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(54) Title: SYSTEM FOR MICROBIAL SPECIES DETECTION, QUANTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY IDENTIFICATION

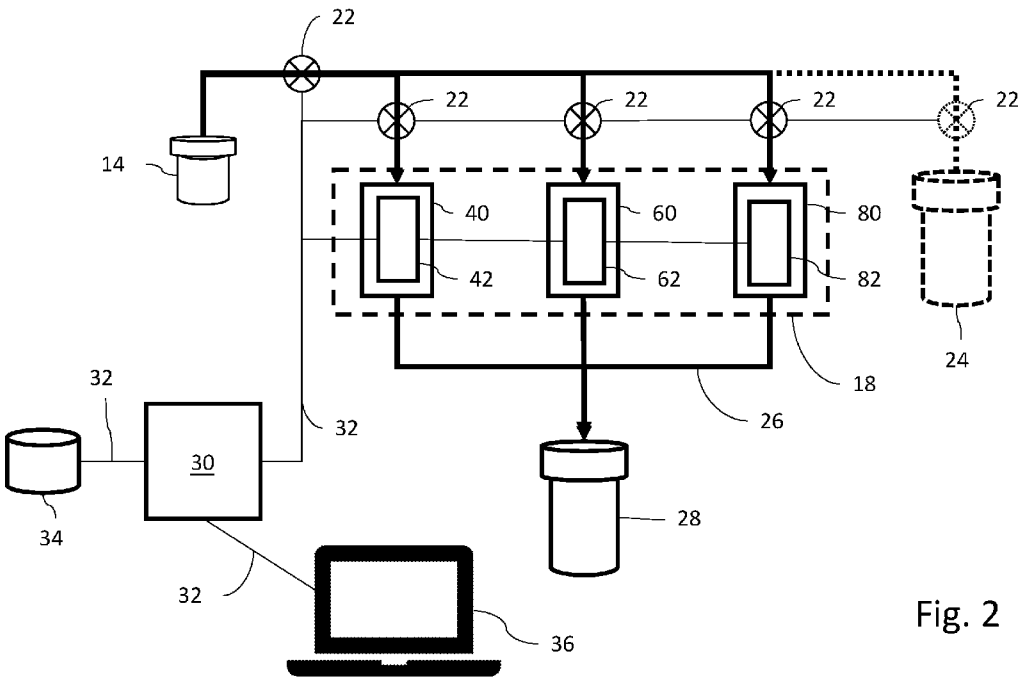


Fig. 2

(57) Abstract: Several microfluidic chips are used to significantly accelerate the time to identify and quantify microbes in a biological sample and test them for antibiotic resistance, particularly for urinary tract infections. A first microfluidic chip uses antibody or similar probes to identify and quantify any microbes present. The same or a similar chip uses antibody or similar probes to identify microbes with DNA or RNA known to indicate antibiotic resistance. Another microfluidic chip tests for antibiotic susceptibility of any microbes by growing them in very small wells in the presence of antibiotics, reducing the time required for such testing by as much as 95%. Another microfluidic chip runs traditional urinalysis or similar tests.

[Continued on next page]

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SYSTEM FOR MICROBIAL SPECIES DETECTION, QUANTIFICATION AND ANTIBIOTIC SUSCEPTIBILITY IDENTIFICATION

CROSS-REFERENCE TO PRIOR APPLICATIONS

[0001] This application claims priority to US Provisional Patent Application Serial No. 62/725,026, filed on 30 August 2018, titled "System for Microbial Species Detection, Quantification and Antibiotic Susceptibility Identification" and to US Patent Application Serial No. 16/551,702, filed on 26 August 2019, titled "System for Microbial Species Detection, Quantification and Antibiotic Susceptibility Identification".

TECHNICAL FIELD

[0002] The present invention relates to a system for detecting and quantifying one or more species of microbe present in a biological sample and identifying antibiotic susceptibility of such microbes. More specifically, the invention relates to a system for detecting, quantifying and identifying microbes as well as antibiotic susceptibility of the microbes in urine samples from patients with possible urinary tract infections.

BACKGROUND ART

[0003] Urinary tract infections (UTIs) are microbial infections which pose a significant public health threat. In 2007, there were 10.5 million outpatient UTI visits for medical care in the United States, and 21.3% of those visits were to emergency. UTIs are also prevalent worldwide, especially in countries with large populations and public sanitation challenges.

[0004] The United States Center for Disease Control and Prevention describes UTIs as infections that occur when a microbe enters the urinary tract. UTIs can arise from poor hygiene, intercourse, abnormal anatomy, and the presence of bacteria, virus or fungus. Some populations are at a higher risk of getting UTIs – women and girls have higher rates of UTIs than men due

to anatomical structure. The elderly and patients with urinary incontinence or catheter implants are also at higher risk for UTIs. UTI symptoms are painful and have been described by patients as causing feelings of pain or burning while urinating, frequent urination and the feeling of a need to urinate despite having an empty bladder. Other common symptoms include low fever (about 38° C), cloudy or bloody urine and pressure or cramping in the groin or lower abdomen. Further complicating accurate and timely diagnosis, UTI symptoms often present differently based on patient age. Infants with UTIs often present with a fever, fussy disposition, or reduced appetite. In contrast, elderly UTI patients may be asymptomatic or may exhibit symptoms resembling dementia, accompanied by excessive fatigue and incontinence. Despite the range of presentation, accurate and timely diagnosis is critical, as untreated UTIs can develop into more severe conditions such as kidney infections or sepsis.

[0005] In current practice, the diagnosis of UTIs involves clinical and physical exams, followed by both a sterile urinalysis and a positive urine culture test that usually takes multiple days to complete and often is not used in outpatient settings. Foxman B., "*Epidemiology of urinary tract infections: incidence, morbidity, and economic costs*", American Journal of Medicine 113(1):5-13 (2002). This 'gold standard' procedure is followed for patients from whom a mid-stream urine sample can be collected. For patients who cannot urinate on their own, urine may be collected via a catheter, which is at the least uncomfortable and can be painful.

[0006] The urinalysis can be done relatively quickly and can tell providers whether an infection may be present in the urinary tract due to reading elevated levels of white blood cells in the sample, but it cannot identify the microbe causing the infection, quantify the level of infection or indicate the appropriate antibiotic.

[0007] The urine culture test detects the specific pathogens present, quantifies the microbial load, and can identify potential antibiotic resistance, but it takes substantial time. In this test, a urine sample is swabbed onto growth medium in a Petri plate. After a suitable incubation period to enable microbial replication, the Petri plate is visually inspected for microbes, which are then identified and quantified by a trained specialist. If looking for antibiotic resistance, multiple test plates may be made, with each subjected to a different antibiotic to test for resistance to that antibiotic. This approach has the advantage that it can find any type of microbe and look for any type of antibiotic resistance, but the disadvantage that the microbes must reproduce long enough to produce colonies visibly differentiable by the human eye. It takes a minimum of 18 hours, and often up to 72 hours, to obtain results from a urine culture test. Notably, when a urine culture test is run to diagnose a suspected UTI, as much as 80% of the time the test results are negative – that is, the test indicates that the patient does not have a UTI.

[0008] The lengthy time required for current urine culture tests results in very unsatisfactory treatment approaches. In most circumstances, and especially in pediatric or geriatric cases, clinicians tend to default-prescribe broad-spectrum antibiotics that cover a range of the microbes most commonly associated with UTIs, long before the urine culture test results are available. Since as much as 80% of the urine culture test results show no infection, this means that 80% of the patients are being prescribed antibiotics when they are of no value whatsoever. If subsequent urine culture test results indicate that the patient has a microbial infection, but not one susceptible to the antibiotic used initially, the original antibiotic will be discontinued and the correct antibiotic prescribed. This assumes the clinician can actually reach the patient at that point, which often is not the case.

[0009] In both the situation when the patient had no UTI, and the one when the patient has an infection that is not susceptible to the initial antibiotic, the patient is prescribed an ineffective antibiotic. This is not helpful for the patient and promotes the growth of antibiotic-resistant microbes.

[0010] In summary, UTIs are extremely common, but the current diagnosis and treatment processes are widely perceived as flawed by practitioners. In particular, the slow detection and quantification of the microbial load in a given infection by a urine culture test means that antibiotics are often prescribed to patients who do not actually have a UTI, and even in patients who do have a UTI, the initially prescribed antibiotics may target the wrong microbe or the microbe may be resistant to that antibiotic, requiring a post-test change in antibiotics. This extremely common issue contributes to antibiotic resistance, which increasingly threatens public health.

[0011] To improve treatment, a novel method of microbe detection, quantification and identification of antibiotic resistance is needed that works in hours instead of days, and that finds the correct treatment for at least the microbes known to frequently cause such infections, all at a reasonably low cost due to the high volume of tests required.

SUMMARY OF THE INVENTION

[0012] The present invention is based on the recognition that the current gold standard urine culture test is excessive. Specifically, the current test assumes that there is no advance knowledge of what microbes may be causing a UTI, so it is necessary to look for all possible microbes. But that assumption is very far from reality. Instead, nearly all UTIs are caused by a surprisingly limited number of microbes.

[0013] For example, in the United States, just one microbial family causes 75% of all uncomplicated UTIs, and 9 microbial families cause nearly all uncomplicated UTIs. Specifically, in uncomplicated UTIs, uropathogenic *Escherichia coli* (UPEC) causes 75% of all uncomplicated UTIs, *Klebsiella pneumoniae* causes 6%, *Staphylococcus saprophyticus* (6%), *Enteroccus* spp. (5%), group B *Streptococcus* (GBS) (3%), *Proteus mirabilis* (2%), *Pseudomonas aeruginosa* (1%), *Staphylococcus aureaus* (1%) and *Candida* spp (1%). For complicated UTIs, the frequencies per microbe are slightly different, but just 4 microbial families cause over 90% of all complicated UTIs, and the same microbial families causing uncomplicated UTIs cause nearly all complicated UTIs. Specifically, UPEC causes 65% of all complicated UTIs, *Klebsiella pneumoniae* causes 8%, *Enteroccus* spp. (11%), GBS (2%), *Proteus mirabilis* (2%), *Pseudomonas aeruginosa* (2%), *Staphylococcus aureaus* (3%) and *Candida* spp (7%). Ana L. Fores-Mireles et al., "*Urinary tract infections: epidemiology, mechanisms of infection and treatment options*", Nature Reviews Microbiology 13: 269-284 (2015). Similarly, in the United Kingdom, depending on how and where UTIs were acquired, the same species cause at least 85% of all UTIs. D.J. Farrell et al., "*A UK Multicentre Study of the Antimicrobial Susceptibility of Microbial Pathogens Causing Urinary Tract Infection*", Journal of Infection 46:94-100 (2003).

[0014] This enables a completely different approach to diagnosing UTIs. Instead of looking for any microbe which might possibly be present, the present invention uses probes to look for the specific microbes which are known to cause nearly all UTIs. The present invention uses DNA, RNA, antibody, aptamer or small molecule probes specifically targeting these common microbes to detect and quantify the microbes much more quickly, without the need to conduct a urine culture test. This new test should take less than hour, which is fast enough for a patient to wait for test results. For the 80% of tests which are negative, this would enable the

clinician to move on to other possible causes of the patient's symptoms and avoid prescribing a completely unnecessary antibiotic. For the 20% of tests which are positive, by diagnosing which microbe is causing the UTI, the test will enable the physician to have a better idea of what antibiotics to prescribe to treat the specific microbe.

[0015] According to a further aspect of the invention, the present invention uses DNA, RNA, antibody, aptamer or small molecule probes specifically targeting the presence of nucleotides or proteins that are known to provide antibiotic resistance, enabling rapid identification of which antibiotics will not work against the microbes that are present. Like the identification and quantification test, this test should take less than hour, which is fast enough for a patient to wait for test results. This ensures that they will be sent home with a prescription for the correct antibiotic, if an antibiotic is appropriate.

[0016] A further aspect of the present invention provides a backup diagnostic antibiotic resistance identification test using an approach more similar to a traditional urine culture test, but in a new system which enables it to be done much faster. This new test is based on the recognition of two problems with the traditional test:

[0017] First, the traditional test requires microbes to replicate enough times to be visible by a human. The time required for such replication is what causes the test to take 18-72 hours. In a system according to the present invention, small wells are used instead of Petri plates and a sensor is used to detect the microbes. The sensor can detect the microbes after far fewer reproduction cycles than the human eye can, shortening the incubation time to at most a few hours.

[0018] Second, the traditional test requires a highly trained person to read the test. This is expensive and can take considerable time, especially if there are multiple varieties of

microbes and/or if tests are run to identify antibiotic resistance, requiring multiple Petri plates. In a system according the present invention, software analyzes the sensor output for each well in at most a few seconds, shortening the time required to read the test to at most a few minutes, even when many cells are used.

[0019] More specifically according to this aspect of the invention, a system identifies antibiotic resistance using a chip with a plurality of wells. Each well has growth medium, antibiotics and a reporter indicating the presence of live microbes. Once provided with a urine sample, the wells are sealed and incubated. A sensor then is used to detect activity from the reporter, which in turn indicates microbe growth in the different wells. But the wells can be much smaller than a Petri dish – less than 1 cc, and preferably less than 0.1 cc – allowing the incubation process to take dramatically less time, since less replication is needed. This test should take 1-7 hours, and with very small wells 1-3 hours. A patient may not want to wait for the test result, but a prescription for the correct antibiotic could be sent to the pharmacy in a few hours, and the patient could be instructed to pick it up then.

[0020] Given that any system providing the tests above will necessarily be handling and testing urine, it also would be convenient if the same system could conduct a conventional urinalysis, which today is done separately from the urine culture test. Test results from a urinalysis typically are ready in less than 1 hour.

[0021] To these ends, the present invention provides a system for conducting urinalysis, detecting and quantifying the presence of the common species of microbes which cause UTIs, identifying any antibiotic resistance as shown by DNA or RNA or the presence of other macromolecules known to provide such, and testing a range of antibiotics for efficacy against

any microbes which are present, whether or not they have such DNA or RNA known to provide antibiotic resistance.

[0022] To conduct these tests, the system has a fixed hardware portion with replaceable microfluidic test chips. The hardware receives a urine sample and delivers it to each of the chips. Various reactants on the chip then conduct the relevant tests. The hardware than measures the results of the tests on the chips, and reports the results, either directly to a screen on the system, or to an associated computer system, or both.

[0023] Preferably, the system uses three chips.

[0024] The first chip is a microbial detection chip (or MDC) with multiple sections, preferably 2-100, more preferably 4-50, more preferably 8-25. Each section is provided with a volume for capturing microbes from a urine sample and for testing them with a probe. Each such probe can test for a specific microbe or for the presence of a specific strand of DNA or RNA indicating antibiotic resistance. In use, the system delivers urine to the different sections, which then identify and quantify the presence of the relevant microbes and/or the relevant DNA or RNA strands. The system them reads the results from each section, preferably optically.

[0025] One notable difference between the MDC and the urine culture test commonly used today is that this system tests for the presence of the specific microbial families known to cause nearly all UTIs v looking for any and all types of microbes which may be present. Similarly, the MDC can test for the specific microbial features known to provide antibiotic resistance. And all of this can be done in a short time, likely under 1 hour.

[0026] The second chip is an antibiotic susceptibility chip (or ASC) with multiple wells, preferably at least 2, more preferably at least 12, more preferably at least 20. In use, the system delivers into each well urine mixed with growth medium and a reporter, such as resazurin,

which will report the presence and amount of living microbes. Alternatively, the growth medium and reporter can be preloaded into each well and just urine added. The wells are then each isolated and incubated to enable any microbes reproduce. Preferably, at least one well contains no antibiotics, as a control. The other sections each are pre-loaded with different antibiotics, to test for resistance to each antibiotic. Alternatively, the antibiotics can be mixed with the urine sample prior to delivery. After a suitable incubation period for growth of any microbes, the system checks and reports on the level of microbes present in each well. Either directly or by comparison to the control well, the results will indicate which antibiotics would be the most effective at controlling any microbial infection.

[0027] The ASC functions as a backup to the MDC. The ASC is agnostic to specific types of microbes – it tests for antibiotic resistance in any microbes that may be present. It does this directly by testing which antibiotics do and do not have an effect, and how much of an effect. The size of the wells can be much smaller than a Petri dish – less than 1 ml and preferably less than 0.1 ml. This dramatically reduced size enables the test to run much faster than a conventional urine culture test, e.g., 1-7 hours, and likely 1-3 hours in small wells. In addition, little skill is required to run or read the test – the urine sample is provided and the test runs automatically.

[0028] The third chip is a urinalysis chip (UC). The UC includes reactants similar to those used today on dip sticks for urinalysis. These reactants change color depending on the amount of a particular chemical in the urine. The system then optically reads the color to determine the test results. This test would take essentially the same amount of time as the current dipsticks, which is a minute or two.

[0029] The chips can each be separate, or they can all be on a replaceable cartridge. Preferably, all portions of the system which contact the urine sample are also part of an easily replaceable cartridge. Alternatively, the system would include provisions for flushing, cleaning and sterilizing any part of the system which contacts the urine sample.

[0030] The system enables identification and quantification of microbes and antibiotic resistance via known DNA or RNA in about one hour, broad-spectrum antibiotic susceptibility testing can be completed in at most a few hours and conventional urinalysis can be completed in a few minutes. The initial time period of about an hour 1 for completion of urinalysis, identification and quantification of microbes and known antibiotic resistance is short enough that it is reasonable for a patient to wait for the test results. This enables the clinician to quickly move on to testing for other hypotheses if the test is negative and the patient does not have a UTI, which is especially useful for patient groups with a high frequency of negative results. The few hour time period for the agnostic antibiotic susceptibility test means the clinician can tell the patient to go to the pharmacy 4 hours after completion of the initial tests to pick up an antibiotic prescription, and it will be an antibiotic that will actually work. This simultaneously will improve patient care and dramatically reduce the prescription of ineffective antibiotics.

[0031] The above summary is not intended to describe each embodiment or every implementation of the invention. Other embodiments, features and advantages of the invention will be apparent from the following detailed description thereof, from the drawings and from the claims.

BRIEF DESCRIPTION OF DRAWINGS

[0032] Fig. 1 is projection view of an embodiment of a system according to the present invention;

[0033] Fig. 2 is block diagram of the system of Fig. 1.

[0034] Fig. 3 is a microbe detection chip (or MDC) for use in the system of Fig. 1.

[0035] Fig. 4 is an antibiotic susceptibility chip (or ASC) for use in the system of Fig. 1.

[0036] Fig. 5 is urinalysis chip (or UC) for use in the system of Fig. 1.

DESCRIPTION OF EMBODIMENTS

[0037] As shown in Fig. 1, a first embodiment according to the present invention includes a system 10 having therein a sample receiving area 12. A urine sample vial 14 containing urine received from a patient can be placed in the sample receiving area 12. The system 10 further includes a sample withdrawal pipette 16 which can be inserted into the urine sample vial 20 to withdraw urine therefrom. The system 10 also includes a cartridge 18 replaceably insertable into the apparatus 10, which contains microfluidic chips to be further described below.

[0038] Referring now to Fig. 2, cartridge 18 contains three microfluidic chips, a microbe detection chip (MDC) 40, an antibiotic susceptibility chip (ASC) 60 and a urinalysis chip (UC) 80. The sample withdrawal pipette 16 is connected via sample distribution lines 20 to provide urine samples to each of the microfluidic chips 40, 60, 80. Flow of sample from the vial 14 to the chips 40, 60, 80 is controlled by a series of pumps 22. If desired, various other fluids, such as buffer or cleansing solution can be provided in one or more solution vials 24, which also are connected to the chips 40, 60, 80 via distribution lines 20 and controlled by pumps 22. Liquid flowing out of the chips 40, 60, 80 is collected via waste collection lines 26 and delivered to a waste collection vial 28. Three sensors are provided in the systems 10, an MDC sensor 42 for MDC 40, an ASC MDC sensor 62 for ASC 60 and a UC MDC sensor 82 for UC 80. In each case, the relevant MDC sensor 42, 62, 82 is positioned to make measurements of the associated

chip 40, 60, 80. A programmed CPU 30 is connected via electrical lines 32 to control operation of the pumps 22 and to operate and collect data from the sensors 42, 62, 82. The CPU 30 also is connected via electrical lines 32 to mass storage 34 and one or more I/O devices 36, which may be a screen on the system 10 or an independent laptop, mobile device, centralized medical record system or the like. Operation of the system will be described further below in connection with each chip 40, 60, 80. The chips 40, 60, 80 preferably are all in replaceable cartridge 18, so they may easily be replaced after each use.

MDC structure and operation

[0039] MDC 40 is shown in greater detail in Fig. 3. Inlets 44 are provided on the chip 40 to receive the urine sample from sample distribution line 20. The urine sample then is distributed via distribution channels 46 and branch structures 48 to a series of microfluidic channels 50. Each microfluidic channel 50 is coated with materials to which microbes will adhere, such as, but not limited to antibodies, proteins, double-stranded DNA, single-stranded DNA, double-stranded RNA, single-stranded RN and aptamers, any of which may comprise 100% natural amino acids, a mix of natural and un-natural amino acids, or 100% un-natural amino acids. The coatings may attach to all forms of microbes, or may target a specific species or family, matching the relevant probes discussed further below.

[0040] A series of probe reservoirs 52 each contain a probe for a different microbe, which will be described in more detail below, and which is held in place in the probe reservoir by a sealant plug 53. Each probe reservoir 52 is connected into the branch structure 48 at a sub-branch level 49 such that it will connect to a specific cluster 51 of microfluidic channels 50. When each probe is released from its probe reservoir 52 as described below, it will flow into its associated cluster 51 and not into the other clusters. At the other end of each microfluidic

channel 50 is a collection channel 56 connect to outlets 57, through which fluids flowing through the MDC 40 can be removed from the MDC 40. Optionally, fluid reservoir 58 may also be provided on the MDC 40, which can contain a variety of fluids for use in the MDC 40, such as, but not limited to, buffering, lysing agent or the like. Such fluids can thereby be provided from the reservoirs 60 on the chip, or from the external solution vial 24, as most convenient for the design of a particular embodiment of the system 10.

[0041] As will be apparent from an inspection of Fig. 3, two sets of channels 50 are shown, each with four corresponding reservoirs 52. The number of sets, the number of reservoirs and the number of channels in each set can be expanded or reduced, as needed for the design of a particular embodiment of the system 10.

[0042] In use, urine is provided to the inlets 44, which will then flow through the distribution channels 46, branches 46, microfluidic channels 50 and collection channels 56 to the outlets 57. As the urine passes through the microfluidic channels 50, it will adhere to the walls due to the coatings. Urine input then is halted and the channels 50 are flushed with a lysing solution, either from an on-chip reservoir 58 or the external solution vial 24. As the lysing solution flows through the microfluidic channels 50, it will lyse all microbes present on the walls of the channels. The lysing solution is halted, and a buffer solution then is provided either from an on-chip reservoir 58 or an external solution vial 24. As this buffer solution flows through the microfluidic channels 50, it will flush out the lysing solution and any other materials not bound to the channel walls.

[0043] Preferably, the material of the sealant plugs 53 closing the probe reservoirs 52 is selected such that the buffer solution will dissolve the sealant plugs 53. If not, then a separate solution can be run through the MDC 40 to dissolve the sealant plugs 53.

[0044] When the sealant plugs 53 dissolve, the probes will then flow out of each probe reservoir 52 into the associated cluster 51 of microfluidic channels 50. The probes will bind to the aspects of each microbe in each channel for which they probe, if present. After an appropriate time period for the probes to bind, a washing solution may be flushed through the system to wash away any unbound materials, leaving only bound probes. The presence and number of bound probes is measured using the MDC sensor 42, and the resultant data is provided to the CPU 30, which stores it in the mass storage 32.

[0045] The MDC structure as described above is best used in situations where the probes need target intracellular material, e.g., DNA. If the probes target only proteins or other compounds expressed on the surface of the microbes, then the structure and method can be simplified. Specifically, according to this embodiment of the invention, probe reservoirs 52 can be omitted, and the probes are used as the coating on the insides of the channel sets. The lysing step described above can be skipped, since the probes will adhere to the surface of the microbes and lysing is unnecessary.

MDC probes

[0046] The probes used in the MDC generally take the form of an attacher-reporter complex, that is, they have an attacher part which is configured to attach to a specific microbe or portion of a microbe, and a reporter part bound to the attacher part which is readily detectable by an external device. Creating such attacher-reporter probes is a well known process to one of ordinary skill in the art, who is aware of many approaches to achieve this end.

[0047] Among the most common attachers is a single-stranded or double-stranded DNA or RNA sequence which will bind to a genus-specific, species-specific or subspecies-specific

DNA, RNA, oligonucleotide, peptide or similar sequences from a microbe. For example, such attachers can target attached DNA Sequences Nos. 1 and/or 2 to identify *Escherichia coli*, Sequences Nos. 3, 4, 5 and/or 6 to identify *Klebsiella pneumoniae*, Sequences Nos. 7 and/or 8 to identify *Staphylococcus saprophyticus*, Sequences Nos. 9 and/or 10 to identify *Enterococcus spp.*, Sequences Nos. 11 and/or 12 to identify *Proteus mirabilis*, Sequences Nos. 13 and/or 14 to identify *Pseudomonas aeruginosa*, Sequences Nos. 15 and/or 16 to identify *Staphylococcus aureus*, Sequences Nos. 17 and/or 18 to identify *Candida spp.*, Sequences Nos. 19 and/or 20 to identify *Candida albicans*, Sequences Nos. 21 and/or 22 to identify *Chlamydia trachomatis*, and Sequences Nos. 23 and/or 24 to identify *Mycoplasma genitalium*. Similarly, attached Sequences Nos. 25 and/or 26 can be used to identify microbes which carry indicia of resistance to the penicillin group of antibiotics, Sequences Nos. 27 and/or 28 to ciproflaxin, Sequences Nos. 29 and/or 30 to levofloxacin, and Sequences Nos. 31 and/or 32 to cephalexin.

[0048] Another well known approach to designing attachers is to fabricate aptamers, which are specific oligonucleotide or peptide molecules that bind to a specific target molecule. Aptamers can be engineered to bind to a known surface marker that is present on a specific microbial species, hence achieving the objective of attaching to only one species of microbe. Aptamers can also be designed to target the virulence factors present on any and all bacterial species and subspecies.

[0049] Suitable aptamers can be designed to target specific proteins that are present on the surface of the organism (such as outer membrane proteins (OMPs), virulence factors, IgGs, etc.) or the proteins and target sequences (such as DNA, mRNA, tRNA, sRNA) inside the cells of the organisms. An example of a surface marker that the antibodies and/or aptamers could bind to is the O Antigen present on the microbial surface. An example of a target sequence is

the 16S ribosomal RNA sequence highly abundant in bacterial species. The 16S sequence has been identified for many species. For example, Sequence No. 1 is the 16S sequence for *Escherichia coli*.

[0050] While such DNA, RNA and aptamer attachers are the most common, many other forms of attachers are well known and can be used in system 10 according to the present invention. For example, and without limitation, such attachers could be natural or synthetic DNA, RNA, antibodies, aptamers or other amino acid structures, using natural and/or non-naturally occurring amino acids, which recognize microbial surface molecules, microbial intracellular proteins, and/or microbial DNA or RNA. All such compounds may be truncated (such as Fab, Fab'2, scFv), engineered for multivalency or otherwise to detect more than one target. All such attachers may also be engineered to resist enzymatic or non-enzymatic degradation.

[0051] Any of these attachers can suitably be selected or designed to target species and subspecies including, but not limited to: *Acetobacter aurantius, Acinetobacter baumannii, Actinomyces israelii, Agrobacterium radiobacter, Agrobacterium tumefaciens, Anaplasma phagocytophilum, Azorhizobium caulinodans, Aztobacter vinelandii, Bacillus anthracis, Bacillus brevis, Bacillus cereus, Bacillus fusiformis, Bacillus licheniformis, Bacillus megaterium, Bacillus mycoides, Bacillus stearothermophilus, Bacillus subtilis, Bacillus thuringiensis, Bacteroides fragilis, Bacteroides gingivalis, Bacteroides melaninogenicus, Bartonella henselae, Bartonella quintana, Bordetella bronchiseptica, Bordetella pertussis, Borrelia burgdorferia, Brucella, Brucella abortus, Brucella melitensis, Brucella suis, Burkholderia mallei, Burkholderia pseudomallei, Burkholderia cepacia, Calymmatobacterium granulomatis, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni,*

Campylobacter pylori, Chlamydia trachomatis, Chlamydophila pneumoniae, Chlamydophila psittaci, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium tetani, Corynebacterium diphtheriae, Corynebacteriym fusiforme, Coxiella burnetti, Ehrlichia chaffeensis, Enterobacter cloacae, Enterococcus avium, Enterococcus durans, Enterococcus faecalis, Enteroccous faecium, Enterococcus galllinarum, Enterococcus maloratus, Eschericichia coli, Francisella tularenisis, Fusobacterium nucleatum, Gardnerella vaginalis, Haemophilus ducreyi, Haemophilus influenzae, Haemophilus parainfluenzae, Haemophilus pertussis, Hamephilus vaginalis, Helicobacter pylori, Klebsilla pneumoniae, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactococcus lactis, Legionella pneumophila, Listeria monocytogenes, Methanobacterium extroquens, Microbacterium multiforme, Micrococcus luteus, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium diphtheriae, Mycobacterium intracellulare, Mycobacterium leprae, Mycobacterium lepraemurium, Mycobacterium phlei, Mycobacterium smegmatis, Mycobacterium tuberculosis, Mycoplasma fermentans, Mycoplasma genitalium, Mycoplasma hominis, Mycoplasma penetrans, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Pasteurella tularensis, Peptostreptococcus, Porphyromonas gingivalis, Prevotella melaninogenica, Pseudomonas aeruginosa, Rhizobium radiobacter, Rickettsia prowazekii, Rickettsia psittaci, Rickettsia quintana, Rickettsia rickettsii, Rickettsia trachomae, Rochalimaea henselae, Rochalimaea quintana, Rothia dentocariosa, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis, Stenotrophomonas maltophillia, Streptococcus agalactiae, Streptococcus avium, Streptococcus bovis, Streptococcus cricetus, Streptococcus faecium, Streptococcus faecalis, Streptococcus ferus, Streptococcus gallinarum, Streptococcus lactis, Streptococcus mitior,

Streptococcus mutans, Streptococcus oralis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus rattus, Streptococcus salivarius, Streptococcus sanguis, Streptococcus sobrinus, Treponema pallidum, Treponema denticola, Vibrio cholerae, Vibrio comma, Vibrio parahaemolyticus, Vibrio vulnificus, Viridans streptococci, Wolbachia, Yersinia enterocolitica, Yersinia pestis, and Y*ersinia pseudotuberculosis.*

[0052] The most commonly used reporters are fluorescent molecules, which either naturally emit light when bound or are stimulated to fluoresce. Examples include but are not limited to naturally occurring fluorescein, synthetic Texas red dye, fluoro-max red and green dye, fluorescent carboxylate-modified particles with europium chelators, fluorescent streptavidin coated particles with europium chelators, and dry fluorescent particles.

[0053] Alternatives reporters include:

[0054] a. Chemiluminescent molecules and visible spectrum luminescence detected via means of visible-spectrum microscopy and imaging.

[0055] b. Radioactive nuclides detected via means of radioactive emissions of alpha and beta particles.

[0056] c. Giant magnetoresistance-based magnetic nanoparticles detected using an applied magnetic field and/or detected using an electrical signal via means of attachment to a GMR-sensitive surface coating.

[0057] d. Magnetic nanoparticles (MNPs) including but not limited to: iron oxide MNP, iron nickel MNP, iron cobalt MNP and other MNP materials based on iron, nickel, cobalt and other ferromagnetic elements or compounds. Such magnetic nanoparticles sometimes are coated with an organic and/or inorganic material such as streptavidin, oleic acid, oleylamine, polyethylene glycol, polysaccharide, polyhydroxybutyrate, biopolymers, iron oxide and like.

[0058] e. Surface plasmon structures which fluoresce or otherwise are readily detectable.

[0059] The attachers and reporters may be joined together by any suitable means, such as ligation, conjugation or via an intermediary structure, such as a bead or iron oxide nanoworm.

[0060] The attachers, reporters and/or attacher reporter complex may also be ligated with a peptide, conjugated to a protein, or otherwise modified for enhanced stability, such as by PEGylation.

[0061] As a specific example, the MDC 40 can be provided with 32 probe reservoirs 52. Each probe reservoir contains a probe formed by a fluorescent reporter and an antibody attacher selected to attach to one of the attached Sequences Nos. 1-32. Each MDC sensor 42 is a fluorescent sensor. The luminescent reporter preferably is the same for each probe, so that a single MDC sensor 42 can be used and moved from one channel 50 to the next. Alternatively, an MDC sensor 42 can be aligned with each channel 50. Results from the probes targeting sequences 1-24 will indicate the presence of the related species. Results from the probes targeting sequences 25-32 will indicate antibiotics which would be ineffective.

[0062] Note that using all of the Sequences Nos. 1-32 as described results in redundancy, since at least two sequences have been provided for each target. While it is not always necessary to have such redundancy, doing so will enhance the accuracy of the test.

ASC structure and operation

[0063] The ASC 60 is shown in greater detail in Fig. 4. An inlet 64 at one end of the chip connects via main channel 66 to the outlet 68 at the opposite end. A plurality of wells 70 are provided along the length of and connected to the main channel 66. Each of the wells 70 is pre-loaded with a different antimicrobial or antibiotic 72, except that one or more wells 74 may

be left empty of antimicrobials and antibiotics to serve as a control. Preferably, the wells 70, 74 also are pre-loaded with a growth medium and a reporter compound, such as, but not limited to, resazurin, which will fluoresce in the presence of living microbes.

[0064] Examples of possible antimicrobials and antibiotics 72 to be used include, without limitation, amikacin, aminoglycosides, amoxycillin, amoxycillin-clavulanate, aztreonam, β-lactams, carbapenems, carbenicillin, ceffriaxone, cefixime, cefoperazone, cefotaxime, cefpodoxime, cefprozil, ceftazidime, cefuroxime, coamoxiclav, cephalexin, cephalosporins, chloramphenicols, ciprofloxacin, clindamycin, colistin, cotrimoxazole, doxycycline, erythromycin, flucloxacillin, fluoroquinolones, folic acid inhibitors, foloxacin, fusidic acids, gentamicin, glycopeptides, kanamycin, lipopeptides, lyncosamides, macrolides, meropenem, metronidazoles, monobactams, moxifloxacin, mupirocin, nalidixic acid, neomycin, nitrofurantoins, norfloxacin, ofloxacin, oxazolidinones, penicillin, piperacillin-tazobactam, pivmecillinam, polymyxin b, quinolones, rifampicin, streptogramins, sulfamethoxazole, sulfonamides, tetracyclines, trimethoprim, vancomycin.

[0065] In use, if the wells 70, 74 are not pre-loaded with growth medium and a reporting compound, urine is mixed with growth medium and a reporting compound. Urine or the urine/growth medium/reporting compound mixture is supplied into inlet 64, flows down the main channel 66, into the wells 70, 74, with any excess exiting through outlet 68. Once the wells 70, 74 are filled, a high viscosity oil, such as, but not limited to, FC-40, is supplied into inlet 64. Due to its viscosity, the oil will flow down the main channel 66, but will not meaningfully enter the wells 70, 74, thus forming an oil plug which effectively seals each of the wells 70, 74 from the other wells 70, 74. The ASC 60 then is incubated for an appropriate time period to allow microbial replication.

[0066] Following incubation, the ASC sensor 62 will measure the amount of microbe in each well by detecting the reporter compound, provide the resultant data to the CPU 30, which then stores the data in mass storage 34. The CPU 30 then can evaluate the level of efficacy of each antimicrobial or antibiotic 72 against microbes in the urine sample either directly by determining lack of any microbe in a well 70 or indirectly by comparing growth rates between cells with antimicrobials or antibiotics 70 and the control well 74. The CPU 30 stores the analytic results in the mass storage 34. By providing levels of efficacy for each antibiotic, the lowest cost, narrowest spectrum antibiotic can be selected which will still be effective.

[0067] To minimize time for completion of the test, the wells 70, 74 should be made as small as is consistent with distinguishing efficacy of the relevant antibiotics, for example, < 1 ml, and preferably < 0.1 ml. This will minimize the amount of microbial replication required before the measurements can be taken.

[0068] While the ASC 60 shown in Fig. 4 has 40 wells 70, 74, it will be understood that the number of wells 70, 74 can be increased or decreased depending on the number of antimicrobials or antibiotics to be tested, and the level of redundancy desired in the design of a specific embodiment of the system 10.

[0069] As a specific example, the ASC 60 has 44 wells 70, 74. Two control wells 74 are provided, and two wells 70 each are pre-loaded with amikacin, amoxicillin-clavulanate, ampicillin, cefotaxime, cefixime, ceftriaxone, cephalexin, cefpodoxime, ciprofloxacin, cefprozil, coamoxiclav, fosfomycin, gentamicin, levofloxacin, nitrofurantoin, norfloxacin, ofloxacin, pivmecillinam, sulfamethoxazole, and trimethoprim. The reporter compound is resazurin and the ASC sensor 72 is a fluorescence sensor. Preferably, a single ASC sensor 72 can be moved to measure each well 70, 74. Alternatively, an ASC sensor 72 can be aligned

with each well 70, 74. Results from the test will indicate which of the antibiotics are most and least effective against the microbes which are present.

[0070] Two wells 70, 74 for the control and each antibiotic may be used to provide redundancy. It is not necessary to have such redundancy but doing so should increase accuracy of the test.

UC structure and operation

[0071] UC 80 is shown in greater detail in Fig. 5. UC 80 includes a channel 84 extending from the top to the bottom of the chip as shown in the drawing, with UC measurement sections 86 provided to sense various compounds in the urine. Preferably, these sections match the measurements typically used for urinalysis, e.g., sections to measure leukocytes, nitrites, urobilinogen, proteins, pH, hemoglobin, specific gravity, ketones, bilirubin and glucose. To achieve this, each section is pre-coated with the same materials as are used on commercially available dipsticks to make the measurements with current products today, such as those provided by Siemens Multistix®, Roche Chemstrip®, McKesson Consult® and Boehringer Combur-Test® urine test strips. These dipsticks typically test parameters such as leukocytes, nitrites, urobilinogen, proteins, pH, hemoglobin, specific gravity, ketones, bilirubin and glucose via colorimetric measurement.

[0072] In use, urine is supplied at one end of channel 84, flows through the channel 84 and out the other end. The relevant colorimetric chemicals in sections 86 will then react with the urine, changing color to indicate the measurements. If desired, a buffer or similar solution may be provided from solution vial 21 to flow through channel 84 to remove any remaining urine. In this configuration, the UC sensor 82 is a colorimeter and is positioned to be able to

read the colors of the various sections 86. When the measurement is complete, UC sensor 82 provides the data to the CPU 30, which stores it in the mass storage 34.

[0073] Once the various tests are completed, the CPU 30 generates and provides at least one report to the I/O device 36. Preferably, the CPU 30 generates at least two reports: The first one is generated after completion of the urinalysis and microbial detection by the MDC 40 and UC 80, since they do not require incubation and can be run quickly. The second report is generated after completion of the antibiotic susceptibility test by the ASC 60, which takes longer due to the required incubation period.

[0074] As will be apparent to one of skill in the art, the system has been described with reference to urine samples to test for UTIs but could readily be adapted to use with other biologic samples to test for other problems. For example, blood, sputum, saliva, mucous or even swabs from the cheek or a wound could be used as the initial biological sample for the system. Depending on the sample, it may be necessary to first add sterile water or saline to the sample to make it sufficiently liquid to flow through the system described. In addition, it may be desirable to change the specific detection compounds used for identification and quantification of microbes to match those likely to cause infections at the biologic location being tested, and other characteristics, e.g., blood chemistry, may be tested instead of conducting a urinalysis. Similarly, the specific microbes described and for which sequences have been provided are bacteria, but the same approach can be used to analyze possible viral, fungal, prion and other infections. But it will be seen that the system as a whole is readily adaptable for a wide variety of tests, sometimes just by substituting different chips.

[0075] One skilled in the art will appreciate that the present invention can be practiced with embodiments other than those disclosed. The disclosed embodiments are presented for

purposes of illustration and not limitation, and the present invention is limited only by the claims that follow.

CLAIMS

What is claimed is:

1. An apparatus for analyzing a biological sample, the apparatus having a sample receiving mechanism, a programmed control system for operating the system and a reporting system, and further characterized by comprising:

    a. a microfluidic microbe detection chip (MDC), the MDC comprising:

        i. a plurality of microfluidic branches, an internal surface of each branch comprising a substance to which microbes will adhere;

        ii. inlets connected to each said microfluidic branch for receiving the biological sample and delivering it to said microfluidic branches, a plurality of probe reservoirs, each said reservoir containing a probe supply and connected to a subset of said microfluidic branches to deliver said probe to said subset of microfluidic branches; and

        iii. outlets connected to said microfluidic branches for removing fluid therefrom;

    b. a sample transfer mechanism for receiving the biological sample and delivering it to said inlets of said MDC;

    c. at least one MDC solution reservoir, each said reservoir containing a solution and connected to said microfluidic branches to deliver said solution to said microfluidic branches;

    d. at least one MDC sensor adjacent to said microfluidic branches to measure the presence of said probes therein; and

e. further characterized in that the programmed control system receives measurements from said at least one MDC sensor and reports the results to the reporting system.

2. The apparatus of claim 1, further characterized in that said biological sample is selected from the group consisting of urine, blood, sputum, saliva, mucous and swabs from solid tissue.

3. The apparatus of claims 1 - 2, further characterized in that said biological sample is urine.

4. The apparatus of claims 1 - 3, further characterized in that said MDC is part of a replaceable cartridge.

5. The apparatus of claim 3, further characterized in that said at least one MDC solution reservoir is part of said replaceable cartridge.

6. The apparatus of claims 1 - 5, further characterized in that each said probe comprises an attacher-reporter complex.

7. The apparatus of claims 6, further characterized in that an attacher portion of each attacher-reporter complex is selected from the group consisting of natural or synthetic DNA, RNA, antibodies, aptamers or other amino acid structures, using natural and/or non-naturally occurring amino acids and which recognize microbial surface molecules, microbial intracellular proteins or microbial DNA or RNA.

8. The apparatus of claims 6 - 7, further characterized in that each said attacher-reporter complex comprises:

a. an attacher portion comprising a microbe-specific antibody which will attach to a specific target portion of DNA or RNA in a microbe;

b. a reporter portion connected to the attacher portion, said reporter portion reporting to said at least one MDC sensor when said attacher is bound to said target portion of DNA or RNA.

9. The apparatus of claims 6 - 8, further characterized in that each said attacher portion attaches to a portion of the microbial surface molecules, microbial intracellular proteins or microbial DNA or RNA which identifies a specific microbe, the microbe being selected from the group consisting of the microbes most likely to cause an infection in the type of biological sample being tested.

10. The apparatus of claim 9, further characterized in that said probes are selected to match those most likely to cause a urinary tract infection.

11. The apparatus of claim 10, further characterized in that said microbes targeted by said probes are selected to match those most likely to cause a urinary tract infection in a specific geography.

12. The apparatus of claims 6 - 11, further characterized in that each said probe targets a microbe selected from the group consisting of *Acetobacter aurantius, Acinetobacter baumannii, Actinomyces israelii, Agrobacterium radiobacter, Agrobacterium tumefaciens, Anaplasma phagocytophilum, Azorhizobium caulinodans, Aztobacter vinelandii, Bacillus anthracis, Bacillus brevis, Bacillus cereus, Bacillus fusiformis, Bacillus licheniformis, Bacillus megaterium, Bacillus mycoides, Bacillus stearothermophilus, Bacillus subtilis, Bacillus thuringiensis, Bacteroides fragilis, Bacteroides gingivalis, Bacteroides melaninogenicus, Bartonella henselae, Bartonella*

quintana, Bordetella bronchiseptica, Bordetella pertussis, Borrelia burgdorferia, Brucella, Brucella abortus, Brucella melitensis, Brucella suis, Burkholderia mallei, Burkholderia pseudomallei, Burkholderia cepacia, Calymmatobacterium granulomatis, Campylobacter coli, Campylobacter fetus, Campylobacter jejuni, Campylobacter pylori, Chlamydia trachomatis, Chlamydophila pneumoniae, Chlamydophila psittaci, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium tetani, Corynebacterium diphtheriae, Corynebacteriym fusiforme, Coxiella burnetti, Ehrlichia chaffeensis, Enterobacter cloacae, Enterococcus avium, Enterococcus durans, Enterococcus faecalis, Enteroccous faecium, Enterococcus galllinarum, Enterococcus maloratus, Eschericichia coli, Francisella tularenisis, Fusobacterium nucleatum, Gardnerella vaginalis, Haemophilus ducreyi, Haemophilus influenzae, Haemophilus parainfluenzae, Haemophilus pertussis, Hamephilus vaginalis, Helicobacter pylori, Klebsilla pneumoniae, Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactococcus lactis, Legionella pneumophila, Listeria monocytogenes, Methanobacterium extroquens, Microbacterium multiforme, Micrococcus luteus, Moraxella catarrhalis, Mycobacterium avium, Mycobacterium bovis, Mycobacterium diphtheriae, Mycobacterium intracellulare, Mycobacterium leprae, Mycobacterium lepraemurium, Mycobacterium phlei, Mycobacterium smegmatis, Mycobacterium tuberculosis, Mycoplasma fermentans, Mycoplasma genitalium, Mycoplasma hominis, Mycoplasma penetrans, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Pasteurella tularensis, Peptostreptococcus, Porphyromonas gingivalis, Prevotella melaninogenica, Pseudomonas aeruginosa, Rhizobium radiobacter, Rickettsia prowazekii, Rickettsia psittaci, Rickettsia quintana,

Rickettsia rickettsii, Rickettsia trachomae, Rochalimaea henselae, Rochalimaea quintana, Rothia dentocariosa, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Staphylococcus aureus, Staphylococcus epidermidis, Stenotrophomonas maltophillia, Streptococcus agalactiae, Streptococcus avium, Streptococcus bovis, Streptococcus cricetus, Streptococcus faecium, Streptococcus faecalis, Streptococcus ferus, Streptococcus gallinarum, Streptococcus lactis, Streptococcus mitior, Streptococcus mutans, Streptococcus oralis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus rattus, Streptococcus salivarius, Streptococcus sanguis, Streptococcus sobrinus, Treponema pallidum, Treponema denticola, Vibrio cholerae, Vibrio comma, Vibrio parahaemolyticus, Vibrio vulnificus, Viridans streptococci, Wolbachia, Yersinia enterocolitica, Yersinia pestis, and *Yersinia pseudotuberculosis.*

13. The apparatus of claims 6 - 12, further characterized in that said probes target DNA selected from the group consisting of attached Sequences Nos. 1 – 24.

14. The apparatus of claims 6 - 13, further characterized in that said attacher portion attaches to a portion of the DNA or RNA of said microbe which identifies resistance to a specific antibiotic or antimicrobial.

15. The apparatus of claim 14, further characterized in that said resistance identifying DNA or RNA identifies resistance to antimicrobials in gram positive and gram negative bacteria.

16. The apparatus of claims 14 - 15, further characterized in that said resistance identifying DNA or RNA identifies resistance to antibiotics or antimicrobials which interfere with

or inhibit cell wall synthesis, protein synthesis, DNA synthesis and integrity, or RNA synthesis and integrity.

17. The apparatus of claims 14 - 16, further characterized in that said resistance identifying DNA or RNA identifies resistance to antimicrobials from the group consisting of aminoglycosides, ansamycins, β-lactams, carbapenems, cephalosporins, chloramphenicols, fluoroquinolones, folic acid inhibitors, fusidic acids, glycopeptides, lipopeptides, lyncosamides, macrolides, metronidazoles, monobactams, nitrofurantoins oxazolidinones, quinolones, streptogramins, sulfonamides, tetracyclines.

18. The apparatus of claims 14 - 17, further characterized in that said resistance identifying DNA or RNA identifies resistance to antibiotics from the group consisting of amikacin, amoxycillin, amoxycillin- aztreonam, carbenicillin, cefixime, cefotaxime, ceffriaxone, cefoperazone, cefpodoxime, cefprozil, ceftazidime, cefuroxime, ceftriaxone, clavulanate, cephalexin, clindamycin, ciprofloxacin, coamoxiclav, colistin, cotrimoxazole, doxycycline, erythromycin, foloxacin, flucloxacillin, fusidic acid, gentamicin, kanamycin, meropenem, moxifloxacin, mupirocin, nalidixic acid, neomycin, nitrofurantoin, norfloxacin, ofloxacin, penicillin, piperacillin-tazobactam, pivmecillinam, polymyxin b, rifampicin, sulfamethoxazole, tetracycline, trimethoprim, and vancomycin.

19. The apparatus of claims 14 - 18, further characterized in that said resistance identifying DNA or RNA identifies resistance to amikacin, amoxicillin-clavulanate, ampicillin, cefotaxime, cefixime, ceftriaxone, cephalexin, cefpodoxime, ciprofloxacin, cefprozil,

coamoxiclav, fosfomycin, gentamicin, levofloxacin, nitrofurantoin, norfloxacin, ofloxacin, pivmecillinam, sulfamethoxazole, and trimethoprim.

20. The apparatus of claims 14 - 19, further characterized in that said probes target DNA selected from the group consisting of attached Sequences Nos. 25 – 32.

21. The apparatus of claims 6 - 20, further characterized in that said reporter portion is selected from the group consisting of fluorescent structure, chemiluminescent structure, radioactive nuclides, magnetic nanoparticles, giant magnetoresistance-based magnetic nanoparticles, coated magnetic nanoparticles and surface plasmon structures.

22. The apparatus of claim 21, further characterized in that said reporter portion comprises said fluorescent structure, which is selected from the group consisting of naturally occurring fluorescein, synthetic Texas red dye, fluoro-max red and green dye, fluorescent carboxylate-modified particles with europium chelators, fluorescent streptavidin coated particles with europium chelators, and dry fluorescent particles.

23. The apparatus of claims 1 - 22 further characterized by having at least 2-100 said probes.

24. The apparatus of claims 1 - 23, further characterized by having at least 4-50 said probes.

25. The apparatus of claims 1 - 24, further characterized by having at least 8-25 said probes.

26. The apparatus of claims 1 - 25, further characterized in that said programmed control system is programmed to operate the apparatus according to the method comprising:

    a. causing said sample transfer mechanism to receive said biological sample and deliver it to said MDC inlets, and thereby to said microfluidic branches such that at least some of any microbes in said biological sample will adhere to said

internal surfaces of said microfluidic branches and any excess biological sample will exit said microfluidic channels via said outlets;

b. causing a lysing solution from a first said MDC solution reservoir to be delivered to said microfluidic branches to lyse microbes adhered therein;

c. causing a buffer solution from a second said MDC solution reservoir to be delivered to said microfluidic branches to substantially flush out through the outlets both the lysing solution and any microbes not adhered to the microfluidic branches;

d. causing each said probe to be delivered to its said connected subset of microfluidic branches to bind to any of the probe's target DNA or RNA adhered inside the microfluidic branches;

e. causing a washing solution from a third said MDC solution reservoir to be delivered to said microfluidic branches to flush unbound probes out through the outlets;

f. causing the MDC sensor adjacent to each said subset of microfluidic branches to measure the level of probes bound inside the microfluidic branches; and

g. reporting the level of probes bound inside the microfluidic branches.

27. The apparatus of claim 26, further characterized in that the MDC further comprises a plug between each probe reservoir and its said connected subset of said microfluidic channels, and wherein said buffer solution dissolves said plug to cause said probes to be delivered.

28. The apparatus of claims 26 - 27, further characterized in that said buffer solution and said washing solution are the same and said second and third reservoirs are the same.

29. The apparatus of claims 1 - 28, further characterized in that all of said reservoirs are on a replaceable cartridge.

30. The apparatus of claims 1 - 29, further characterized in that some of said reservoirs are on a replaceable cartridge and some are not.

31. The apparatus of claims 29 - 30, further characterized in that said MDC is on said replaceable cartridge.

32. The apparatus of claims 1 - 31, further characterized in that the apparatus further comprises:

    a. an antibiotic susceptibility chip (ASC), the ASC comprising:

        i. a main channel;

        ii. a plurality of wells formed adjacent to and connected to said main channel, each said well comprising a volume to receive said biological sample and at least some of said cells pre-coated with at least one antibiotic;

        iii. an inlet near one end of said main channel;

        iv. an outlet near an opposite end of said wells;

    b. said sample transfer mechanism further being connected to said ASC inlet in parallel to said MDC inlet;

    c. at least one ASC solution reservoir containing a solution and connected to said ASC inlet;

d. a reporter in each said well to report the presence and amount of microbes in said well;

e. at least one ASC sensor adjacent to said ASC wells to measure said reporter; and

f. wherein said programmed control system is further configured to operate said ASC, to receive measurements from said at least one ASC sensor and to report the results.

33. The apparatus of claim 32, further characterized in that said programmed control system is programmed to operate the apparatus according to the method comprising:

a. causing said sample transfer mechanism to receive said biological sample and deliver it to said ASC inlet to substantially fill said ASC main channel and wells, and with any excess biological sample exiting said ASC main channel via said ASC outlet;

b. causing a high viscosity solution from a first said ASC solution reservoir to be delivered to said ASC main channel to block off each said well from the other said wells;

c. incubating said ASC for a time period and under environmental conditions conducive to microbial growth;

d. causing said at least one ASC sensor adjacent to said wells to measure the level of microbes in each said well as indicated by said reporter; and

e. reporting the amount of microbes found inside each said well.

34. The apparatus of claim 34, further characterized in that the programable control system further is programmed to add a growth compound and said reporter from a second said ASC solution reservoir to the biological sample prior to delivery of the biological sample to said ASC inlet.

35. The apparatus of claim 34, further characterized in that each said well further is pre-loaded with a growth compound and said reporter prior to introduction of said biological sample thereto.

36. The apparatus of claims 32 - 35, further characterized in that said reporter comprises resazurin and wherein said at least one ASC sensor comprises a fluorescent sensor for the detection of resazurin combined with a living microbe.

37. The apparatus of claims 1 - 36, further characterized in that the system further comprises:

    a. a microfluidic urinalysis chip (UC), the UC comprising:

        i. a channel having a plurality of UC sensor areas along a length thereof, each UC sensor area having a surface which will bind to or react with a specific constituent of said biological sample;

        ii. an inlet along said channel at one end of said UC sensor areas;

        iii. an outlet along said channel on an opposite end of said UC sensor areas;

    b. said sample transfer mechanism further being connected to said UC inlet in parallel to said MDC inlets;

    c. at least one UC sensor adjacent to said UC sensor areas to measure the value of said specific constituent of said biological sample in said UC sensor area; and

d. further characterized in that said programmed control system is further configured to operate said UC, to receive measurements from said at least one UC sensor and to report the results.

38. The apparatus of claim 37, further characterized in that said UC sensor areas comprise areas to measure characteristics selected from the group consisting of bilirubin, glucose, hemoglobin, ketones, leukocytes, nitrites, pH, protein, specific gravity, urobilinogen.

39. The apparatus of claims 37 - 38, further characterized in that said UC sensor areas each change color to varying degrees depending on the amount of said constituent in said biological sample and wherein said at least one UC sensor comprises a colorimeter which measures the degree of color change in each said UC sensor area.

40. The apparatus of claims 37 - 39, further characterized in that said programmed control system further is programmed to operate the apparatus according to the method comprising:

a. causing said sample transfer mechanism to receive said biological sample and deliver it to said UC inlet, and thereby to said UC channel and UC sensor areas with any excess biological sample exiting said UC channel via said UC outlet;

b. causing said at least one UC sensor adjacent to said sensor areas to measure the value of constituents bound in the sensor area; and

c. reporting the value of constituents bound in the sensor areas.

41. An apparatus for diagnosing a urinary tract infection using a urine sample, the apparatus having a sample receiving mechanism, a programmed control system for operating the system and a reporting system, and further characterized by comprising:

a. a microfluidic microbe detection chip (MDC), the MDC comprising:

   i. a plurality of microfluidic branches, an internal surface of each branch comprising a substance to which microbes will adhere;

   ii. inlets connected to each said microfluidic branch for receiving the urine sample and delivering it to said microfluidic branches, a plurality of probe reservoirs, each said reservoir containing a probe supply and connected to a subset of said microfluidic branches to deliver said probe to said subset of microfluidic branches, wherein each said prope attaches to a portion of the microbial surface molecules, microbial intracellular proteins or microbial DNA or RNA which identify a specific microbe, the microbes being selected from the group consisting of the microbes most likely to cause a urinary tract; and

   iii. outlets connected to said microfluidic branches for removing fluid therefrom;

b. an antibiotic susceptibility chip (ASC), the ASC comprising:

   i. a main channel;

   ii. a plurality of wells formed adjacent to and connected to said main channel, each said well comprising a volume to receive the urine sample and at least some of said cells pre-coated with at least one antibiotic;

   iii. a reporter in each said well to report the presence and amount of living microbes in said well;

   iv. an inlet near one end of said main channel;

v. an outlet near an opposite end of said wells;

c. a sample transfer mechanism for receiving the urine sample and delivering it to said inlets of said MDC and said ASC;

d. at least one solution reservoir, each said reservoir containing a solution and connected to said microfluidic branches or said main channel to deliver said solution to said microfluidic branches;

e. at least one MDC sensor adjacent to said microfluidic branches to measure the presence of said probes therein;

f. at least one ASC sensor adjacent to said ASC wells to measure said reporter; and

g. further characterized in that the programmed control system is programmed to operate the apparatus to deliver the urine sample and solutions from said reservoirs to said MDC and ASC, receiving measurements from said at least one MDC sensor and said at least one ASC sensor and to report the results.

42. A method for diagnosing a urinary tract infection using a urine sample, characterized by:

a. Providing a microfluidic microbe detection chip (MDC), the MDC comprising a plurality of microfluidic branches and a plurality of different probes associated therewith, each branch configured to receive an aliquot of the urine sample, and each probe comprising a structure which attaches to a portion of the microbial surface molecules, microbial intracellular proteins or microbial DNA or RNA which identify a specific microbe, the microbes being selected from the group

consisting of the microbes most likely to cause a urinary tract and DNA or RNA, or which identify presence of DNA or RNA indicated resistance to a particular antibiotic;

b. Providing an antibiotic susceptibility chip (ASC), the ASC for receiving an aliquot of the urine sample and comprising a plurality of wells in which to test the sample, each well being less than about 1 cm in volume and containing a different version of antibiotics to be tested for efficacy;

c. Delivering the urine sample to said MDC and said ASC for testing;

d. Measuring the volume of probes in each microchannel branch which positively indicate presence the microbe or antibiotic tested for by each said probe;

e. Incubating the ASC in conditions conducive to microbial growth for a suitable period of time, then measuring the volume of microbes in each well; and

f. Reporting the results of such measurements.

Fig. 1

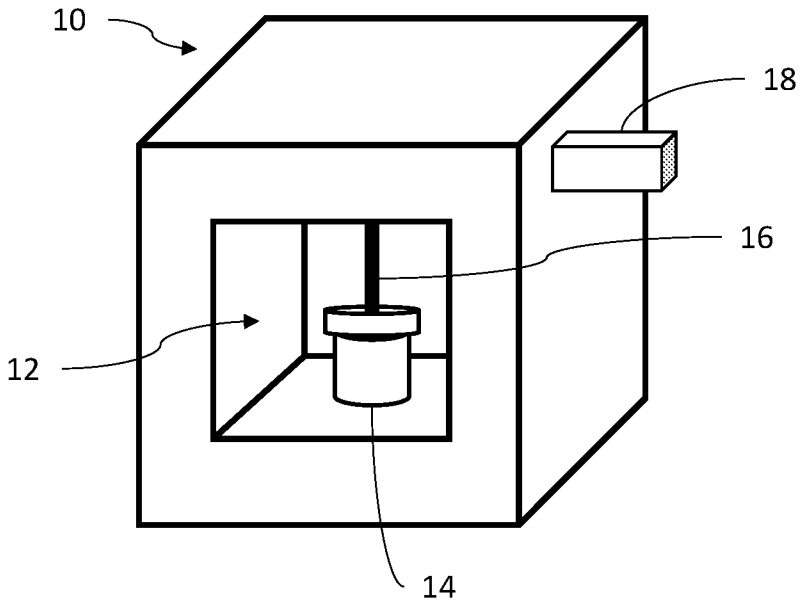
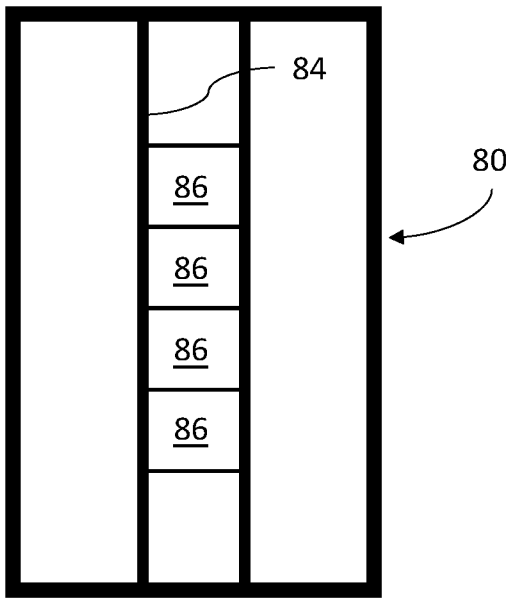


Fig. 5



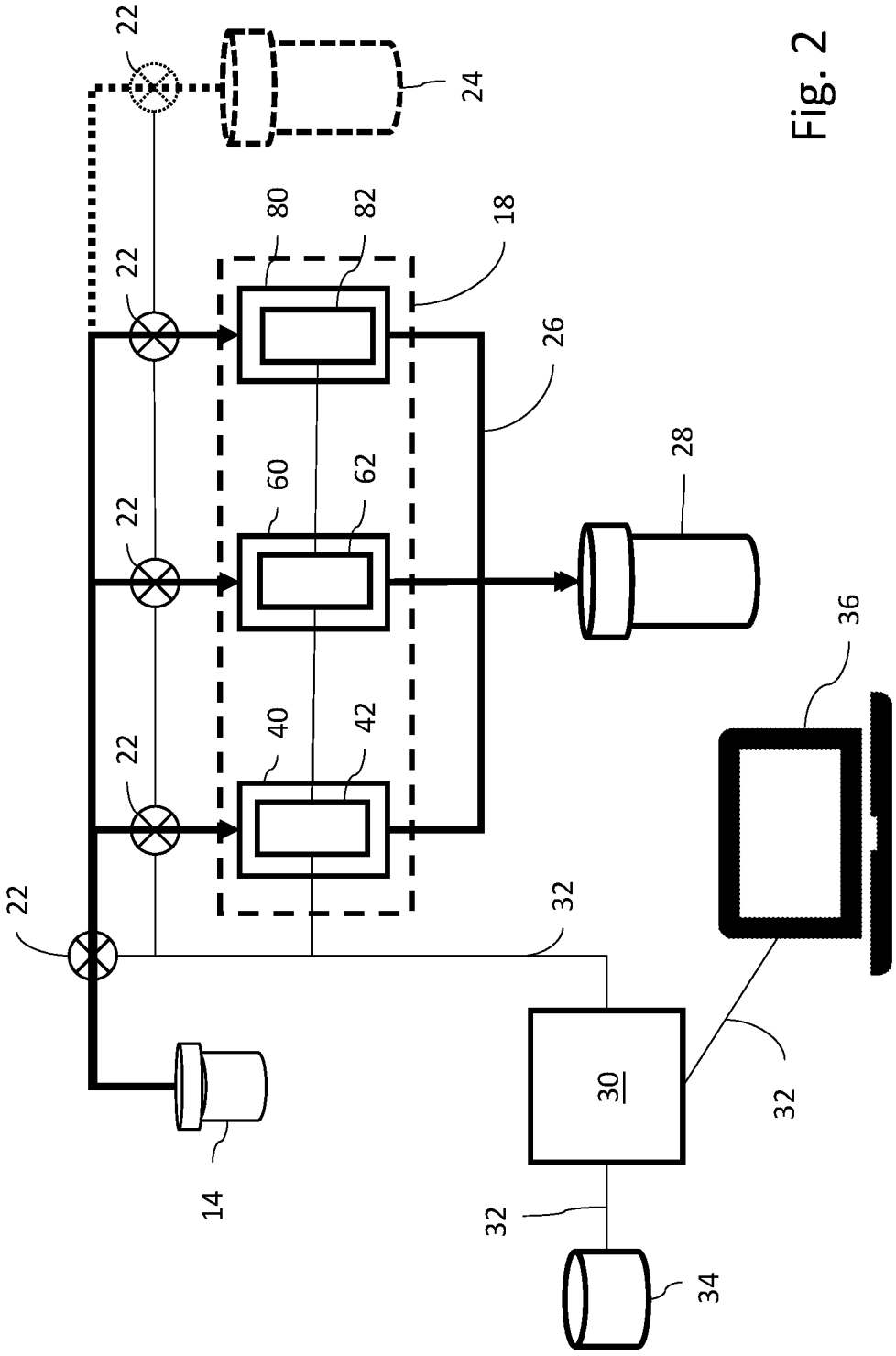


Fig. 2

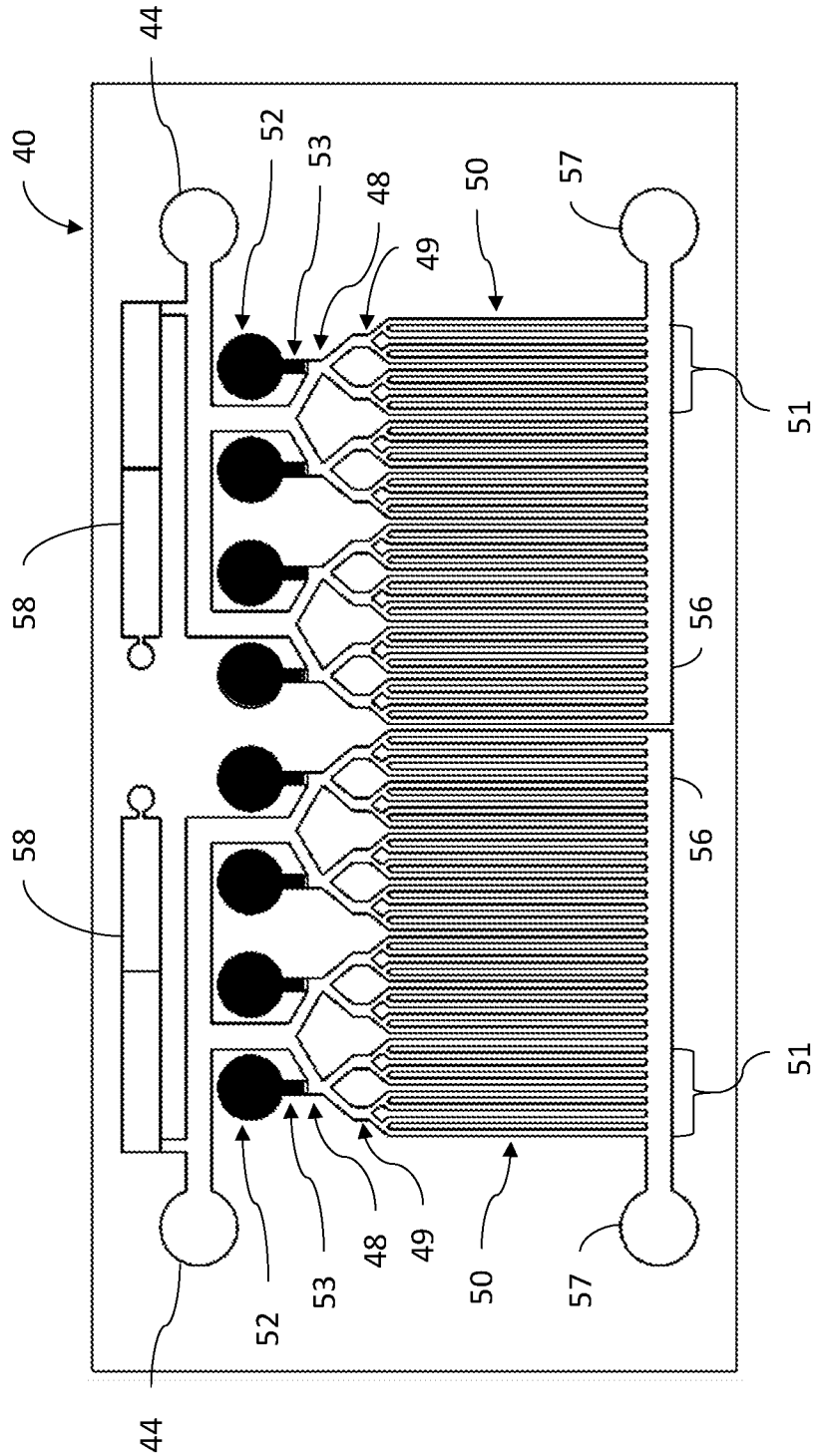
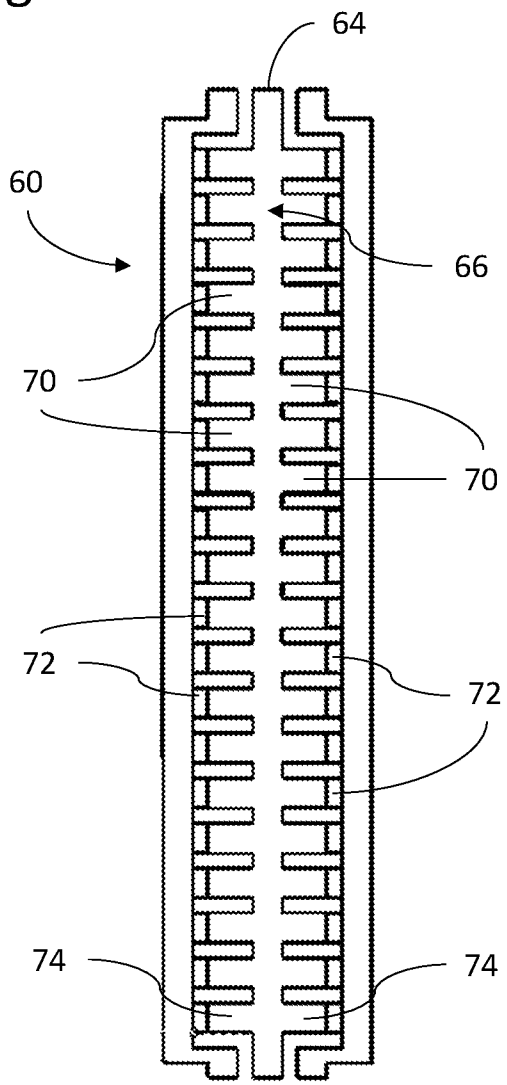


Fig. 3

Fig. 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/048649

A. CLASSIFICATION OF SUBJECT MATTER

G01N 33/569(2006.01)i, G01N 33/53(2006.01)i, B01L 3/00(2006.01)i, C12Q 1/18(2006.01)i, C12Q 1/689(2018.01)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 33/569; B01L 3/00; C12Q 1/18; C12Q 1/24; C12Q 1/70; G01N 33/53; C12Q 1/689

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:Urinary tract infection(UTIs), microbe, detection, antibiotic susceptibility

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2017-0298456 A1 (THE CHARLES STARK DRAPER LABORATORY, INC.) 19 October 2017 See abstract; figures 1, 2A; paragraph [0055]; claims 1-2, 11, 17, 19.	1-2
Y		41-42
Y	US 2016-0215324 A1 (SRINIVASAN, A. et al.) 28 July 2016 See abstract; figures 2B, 4A-4B; claims 1, 32-33, 36.	41-42
Y	AVESAR, J. et al., `Rapid phenotypic antimicrobial susceptibility testing using nanoliter arrays`, Proceedings of the National Academy of Sciences, 26 June 2017, Vol.114, pages E5787-E5795 See abstract; figure 1.	41-42
A	ALTOBELLI, E. et al., `Integrated biosensor assay for rapid uropathogen identification and phenotypic antimicrobial susceptibility testing`, European urology focus, 2017, Vol.3, No.2-3, pages 293-299, HHS Public Access Author Manuscript Version internal page 1-13 See abstract; figure 1; page 5.	1-2,41-42
A	US 2016-0032350 A1 (THE BROAD INSTITUTE, INC. et al.) 04 February 2016 See the whole document.	1-2,41-42

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

☒ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"D" document cited by the applicant in the international application
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"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
17 December 2019 (17.12.2019)	**17 December 2019 (17.12.2019)**

Name and mailing address of the ISA/KR
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/048649

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 5,7,10-11,15,22,27,33-35,38
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
Each of claims 5, 7, 10-11, 15, 22, 27, 33-35, 38 refers to a claim which is not drafted in accordance with the third sentence of Rule 6.4(a).

3. ☒ Claims Nos.: 3-4,6,8-9,12-14,16-21,23-26,28-32,36-37,39-40
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required addtional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of any additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/US2019/048649

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 2017-241592 A1	18/10/2018
		CA 3019362 A1	05/10/2017
		CA 3019532 A1	05/10/2017
		EP 3436196 A2	06/02/2019
		EP 3436592 A2	06/02/2019
		JP 2019-509746 A	11/04/2019
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US 2016-0032350 A1	04/02/2016	US 10077462 B2	18/09/2018
		WO 2014-152643 A2	25/09/2014
		WO 2014-152643 A3	11/12/2014