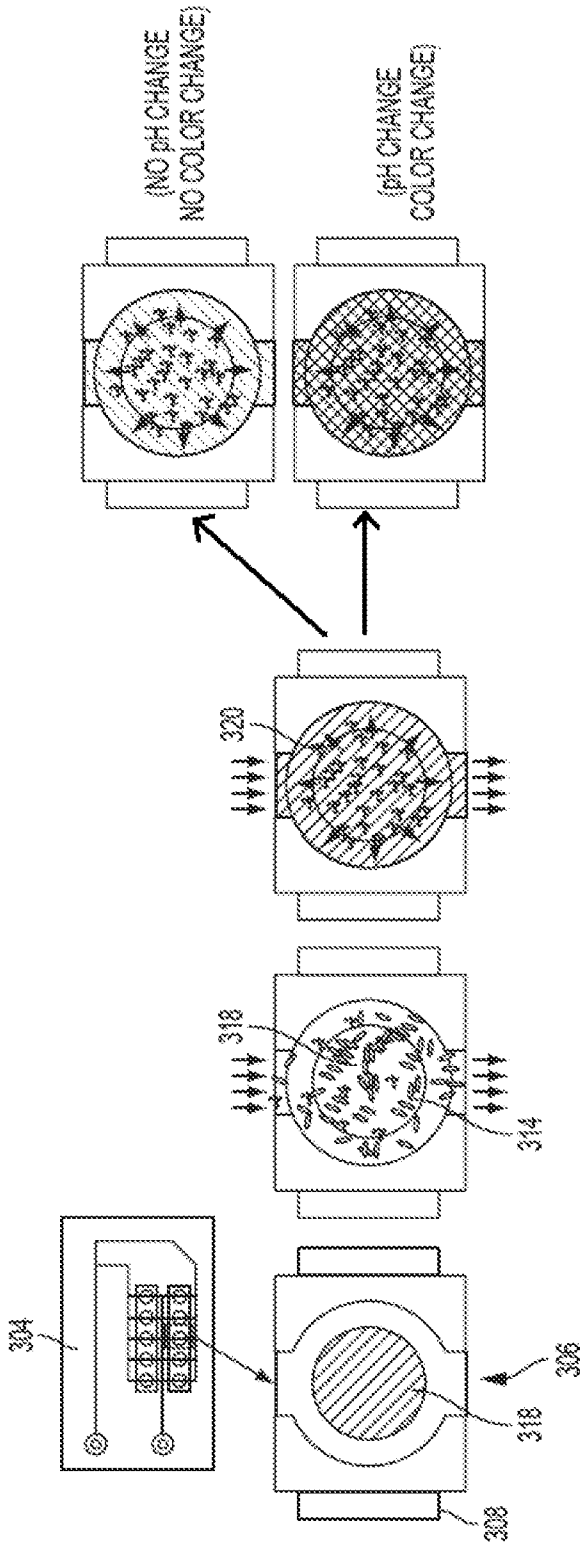


FIG. 6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NO COLOR CHANGE)
(pH CHANGE
COLOR CHANGE)
320
318
314
304
306
318
308

FIG. 7

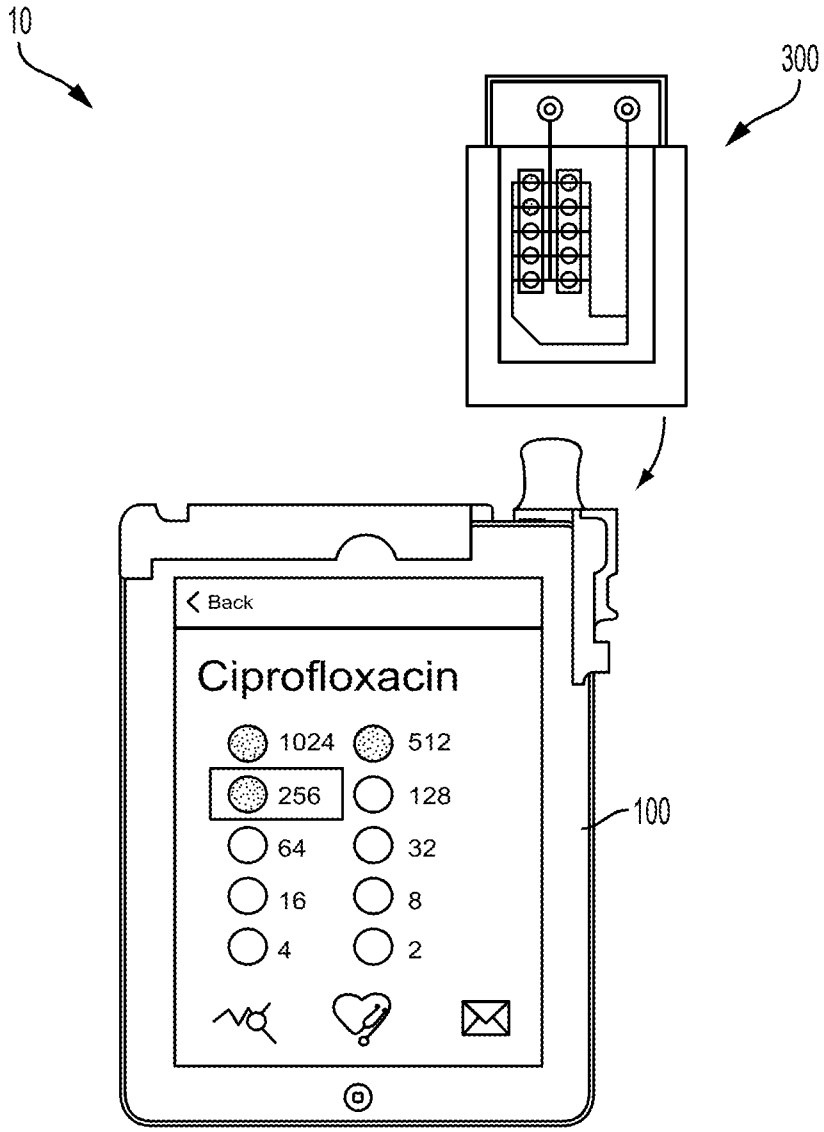

FIG. 8

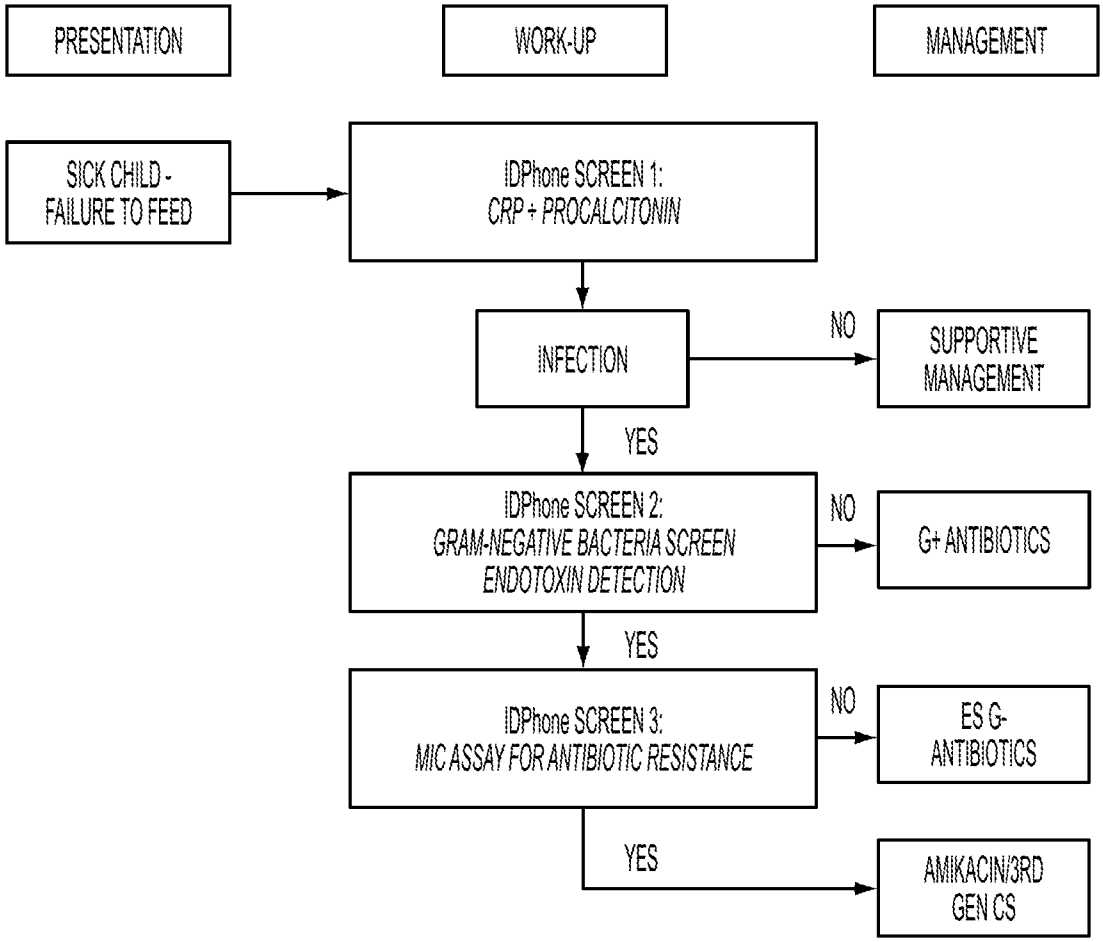
PRESENTATION
WORK-UP
MANAGEMENT
SICK CHILD - FAILURE TO FEED
IDPhone SCREEN 1: CRP + PROCALCITONIN
INFECTION
NO
SUPPORTIVE MANAGEMENT
YES
IDPhone SCREEN 2: GRAM-NEGATIVE BACTERIA SCREEN ENDOTOXIN DETECTION
NO
G+ ANTIBIOTICS
YES
IDPhone SCREEN 3: MIC ASSAY FOR ANTIBIOTIC RESISTANCE
NO
ES G- ANTIBIOTICS
YES
AMIKACIN/3RD GEN CS

FIG. 9

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/043102

A. CLASSIFICATION OF SUBJECT MATTER

G01N 35/08(2006.01)i, G01N 33/558(2006.01)i, G01N 33/543(2006.01)i, G01N 33/58(2006.01)i, B01L 3/00(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)
G01N 35/08; C12M 1/00; C12Q 1/18; G01N 33/00; G01N 35/00; G01N 33/558; G01N 33/543; G01N 33/58; B01L 3/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: detection, target, microfluidic channel, test trip, longer pathway, MIC chip, well, magnet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2010-0035349 A1 (BAU, HAIM H. et al.) 11 February 2010 See paragraphs [0033]-[0121]; claims 1-32; and figures 1A-18C.	1-15
A	US 2009-0325276 A1 (BATTRELL, C. FREDERICK et al.) 31 December 2009 See paragraphs [0051]-[0221]; claims 65-84; and figures 1-12B.	1-15
A	US 2013-0196364 A1 (SNU R&DB FOUNDATION et al.) 01 August 2013 See paragraphs [0054]-[0175]; claims 1-30; and figures 1-31.	1-15
A	HART, R. W. et al., Point-of-care oral-based diagnostics, Oral Diseases, 2011, Vol. 17, pp. 745-752 See the whole document.	1-15
A	CIRA, NATE J. et al., A self-loading microfluidic device for determining the minimum inhibitory concentration of antibiotics, Lab on a chip, 2012, Vol. 12, pp. 1052-1059 See the whole document.	1-15

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 October 2016 (12.10.2016)	**12 October 2016 (12.10.2016)**

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2016/043102

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2010-0035349 A1	11/02/2010	US 8697007 B2	15/04/2014
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		WO 2008-147382 A8	15/01/2009
US 2013-0196364 A1	01/08/2013	KR 10-1290648 B1	29/07/2013
		KR 10-1461662 B1	04/12/2014
		KR 10-2013-0089619 A	12/08/2013
		US 2015-0315625 A1	05/11/2015
		US 2015-0337352 A1	26/11/2015
		US 2015-0337353 A1	26/11/2015
		US 9133498 B2	15/09/2015