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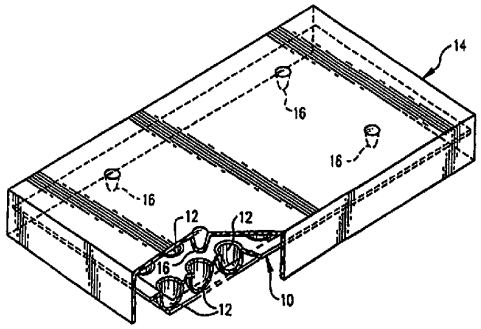

(57) Abstract

Method and assay devices for the detection of the presence or amount of biological material, analyte(s), or microorganism(s) in a sample. The method includes the steps of liquefying the sample (if necessary) and distributing the liquefied sample over the surface of the assay device. The device may comprise an incubation plate, a dip stick device, or other devices. The devices have at least one reagent provided within the devices. Some devices have a generally flat horizontal surface which is divided into a plurality of recessed wells. Others have one or more surfaces with reagent island(s) immobilized thereon. Each well or reagent island is adapted to hold an aliquot of liquid. The wells or reagent islands are sized and shaped, and formed of a suitable material, to hold the aliquot within the well or reagent island by surface tension. Any excess liquid from the liquefied sample is drained from the surface of the device. The method then involves incubating the assay device until the presence or amount of the biological material, analyte, or microorganism is determined.

Device and Methods for Determination of Analyte in a Solution

Background of the Invention

This invention relates to the field of assay technology, and in particular embodiments, to devices and methods for quantification of analytes, eg., biological material, in a sample.

Many industries need to detect and quantify the concentration and level of biological material or other analyte in a sample. For example, the determination of bacterial concentration in food and water is an essential part of food and water quality testing. EPA regulations require that no coliform such as *Escherichia coli* can be present in potable water. The "presence/absence" format of a testing medium, such as Colilert® chemical mixture (IDEXX Laboratories, ME) which is used as a testing medium for *Escherichia coli* and all coliform bacteria, is very useful in making this determination. Colilert® chemical mixture is based on the Defined Substrate Technology described in Edberg, "Method and Medium for use in Detecting Target Microbes *In Situ* in A Specimen Sample of A Possibly Contaminated Material," US 4 925 789 and 5 492 933.

However, there are areas where the quantification, not just the detection, of bacterial concentration is important. Examples of such areas include waste water, incoming water in water purification systems, surface water, and food testing. For example, numerous restaurant chains will only accept raw ground beef or poultry that contains less than a certain concentration of bacterial contamination. Therefore, food processing plants must carry out the necessary microbiological tests to determine the bacterial concentration of these food items before they can be released to customers.

The classical methods of quantification of biological material are the standard plate count method or the multiple tube fermentation (MTF) method. A quantity of sample being tested for microbial contamination is first dispensed in a Petri dish. Then 15mL of the appropriate media is poured over the sample. The Petri-dish is then swirled to mix the sample in the medium and the Petri-dish is left to solidify at room temperature for approximately 20 minutes. The medium is then incubated at a specific temperature for a specific time, and any resulting colonies are counted.

The multiple tube fermentation method is described in Recles *et al.*, "Most Probable Number Techniques" published in "Compendium of Methods for the Microbiological Examination of Foods", 3rd ed. 1992, at pages *105-199*, and in Greenberg *et al.*, "Standard Methods For the Examination of Water and Wastewater" 8th ed. 1992). In this method, a volume of sample is dispensed into several tubes representing this dilution range. The tubes are then incubated at the appropriate temperature so that the bacteria in each tube are allowed to grow. After incubation at a specific temperature for a specific time, the number of positive tubes is counted. The most probable number can be determined from the formula described in Recles *et al.*, *supra*.

Water testing is mostly done by membrane filtration, where a certain volume of water is passed through the membrane and the membrane is incubated in a medium for a certain period of time. After appropriate incubation, the colonies are counted.

In many industries there is also a need to qualitatively and/or quantitatively detect the presence of an analyte in a liquid solution. For example, the detection of inorganic ions may be important in a manufacturing process using a test solution.

Heretofore, the methods and devices generally relied upon for measuring an analyte in solution have either required removing all of an aliquot from the test solution or exposing a dip stick to a test solution. Although these methods and devices can detect an analyte in solution, they suffer from a number of disadvantages.

The dip stick-related methods are not quantitative. For example, in the general dip stick embodiments there is no capability for determining or quantifying the presence of an analyte in a unit volume of that solution. Instead, the dip stick is simply brought into contact with the test solution.

Other methods require a user to manually remove an aliquot from a test solution and transfer it to a separate device for detection or quantification of the analyte.

Therefore, despite the ability of these methods and devices to detect an analyte in solution, the methods and devices currently used have proven to have limited accuracy; and/or to be costly, complicated, time consuming; and/or to have a limited range of uses because of the particular assay technology.

Thus, there exists a need for a simple, accurate, and inexpensive method for the determination of an analyte in solution without the disadvantages known in the prior art. In particular, there is a need for devices and methods capable of providing a quantitative assay of an analyte in a particular volume of a solution.

Summary of Invention

The present invention provides devices and methods for detecting and enumerating the presence or absence of biological materials, analytes, and microorganisms in sample solutions.

In one aspect, the invention describes a device for determining the presence or amount of an analyte(s) or microorganism(s) in a test solution. The device features a substantially hydrophobic support structure with at least one reagent island immobilised on the support structure which is capable of absorbing a predetermined volume of test solution. The device also comprises a means for determining the presence or amount of the analyte or microorganism, the means being positioned on or within the reagent island(s). This aspect of the invention may take the form of a plastic or polymer dip stick with one or more reagent island(s) immobilised on it. The reagent island may be made of any absorbent material, for example cellulose.

The support structure may have multiple reagent islands immobilised on it. The means for determining the presence or amount of microorganisms or analyte(s) may comprise a powder which is incorporated onto or within the material from which the reagent island is prepared. The means may be a reagent or a combination of reagents which leads to a production of a detectable signal when target analyte(s) or microorganism(s) is present. The device may contain multiple reagent islands which can contain different reagents or combinations of reagents so that numerous assays can be conducted on a single device. In a preferred embodiment, the reagent islands are comprised of cellulose. The support structure can be constructed of plastic, polymer, or any suitable materials onto which a reagent island can be mounted and which will not interfere with the assay. In another embodiment, the device also comprises a container for holding the support structure before, during, and after conducting the assay. The container will typically comprise a test tube and may further comprise a cap for the test tube to further protect the device.

In another aspect, the invention provides an assay device for determining the presence or amount of an analyte(s) or microorganism(s) in a sample which comprises a substantially hydrophobic solid support structure with at least one discrete reagent island immobilised thereon which is capable of absorbing and holding a predetermined volume of test solution. Positioned on or within the reagent island is a means for determining the presence or absence of a target analyte or microorganism. This aspect of the device further comprises a container which is open at opposite ends. The container may typically take the form of a laboratory pipette. This aspect of the invention may comprise any of the various embodiments described with respect to the device described above. The reagent islands can also be arranged in zones such that each zone provides a separate assay. The reagent islands can be adapted to retain aliquots of liquid by surface tension or by absorption. The device may typically comprise an elongated strip. The reagent comprised in the reagent islands may be a growth medium or an indicator growth medium. The reagent may also be an enzyme or an enzyme substrate.

In another aspect, the invention provides an assay device for determining the presence or amount of an analyte(s) or microorganism(s) in a sample comprising a solid support structure with at least one reagent island immobilised thereon. Each reagent island is adapted to hold an aliquot of liquid and is of a size and shape and made of a material suitable to hold the aliquot within the reagent island. At least one of the reagent islands contains at least one reagent for the detection of the analyte(s) and/or microorganism(s) of interest. The device does not provide a positive response for the analyte(s) or microorganism(s) when the analyte(s) or microorganism(s) is not present in the test sample.

In a preferred embodiment, the reagent islands of the device may take up a preselected total volume. In one embodiment the device may comprise a combination of reagents located in separate reagent islands. In another embodiment, the reagent islands are arranged in zones, wherein each zone provides a separate assay so that multiple assays may be conducted on a single device. The reagent islands may be adapted to retain aliquots of liquid sample by surface tension or by absorption. The reagent islands may be adapted to each take up equal volumes of liquid. Or the reagent islands may exist in subsets of islands, wherein each subset consists of at least one island, and each subset takes up different volumes from the other subsets. The device may be constructed so that the reagent islands are immobilised, on more than one surface. In different embodiments, the device may be a plate or an elongated strip. In a preferred embodiment, the at least one reagent may be a growth medium. In various embodiments, the at least one reagent may be cells of at least one bacterial strain, an enzyme, or an enzyme substrate.

The invention also provides methods for detecting the presence or amount of biological material in a sample. In one aspect, the invention provides a method of manufacturing a device useful for detecting an analyte(s) or microorganism(s) in a test solution, the method comprising the steps of 1) providing a material capable of absorbing a predetermined 'volume of liquid per amount of material; 2) preparing at least one reagent island from the material; 3) combining the material with a means for detecting the presence or amount of the analyte or microorganism; and 4) securing the reagent island to a substantially hydrophobic support structure.

In various embodiments, the preparing step may comprise preparing multiple reagent islands. In another embodiment, the reagent islands may be capable of absorbing a predetermined volume of solution.

In another aspect, the invention provides a method of detecting an analyte or microorganism in a test sample comprising the steps of I) contacting the test sample with at least one reagent island capable of absorbing a predetermined volume of test solution and immobilised on a support structure, and which comprises a means for determining the presence or absence of an analyte or microorganism positioned on or within the reagent island(s); 2) separating the test sample from the reagent island(s) after the reagent island(s) has absorbed a predetermined amount of sample; 3) subjecting the reagent island(s) to reaction parameters which allow development of the reagent and generation of a sensible signal; and 4) determining the presence or amount of an analyte or microorganism.

In another aspect, the invention provides a method for detecting an analyte or microorganism in a test sample which comprises the steps of 1) selecting a test solution for the detection of the analyte or microorganism; 2) providing a device which comprises a substantially hydrophobic support structure and at least one reagent island(s) immobilised on the support structure, and which is capable of absorbing a predetermined volume of the test solution and comprises a means for determining the presence or amount of the analyte(s) or microorganism(s); 3) contacting the device with the test solution for a time sufficient to allow the reagent island(s) to absorb the predetermined volume; and 4) allowing the means for determining the presence or amount of an analyte(s) or microorganism(s) to determine the presence or amount of the analyte(s) or microorganism. In one embodiment, the contacting step may involve introducing the device into the test solution and removing it from the test solution. In another embodiment, the providing step may comprise providing a determining means which comprises a reagent which produces a sensible signal that signifies the presence or amount of an analyte(s) or microorganism(s). In another embodiment, the allowing step may comprise subjecting the device to reaction parameters sufficient to allow development of the reagent. Another step may be added to the method comprising observing the determining means, or a step of determining the presence or amount of an analyte(s) or microorganism(s), or a step of determining the quantity of the analyte or microorganism.

Accordingly, in a first embodiment of the invention there is provided a device for determining the presence or amount of an analyte or microorganism in a test solution, said device comprising: a substantially hydrophobic support structure, at least one reagent island formed of a single layer hydrophilic pad immobilised on said support structure, said at least one reagent island being constructed to absorb a predetermined volume of the test solution, wherein the at least one reagent island comprises at least one reagent; and, means for determining the presence or absence of an analyte or microorganism positioned on or within said reagent island.

According to a second embodiment of the invention there is provided a device for determination of the presence or amount of a microorganism or an analyte in a sample, said device comprising a solid, substantially hydrophobic support structure comprising at least one surface, said at least one surface defining a plurality of discrete reagent islands formed of single layer hydrophilic pads, each of

said reagent island being constructed to absorb a predetermined volume of test solution and being adapted to hold an aliquot of liquid and being sized, shaped, and formed of a material suitable to hold said aliquot within said reagent islands at least one of said reagent islands containing at least one reagent for the detection of a microorganism or analyte such that said device will not provide a positive response for an analyte or microorganism in the absence of a microorganism or analyte present in a sample applied to said device.

According to a third embodiment of the invention there is provided a method of manufacturing a device useful for detecting an analyte or microorganism in a test solution, said method comprising the steps of: providing a hydrophilic material constructed to absorb a predetermined volume of liquid per amount of the material; preparing at least one reagent island formed of a single layer from said material; combining said material with a means for detecting the presence or amount of an analyte or microorganism; and, securing said island to a substantially hydrophobic support structure.

According to a fourth embodiment of the invention there is provided a method of detecting the presence or amount of an analyte or microorganism in a test solution comprising the steps of: contacting a test solution with at least one reagent island formed of a single layer hydrophilic pad immobilised on a substantially hydrophobic support structure, said at least one reagent island being constructed to absorb a predetermined volume of test solution and including means for determining the presence or amount of an analyte or microorganism positioned on or within said reagent island; separating the test solution from said reagent island immobilised on a support structure after said reagent island has absorbed a predetermined amount of test sample; subjecting said reagent island to reaction parameters which allow development of the reagent whereby a sensible signal is provided; and determining the presence or amount of an analyte or microorganism.

According to a fifth embodiment of the invention there is provided a method for the detection of the presence or amount of an analyte or microorganism in a test solution, said method comprising the steps of: selecting a test solution for the detection of the presence or amount of an analyte or microorganism; providing a device comprising a substantially hydrophobic support structure and at least one reagent island formed of a single layer hydrophilic pad immobilised on said support structure, said reagent island being constructed to absorb a predetermined volume of test solution, said reagent island including means for determining the presence or amount of an analyte or microorganism; contacting said device with a test solution for a time sufficient to allow said reagent island to absorb said predetermined volume; and, allowing said means for determining the presence or amount of an analyte or microorganism to determine the presence or amount of said analyte or microorganism.

According to a sixth embodiment of the invention there is provided a device useful for detecting an analyte or microorganism in a test solution, said device manufactured according to the method of the third embodiment.

Brief Description of the Drawings

Fig. 1 is a plan view of a first embodiment of the present invention employable as a single assay dip stick device;

Fig. 2 is a side view of Fig. 1;

Fig. 3 is a second embodiment of the device of the present invention employable as dip stick assay device having a multiple reagents islands;

Fig. 4 shows the device of Fig. 3 in which multiple assays have been performed;

Fig. 5 is a perspective view of the third embodiment of the device of the present invention in which a dip stick assay device having two planes with multiple reagent islands thereon is removably insertable in a tube with a cap thereon;

Fig. 6 is a perspective view of a fourth embodiment of the device of the present invention in which a plurality of reagent islands are located in more than one plane on a stick and are removably locatable in a tube having a removable cap thereon; and

Fig. 7 is a perspective view of a fifth embodiment of the device of the present invention in which a laboratory pipette contains an elongate block having a plurality of reagent islands on one or more sides thereof.

Description of the Preferred Embodiments

Definitions

"Microorganism" - microorganisms means any microbe, including bacteria, fungi, or protists.

"Bacteria" - all organisms belonging to the Kingdom Monera.

"Biological Material" - any material derived from a biological organism.

"Target Organisms" - any "Microorganism" preferably but not limited to *E. coli*, yeast and/or mould.

"Cell" - any cell found in a plant, animal, bacteria, or any other living organism.

"Analyte" - any atom or molecule or ion which is involved in cellular metabolism in a living organism.

"Target Analyte" - any "Analyte", preferably but not limited to metabolites and/or enzyme activity.

"Proteinaceous Material" - proteins, peptides, enzymes, or amino acids. "Hydrophobic" - having a sufficient degree of hydrophobicity to prevent "crosstalk" or bridging of liquids, such as samples or reagents, or adjacent incubation cells, wells or islands.

Structure

The present invention provides a simpler method for accurate quantification of the number of microorganisms in a sample, or for quantification of any other type of analyte, such as discrete particulate biological material within a sample. Such biological materials include fungi or other living organisms, as well as aggregates of proteins, such as enzymes, or even co-factors, using reaction mixtures well known to those in the art. The invention generally makes use of a novel article which is designed to take-up and hold a pre-selected volume of a liquid sample and to provide a reagent or reagents to such sample volume. In preferred embodiments, the preselected volume is divided into a plurality of sample aliquots. The plurality of sample aliquots may be all of the same volume or may be in sets of aliquots, where each set contains aliquots of different volumes.

The device used is generally in the form of a substantially hydrophobic support structure with at least one reagent island mobilised on the support structure that is capable of absorbing a pre determined volume of test solution. The device is constructed to contain at least one reagent provided that at least one reagent island and preferably to a plurality or all of the individual reagent islands. Because the reagent islands contain the reagent or reagents, the reagent(s) is/are present on the device prior to introduction of a sample. For example, such a reagent may be a specific growth medium for bacteria. The provision of the reagent or reagents within the device eliminates the need for a tester to prepare separately the reagent and then add it to a sample or to the device. In addition, in many applications, the construction of the device is preferably arranged so that no pipetting is

required, the device is simply dipped into the sample, or an approximate quantity of the liquefied sample is poured on to the reagent islands, and a designed volume of sample is retained within the reagent islands. The device therefore provides an auto-dispensing function.

Each reagent island to which a reagent or reagents is provided may receive the same or different reagents. Thus, where a plurality of reagents is used, separate reagent islands may receive different single reagents, different combinations of reagents, or combinations of these possibilities. Specifically, the method of this invention can be used to take the place of a Petri dish. Specifically, the method of this invention can be used to replace those existing tests that are generally run on Petri dishes to score the number of microbial colonies. Since discrete aliquots of the sample are provided in the device, one of ordinary skill in the art need only score the number of positive reagent islands in the device to define the quantity of biological material within the original sample, as with the MPN test discussed above.

As noted above, the biological material that can be detected is any material that forms a discrete particle, such as a microorganism, which may be quantified by determining the presence or absence of such a biological material within each well of the incubation plate. The sample may be any biological sample or environmental sample such as waste water, food, a surface swab, or swabs from other surfaces, such as a throat, or other samples well known to those in the art. This sample may be a liquid sample, or may be dissolved in a liquid to form the liquefied sample. Thus, the term "liquefying" refers to providing the sample in a liquid that can be rapidly aliquotted within the device.

This invention provides an extremely useful device and method which allows unskilled personnel to rapidly determine the quantity of biological material within a sample. Since the sample is readily liquefied by people without significant training in microbiology, and the materials for any specific tests can be provided by the manufacturer, such people can readily perform the tests with accuracy. The device is generally provided in the sterile form so that no inappropriate detection of biological material can occur.

While it is known to provide plastic containers which can hold liquid within a plurality of recesses, this device and method provides an automatic aliquotting (or auto-aliquotting) method in which the steps to be performed by the tester are reduced and simplified by removing the requirement for manual addition of a reagent and preferably also eliminating the need for pipetting a sample or other liquid onto the plate. This is an improvement over the existing products used to detect and quantify microorganisms because the potential for contamination of the reagent or errors in dilution are reduced.

The present device can be used particularly in food analysis and in testing of clinical samples. The separation of the reagent islands of the present device prevents crosstalk or contamination between each aliquot. Because of this, many of the tests can be performed by observing fluorescence (which is not readily performed in an agar-containing Petri dish). The device is particularly useful when there is a large quantity of microorganisms present in a sample, such as more than one organism per 1mL or per 10mL.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Dip Stick Testing Device For Microorganism

Detection and Enumeration

Next referring to Figs. 1-7, the concept is to use small individual absorbent materials which contain lyophilised or "dip-dried" reagent in a setting that allows automatic sample distribution and target microorganism enumeration.

A number of reagent-containing absorbent (hydrophilic;) materials ("reagent islands") are immobilised onto a support structure made of a hydrophobic material to form individual reagent islands. The reagent islands may be embedded in the support structure, for example. However, this should not be construed as limiting as any form of securing the reagent island to the support structure may be used as long as it does not interfere with the assay. The liquid absorbing rate of each reagent island in this embodiment is the same because the reagent islands are made of the same material and have the same size. Further configuration can be made so there are two or more groups of reagent islands in different sizes to form a setting that can be used to achieve higher microorganism quantification without serial dilution based on the principles of the Most Probable Number (MPN) method.

In a preferred embodiment, the reagent is one developed to test for the presence of target microorganisms in a sample using, but not limited to, defined substrate technology (DST) and/or Multiple Enzyme Technology (MET). A positive detection of target microorganisms in such reagents will cause a colour change of the reagent and/or cause the emission of a fluorescent signal from the reagent when viewing under a long-wave UV lamp. Examples of such reagents are Colilert®, Enterolert®, Simplate TPC™, and Simplate CEC™.

Sample inoculum is pre-calculated based on the maximum absorbing rate (saturation point) of a reagent island and the total number of reagent islands on a device. When the predetermined amount of liquid sample (ie. water, milk, juice and food homogenous) is inoculated on to this device, each reagent island absorbs the same amount of sample. Sample can be distributed to each reagent island by simply moving the device in back-forth or circular motion. The area between each reagent island is hydrophobic and when each reagent island absorbs sample to its saturation point, there will be no sample left between the reagent islands and cross contamination between reagent island will be prevented. Liquid sample absorbed by the reagent islands also re-hydrolyses the reagent in the reagent islands to support the growth of microorganisms in the sample.

After incubating the sample-containing device at a pre-determined temperature, reagent islands containing target microorganisms from the sample will have a change in colour and/or emit fluorescent signals (positive); reagent islands lacking target microorganisms from the sample will exhibit no colour change and emit no fluorescent signals (a negative result). Target microorganism concentration in the sample under testing can be calculated based on the number of positive and negative reagent islands observed using the Most Probable Numbers (MPN) method.

Another application of this concept is to have reagent islands lyophilised with different types of reagents. Each reagent is designed to test one specific aspect of a sample, such as microorganisms, chemicals, or any other detectable analyte of interest. Combination of these reagent islands in the

same device forms a test kit providing a one-step test for multiple chemicals, analytes, or biological materials.

Specifically referring to Figs. 1 and 2, there is shown another aspect of the invention which comprises a dip stick 100 containing a reagent island 101. The stick will generally be made of plastic, but its composition is not critical and may be constructed of any hydrophobic material which will not leach into the test sample or interfere with the assay. Figures 8 and 9 depict the stick with a single reagent island.

Figure 3 depicts a preferred embodiment of the dip stick containing multiple reagent islands 102. Zones of reagent islands may be set up on the dip stick with each zone having reagent islands that contain different reagents or different combinations of reagents than the other zones, thereby enabling multiple assays to be performed on a single dip stick, each zone testing for a different analyte or microorganism. Figure 4 depicts the dip stick assay device of Fig. 3 which has undergone the assay procedure and illustrates reagent islands showing both positive results (dark reagent islands) and negative results (white reagent islands).

Figure 5 shows a preferred embodiment of this aspect of the invention. There is shown the dip stick 100 with multiple reagent islands 101 which is folded and inserted into a test tube 103. In this embodiment, the test tube also has a cap 104 which further protects the reagent islands from environmental factors. Of course, the device can be placed in other types of containers such as a plastic sleeve or any container which serves to protect the device. The reagent placed on the reagent island can be any reagent or combination of reagents which can be distributed onto or into the material of the reagent island.

Application of Test Sample to Dip Stick Device

The methods of applying sample to the device are varied. The dip stick can be dipped into the test sample, and left in contact long enough for the reagent islands to absorb a predetermined amount of fluid. This will generally be less than 3s, but may be more depending on what material the reagent islands are constructed from. In a preferred embodiment, the reagent islands are constructed from cellulose. However, they may also be constructed of any material which is capable of absorbing and holding a volume of fluid. The test sample may also be pipetted from the test solution onto the reagent islands with a transfer pipette.

The support structure may also comprise a box made of plastic or other suitable hydrophobic material. The reagent islands may be positioned inside of the box and the box can be opened and test solution applied using a pipette or by pouring, or any suitable means. The box may have a lid to further protect the device secured thereon or as a separate piece.

Referring now to Figure 6, the support structure may also comprise a centre support 104 to which "leaves" 105 are attached and support the reagent islands 106. The leaves can be arranged in a three-dimensional structure as depicted in Figure 6, thereby maximising the number of leaves which can be accommodated on one device. The device can be placed into a circular container 108 and a cap 107 placed thereon to provide Further protection for the device before, during, and after development of the assay.

Pipette Device

Referring now to Figure 7, in another aspect, the invention provides a device comprising a support structure 109 which is contained within a laboratory pipette 111. Multiple reagent islands 110 are contained on or within the support structure 109. Preferably, the support structure 109 has more than one side with reagent islands 110 thereon. The support structure 109 is most preferably formed of a synthetic polymer, such as a plastic, and is inserted into a laboratory pipette 111 which is preferably comprised of glass or plastic and has a tapered end for sample uptake. Sample may be merely drawn into the pipette by an aspiration apparatus known in the art, allowed to remain for a time sufficient for the reagent islands to absorb a predetermined volume of test sample, and expelled from the device. The device is then incubated for a time sufficient to develop the assay, and the results determined. The pipette itself comprises both a simple means of applying sample to the test device, as well as a protective function.

Example 1

A Bacterial Detection System for coliforms and *E. coli* in Milk Using the Dip Stick Device

The following is an example of how the present invention provides a method of coliform detection in milk that is easy, does not comprise many steps, and provides results that are easy to interpret. The assay was conducted using a test device with multiple reagent islands, each of which absorbed 0.033mL of milk. The milk was added to the test device and was auto-aliquotted to all the reagent islands. The sample was incubated for 24h and provided the number of coliforms present by examining how many of the reagent islands change colour. The number of coliforms present is determined based on the MPN chart. The data obtained from the assay are set out in Table 1. The flexibility of the test device's uptake ability is emphasised since volume uptake can be easily adjusted by adjusting the number and/or sizes of the reagent islands. The device also has the capability to have multiple tests on the same device, by impregnating reagent islands with different media.

Table 1

Milk Samples	Dipstick Device (cfu/mL)	Milk Samples	Dipstick Device (cfu/mL)	Milk Samples	Dipstick Device (cfu/mL)
#71 97A	10.3	#7197B	11.8	#7197C	16.4
#710	56.3	#713	18	#3B1	0
#3B2	37	#2B2	0	#2B1	9.3
#618	69.1	#618A	All +	#622A	12
#622B	36	#622C	3	#7697A	56
#7697B	22	#7697C	16	#7697D	31
#811	3	#815	16	#813	6.2
Mean	23.41	Std. Deviation	20.75		

The invention has been described in detail with particular reference to certain preferred embodiments thereof, but it will be understood that variations and modifications can be effected within the spirit and scope of the invention.

The claims defining the invention are as follows:

1. A device for determining the presence or amount of an analyte or microorganism in a test solution, said device comprising: a substantially hydrophobic support structure, at least one reagent island formed of a single layer hydrophilic pad immobilised on said support structure, said at least one reagent island being constructed to absorb a predetermined volume of the test solution, wherein the at least one reagent island comprises at least one reagent; and, means for determining the presence or absence of an analyte or microorganism positioned on or within said reagent island.

2. The device of claim 1, further comprising a plurality of reagent islands immobilised on said support structure.

3. The device of claim 1, wherein said means for determining the presence or amount of an analyte or microorganism is a powder incorporated onto or within said reagent island.

4. The device of claim 1, wherein said means for determining the presence or amount of an analyte or microorganism comprises a reagent that leads to production of a sensible signal when a target analyte or microorganism is present in a sample.

5. The device of claim 1, wherein said at least one reagent island is comprised of cellulose.

6. The device of claim 1, wherein said substantially hydrophobic support structure is comprised of a plastic.

7. The device of claim 1, wherein said at least one reagent comprises cells of at least one bacterial strain.

8. The device of claim 1, further comprising a plurality of different reagent islands.

9. The device of claim 1, wherein said device comprises a plurality of reagent islands and said at least one reagent comprises different combinations of reagents located in different ones of said reagent islands to provide a plurality of different assays in said plate.

10. A device for determination of the presence or amount of a microorganism or an analyte in a sample, said device comprising a solid, substantially hydrophobic support structure comprising at least one surface, said at least one surface defining a plurality of discrete reagent islands formed of single layer hydrophilic pads, each of said reagent islands being constructed to absorb a predetermined volume of test solution and being adapted to hold an aliquot of liquid and being sized, shaped, and formed of a material suitable to hold said aliquot within said reagent islands, at least one of said reagent islands containing at least one reagent for the detection of a microorganism or analyte such that said device will not provide a positive response for an analyte or microorganism in the absence of a microorganism or analyte present in a sample applied to said device.

11. The device of claim 10, wherein said reagent islands together take up a preselected total volume.

12. The device of claim 10, wherein said at least one reagent comprises a plurality of reagents located in separate islands.

13. The device of claim 10, wherein said reagent islands are arranged in a plurality of zones, wherein each said zone provides a separate assay.

14. The device of claim 10, wherein said reagent islands are adapted to retain aliquots of liquid sample by surface tension.

15. The device of claim 10, wherein said reagent islands are adapted to retain aliquots of liquid sample by absorption.

16. The device of claim 10, wherein said reagent islands are designed and adapted to each take up equal volumes.

17. The device of claim 10, wherein said reagent islands are located on more than one surface of said solid support structure.

18. The device of claim 10, wherein said device is a plate.

19. The device of claim 10, wherein said support structure is an elongate strip.

20. The device of claim 10, wherein said support structure is constructed of a hydrophobic material.

21. The device of claim 10, wherein said at least one reagent is selected from the group consisting of growth medium, bacterial cells, enzyme, and enzyme substrate.

22. The device of claim 21, wherein said growth medium is an indicator growth medium.

23. A method of manufacturing a device useful for detecting an analyte or microorganism in a test solution, said method comprising the steps of: providing a hydrophilic material constructed to absorb a predetermined volume of liquid per amount of the material; preparing at least one reagent island formed of a single layer from said material; combining said material with a means for detecting the presence or amount of an analyte or microorganism; and, securing said island to a substantially hydrophobic support structure.

24. A method of detecting the presence or amount of an analyte or microorganism in a test solution comprising the steps of: contacting a test solution with at least one reagent island formed of a single layer hydrophilic pad immobilised on a substantially hydrophobic support structure, said at least one reagent island being constructed to absorb a predetermined volume of test solution and including means for determining the presence or amount of an analyte or microorganism positioned on or within said reagent island; separating the test solution from said reagent island immobilised on a support structure after said reagent island has absorbed a predetermined amount of test sample; subjecting said reagent island to reaction parameters which allow development of the reagent whereby a sensible signal is provided; and determining the presence or amount of an analyte or microorganism.

25. A method for the detection of the presence or amount of an analyte or microorganism in a test solution, said method comprising the steps of: selecting a test solution for the detection of the presence or amount of an analyte or microorganism; providing a device comprising a substantially hydrophobic support structure and at least one reagent island formed of a single layer hydrophilic pad immobilised on said support structure, said reagent island being constructed to absorb a predetermined volume of test solution, said reagent island including means for determining the presence or amount of an analyte or microorganism; contacting said device with a test solution for a time sufficient to allow said reagent island to absorb said predetermined volume; and, allowing said means for determining the presence or amount of an analyte or microorganism to determine the presence or amount of said analyte or microorganism.

26. A device for determining the presence or amount of an analyte or microorganism in a test solution, said device being substantially as hereinbefore described with reference to the accompanying drawings.

27. A device for determination of the presence or amount of a microorganism or an analyte in a sample, said device being substantially as hereinbefore described with reference to the accompanying drawings.

28. A method of manufacturing a device useful for detecting an analyte or microorganism in a test solution, said method being substantially as hereinbefore described with reference to the accompanying drawings.

29. A method of detecting the presence or amount of an analyte or microorganism in a test solution, said method being substantially as hereinbefore described with reference to the example.

30. A device useful for detecting an analyte or microorganism in a test solution, said device manufactured according to the method of claim 23 or 28.

Dated 15 October 2002
IDEXX LABORATORIES, INC.

Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

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FIG. 8

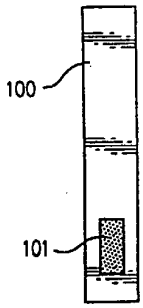

FIG. 9

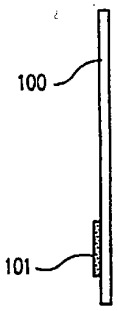

FIG. 10

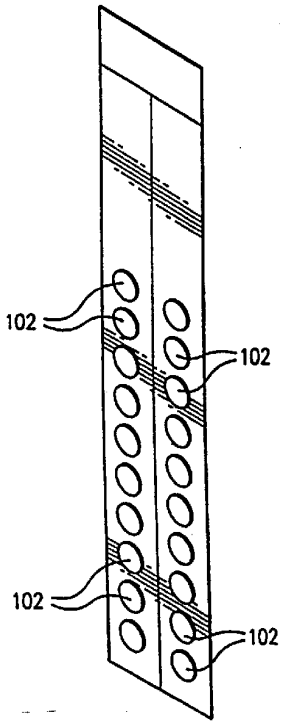

FIG. 11

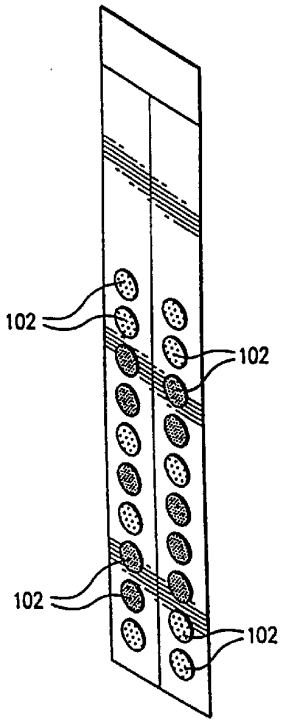

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FIG. 12

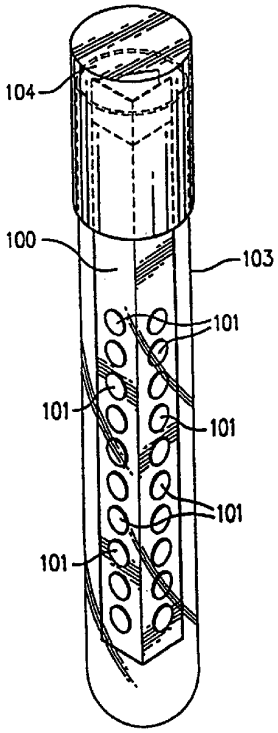

FIG. 13

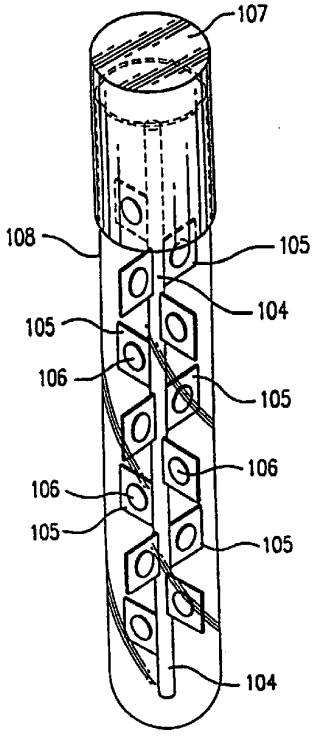

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FIG. 14