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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : G01N 33/569 — A1

(11) International Publication Number: **WO 98/32020**

(43) International Publication Date: 23 July 1998 (23.07.98)

(21) International Application Number: PCT/US97/04289

(22) International Filing Date: 25 March 1997 (25.03.97)

(30) Priority Data:
08/785,833 15 January 1997 (15.01.97) US

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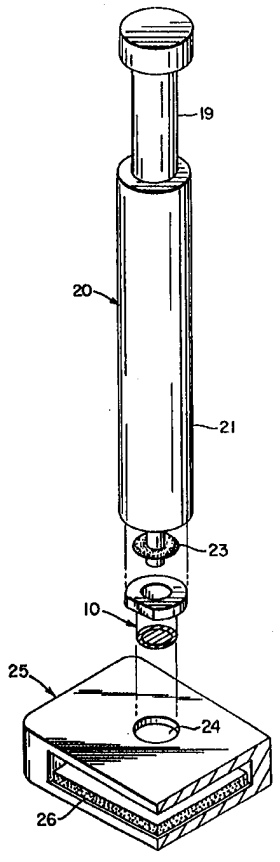
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: METHOD FOR DETECTION OF CONTAMINANTS

(57) Abstract

A method and device for determining the presence and concentration of total microbial contamination or the presence and concentration of a specific microbial species is described. The method consists of a means of collection of the microbes from an air, liquid, surface or other sample and suspending them in fluid phase (3). An aliquot of the fluid phase (3) is introduced into a disposable test device which allows filtration of the sample to concentrate the microbes and to remove extraneous substances including somatic cells, and concentration of the microbes. The total concentration of microbes is determined by adding a somatic and bacterial releasing reagent to a disposable test device (25) which comprises a membrane (26) containing the luminescent reagents luciferin and luciferase, and introducing the disposable test device (25) into a luminometer that can read the luminescence from the underside.



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METHOD FOR DETECTION OF CONTAMINANTS

Field of the Invention

The present invention relates to a method for detecting the presence and determining the quantity of contaminants. More particularly, the invention relates to a method for rapidly determining the total microbial contamination or for determining the presence and quantity of specific microbial or chemical contaminant present on a wide variety of surfaces including surfaces of meat carcasses or other food, surfaces of equipment, surfaces where food is being processed or prepared, and surfaces of equipment, gloves and materials in medical situations. Airborne and liquid contaminants are also detectable. Furthermore, the invention relates to a method for determining the total microbial or specific microbial or chemical contamination by bioluminescence or chemiluminescence.

Background of the Invention

Microbial contamination is a significant cause of morbidity and mortality. Rapid and routine procedures for quantitative determination of bacteria, particularly those present on surfaces, is frequently of vital importance, particularly in food processing and in hospitals. Food poisoning is often a result of microbial contamination of meat or food that occurs during processing. Contamination can be spread through contact of food with surfaces. In addition, spread of disease in hospitals and other facilities often occurs as a result of passage of infectious microbes on the surface of clothes or equipment, or through the air, water or other liquids.

A key feature of these applications is the requirement for rapid testing within minutes, a method that will overcome the potential contaminants from a

variety of surfaces, liquids, and air, a requirement for no cross-over in the results from one test to a second, and a need for both general and specific testing for microbes. There should be the ability to test for contamination by both microbial counts and the ability to test for the presence of specific microbes.

Various methods have been utilized to measure microbial contamination on surfaces. Traditional procedures for assaying bacteria on surfaces are based on swabbing the surface followed by a culture of the swab for 24 to 48 hours in or on media that supports the growth of microbial species. The cultures are observed manually or with automated instrumentation to determine the number of colonies that have formed as an indicator of the number of microbes initially present on the surface. The disadvantages of this methodology are long assay times, requirements for specially trained personnel, and possible inadequate identification of the presence of certain potentially pathogenic microbes whose growth is not supported by the specific media or environment. In particular, it may be difficult to detect fungal contamination by this method. In addition, in many of the potential applications, the method does not give results in the time frame required for effective response.

Luminescent reactions have been utilized in various forms to detect bacteria in fluids and in processed materials. In particular, bioluminescent reactions based on the reaction of adenosine triphosphate (ATP) with luciferin in the presence of the enzyme luciferase to produce light (the "firefly" reaction) have been utilized. Since ATP is present in all living cells including all microbial cells, this method can be used in a rapid assay to obtain a quantitative estimate of the number of living cells in a sample. Early discourses on

the nature of the reaction, the history of its discovery, and its general area of applicability are provided by E.N. Harvey (1957), A History of Luminescence: From the Earliest Times Until 1900, Amer. Phil. Soc., Philadelphia PA and W.D. McElroy and B.L. Strehler (1949) Arch. Biochem. Biophys. 22:420-433. Alternatively, chemiluminescent detection by isoluminol or similar compounds has been used. This method is based on the detection of iron-containing substances in microbes.

Test procedures exemplifying the use of bioluminescent reactions for bacterial determinations and, specialized instrumentation for measurement of the associated light emission, are known and have been disclosed. Plakas (U.S. patent numbers 4,013,418,4,144,134, and 4,283,490) teaches a bioluminescent assay for the detection of bacteria in a sample including the steps of lysing non-bacterial cells, effecting filtration by positive pressure, washing, lysing bacterial cells and detecting ATP released with a luciferin/luciferase/Mg2+ regent. The art in this patent does not deal with the specific problems associated with collection of material from a surface or with the detection of specific bacteria. No issue of the timing is mentioned and the invention as disclosed would require significant time.

Chappelle in U.S. Patent 4,385,112 discloses a method for detection of water based bacteria based on bioluminescence. This test requires several hours to perform and is specifically addressed to the detection of total bacterial content in water.

Clendenning in his U.S. Patent 3,933,592 discusses a method for bioluminescent detection of microbial contamination and in the examples refers to performing the procedure in less than 2 minutes. The procedure does not involve pre-treatment phases and the removal of

somatic cell ATP.

Aegidius (U.S. Patent 5,258,285) discloses a method for detection of bacterial concentration in a sample that utilizes a filtration step, a washing step to remove extraneous material including somatic cell ATP, establishing an extraction chamber in which luciferin/luciferase/Mg2+ is added and the reaction measured. This method does not mention time. In addition, it utilizes separate chambers for washing, extracting the bacterial ATP, and measuring the reaction. This may potentially result in decreased sensitivity due to loss of the material in the process of transferring the solution from chamber to chamber. Further, the method does not describe a means of collecting a sample from a surface.

Detection of bacteria on surfaces poses additional issues not addressed in these previous methods. First and foremost is the method for collecting a sample to be compatible with these test devices and materials. The method must effectively retrieve the bacteria from the surface and result in a liquid suspension of the microbes.

A second issue of main concern is that surfaces or other areas being monitored often are contaminated with materials that may interfere with the detection of the microbes. Interfering materials that can be present on surfaces, air, or liquids are somatic cells, from the food itself and including both animal and plant cells, or from the hands of an individual in contact with the surface. Since all living organisms including somatic cells contain ATP, the presence of these cells can mask or alter the reading obtained.

An additional source of interfering substances are those that interfere with the light producing reaction itself. These substances include a wide range of

chemicals such as chlorine, oxidizing agents, free ATP, heavy metals, and other chemicals. As some of these chemicals are used for disinfecting of a surface, it is obvious that a reliable method for analyzing microbial contamination must include a means of eliminating these substances from the sample.

It is a further requirement in many cases in the food processing and hospital applications that the method for monitoring for microbial contamination of surfaces be rapid. For example, in the processing of beef carcasses, the carcasses are processed on a line and any testing of the material for microbial contamination must be performed within the time frame required for the carcass to move to further processing.

Previously disclosed luminescence based methodologies for microbial detection have not included any means for directly processing a sample from a surface, solid, or gas and making a liquid suspension for testing or directly from an air or liquid sample. Further, the processes have required multiple devices or chambers for containment, filtration, and measurement of the reaction. Finally, the processes have not incorporated a disposable device that allows for minimizing cross-contaminations. Finally, in those assays for detecting specifically microbial ATP and other specific contaminants, previously disclosed inventions have relatively long time frames which are not consistent with on-line processing, quality control, and immediate verification of results.

Summary of the Invention

The present invention is a method and device for determining the presence and/or concentration of total microbial concentration or the presence and/or concentration of a specific target analyte. In one embodiment of the invention, the method comprises

collecting a surface sample by wiping a circumscribed area of a surface in a prescribed fashion using a collection apparatus means comprised of an absorbent or adsorbent material. The collection apparatus means is placed into a container containing a fluid and agitated to release the surface contaminants from the collection apparatus means into the fluid. The collection apparatus means can be in the form of a sponge or a swab and the container can be a bag, tube, or small cup. The fluid may be collected directly, by preparing a liquid suspension from a solid sample, by passing a gas through a collection fluid, or by directly collecting the air on the test device. An aliquot of the fluid phase is subsequently transferred to a disposable test device comprised of a translucent hollow cylinder, open on the top and having a porous filter attached on the bottom. The fluid phase is filtered through the disposable test device comprised of a translucent hollow cylinder, open on the top and having a porous filter attached on the bottom. The fluid phase is filtered through the disposable test device by applying either positive or negative pressure resulting in retention of microbes or target analytes on the surface of the filter. The filtration process results in the concentration of analyte and the removal of any interfering substances from the collectate prior to testing, such as inhibitors or any nonspecific materials to maximize test sensitivity and specificity. The filter retentate can be washed by adding appropriate wash solution and reapplying appropriate pressure to force the fluid phase through the filter.

Another feature of the present invention is that the retentate captured on the filter of the disposable test device can be assayed by a chemiluminescent or bioluminescent test method. The final step of the test

method comprises the addition of luminescent substrate to the retentate resulting in the chemiluminescent reaction and measuring the light output from said chemiluminescent reaction by using a photometer that accommodates the disposable device.

In another embodiment of the invention, a liquid or air sample may be tested to determine the level of contamination. The use of a liquid sample eliminates the need to wipe a surface and/or wash the sample in a fluid. Similarly, an air sample may be tested, using conventional collection means, without the need to swab a surface of any sort.

The present invention allows for a contaminant to be identified and/or concentration determined in less than 1 hour from time of collection to end result, and generally in less than 5 minutes.

More specifically, the present invention comprises a method for performing chemiluminescent assays such as bioluminescent assays for ATP, chemiluminescent immunoassay or DNA probe assays. One embodiment of the present invention is a method for determining the total microbial contamination comprising the steps of:

a) collecting a sample with a collection means;

b) agitating the collection apparatus means with a fluid phase to dislodge the contaminants into a fluid phase, the fluid phase the becoming the collectate;

c) placing an aliquot of the collectate into a disposable test device;

d) adding a washing/lysing reagent that lyses any somatic cells present in the aliquot;

e) applying a positive pressure to the top of the disposable test device or negative pressure to the bottom of the disposable test device to eliminate the liquid phase containing free ATP

and any chemical inhibitors as well as concentrating the bacteria at the interface;

f) adding a bacterial lysing reagent that perforates the bacterial cell walls allowing the release of microbial ATP;

g) adding ATP free luciferin and luciferase reagent; and

h) determining the amount of ATP present by measuring the light emitted through translucent sides of said disposable test device.

The choice of collection fluids is well known to those skilled in the art. Generally, the fluid is comprised of a detergent, salt, or buffer or any combination thereof that maintains the integrity of the microbial cell walls. A fluid consisting of 0.15M sodium chloride containing 0.5% Tween 20 detergent is one such choice. It is possible to use other formulations including phosphate or HEPES buffered saline and other detergents including zwitterionic detergents and non-ionic detergents.

It will be obvious to a person skilled in the art that mixing of reactant(s) could be achieved in any of the steps through the use of a micropipette. The detection method of this invention specifically allows for both the concentration of analyte and any resulting chemiluminescent reaction caused by the presence of said analyte to occur within the chamber of the disposable test device. An added feature of the disposable test device is that the diameter of the filter is from 0.5 to 2.0 cm, preferably about 1.0 cm, so that the volume of bioluminescent or chemiluminescent substrate solution is minimized to maximize signal output to the photodetector means. The final volume of the substrate should be between 20 μl to 1000 μl, most preferably about 60 μl to

100 µl. The disposable test device can be inserted into a complementary device comprising a larger (liquid tight) chamber, comprising at least two components, that can house the disposable test device and through which a volume of collectate greater than 500 µl can pass through the filter under positive or negative pressure and retain the microbes or the analytes of interest onto the surface of the filter. For example, the disposable test device can be inserted into the lower chamber of the two component device, with the lower chamber having an outflow for the filtrate to which is attached a removable upper chamber of the two component device. The upper chamber comprises a liquid tight seal to the lower compartment and has an intake fitting. The intake fitting can be configured for a complementary Luer tip fitting for attachment of a Luer tipped syringe. The syringe may include at least one series of prefilter(s) to remove any larger debris from entering the filter of the disposable test device. At completion of passing the collectate through the filter of the disposable test device, the two component device can be opened, and the disposable test device physically removed. The disposable test device then contains the retentate from a large volume of collectate (i.e. 50 ml). The filtration of the large volume of collectate enables increased sensitivity for analyte detection of the collectate fluid. The disposable test device is then processed as previously described.

The luciferin/luciferase chemiluminescent reactions for ATP are well known. Other chemiluminescent reactions employing bacterial luciferase reactions, or luminols for total microbial determinations, can be easily adapted to the methods and devices of the present invention.

The invention further concerns a detection method in which the presence and quantity of specific microbes on a surface can be detected in a time frame less than one hour. The method comprises the steps of:

a) providing a clean disposable test device comprising an open top, translucent sides and a porous filter attached to the bottom side;

b) adding an aliquot of collectate, said collectate being that described as above;

c) adding an appropriate wash solution comprised of detergent, or buffered salts or a combination thereof;

d) applying positive pressure to the top of the disposable test device, or negative pressure to the bottom of the disposable test device to remove fluid from the device and deposit microbes or target analytes directly or indirectly onto the surface of the porous filter;

e) adding a specific labeled antibody directed against the specific microbes to be detected and incubating for an appropriate period of time;

f) applying positive pressure to the top of the disposable test device, or negative pressure to the bottom of the disposable test device to remove fluid containing unreacted enzyme labeled antibody from the device;

g) adding an appropriate wash solution comprised of detergent and buffered salts;

h) applying positive pressure to the top of the disposable test device, or negative pressure to the bottom of the disposable test device to further remove fluid containing unreacted labeled antibody from the device; and

i) adding a chemiluminescent substrate and determining the amount of light emitted by the chemiluminescent substrate using a photometer that accommodates the disposable test device in a manner which allows its precise positioning with respect to the surface of the photosensor and which precludes any possible loss of the final reaction mixture during and after the measurement cycle.

The method described above can also be modified by

adding capture particles such as latex spheres coated with a binder such as specific antibodies to antigens or antigens to antibodies of the target microbe into the disposable test device prior to performing step (d). The method can also be modified so that capture particles and enzyme labeled antibodies and the collectate are all simultaneously reacted within the disposable test device, prior to performing step (f). The particles may also be coated with a specific antigen which binds a virus specific antibody. The particles are added with the collection fluid to said disposable test device in step (c). The detection reagent may be an enzyme conjugated to the binder, wherein the binder is an ATP enhanced antibody or an ATP encapsulated liposome bound to the binder and where the binder itself may be antibodies, antigens, lectins, DNA fragments, viruses, and combinations thereof. The enzyme may be a peroxidase, a phosphatase, an oxidase, a luciferase, or combinations thereof.

In yet another embodiment of the invention, all of the chemicals and solutions are in a disposable membrane device. Such a device is easier to use, particularly in the field, than the use of a disposable test device or large volume concentration device. Utilization of the membrane device significantly reduces the need for additional reaction reagents, thus resulting in a more accurate and mobile test system. The membrane device also allows for the processing of liquid and air samples directly on the membrane. Virtually all of the elements of the invention are essentially self contained in the disposable membrane device. The membrane device preferably comprises a hinged two sided plastic, cardboard, or paper support having a top and bottom section, and an absorbent pad or disk positioned on top of the inner side of the top section. On top of the absorbent disk is a glass filter membrane, which may be held in place by a plastic or paper rigid layer.

In this embodiment of the invention, the membrane device may serve as a collection device as well as a reaction system. For example, to estimate the concentration of bacteria in a given quantity of an air sample, the glass filter membrane portion of the ticket is inserted into a chamber through which a measured volume of air is passed and the air is allowed to impinge on the surface of the glass membrane. The device is removed and, after addition of a bacterial releasing agent to release the cellular ATP, the device is folded to initiate the reaction. In yet another embodiment of the invention, the bacterial releasing agent is bound to the glass filter membrane or other membranes of the device.

The bottom section of the membrane device preferably comprises a transparent window to which is affixed a semitransparent membrane (when wet) with immobilized luciferin-luciferase.

Various buffers for extracting antigens and washing immune complexes are well known to those skilled in the art.

Brief Description of the Drawings

Figure 1 is a side view of the collection apparatus means comprising a shaft, absorbent tip, and a container with fluid;

Figure 2 is an angular side view of the collection apparatus means comprising a sponge and a bag with fluid;

Figure 3 is a frontal view of a large volume concentrating apparatus;

Figure 4 is an exploded perspective view of a large volume concentrating apparatus;

Figure 5 is a cross-sectional side view of a negative pressure apparatus;

Figure 6 is an exploded perspective drawing of a positive pressure apparatus, disposable test device and holder with absorbent disk;

Figure 7 is a drawing of the disposable test device,

its respective positioning into the complementary draw slide and the relationship to the photosensor means;

Figure 8 is a graph of the total plate count obtained after 48 hours of incubation and the relative light units obtained from the 5 minute bioluminescent procedure outlined in the preferred embodiment with each data point representing a single beef carcass;

Figure 9 is a cross section side view of the membrane device;

Figure 10 is an angular overhead view of the membrane device; and

Figure 11 is a cross-sectional view of the membrane device positioned over the photomultiplier.

Detailed Description of the Preferred Embodiment

Figure 1 is a drawing of a collection apparatus means comprised of a shaft **1** and absorbent tip **2**. The absorbent tip **2** is wetted with an excess of collection fluid **3** and used to wipe a circumscribed area of a surface to be monitored. After wiping the area, the absorbent tip **2** is placed into a container **4** and agitated to release any of the absorbed bacteria into the collection fluid **3**.

Referring to **Figure 2**, the collection apparatus means may be comprised of a sponge **5**. The sponge **5** is wetted with collection fluid **3** and used to wipe a circumscribed area of to be monitored. After wiping the area, the sponge **5** is placed into a plastic bag **6** containing excess fluid and squeezed several times to release any of the absorbed bacteria into the collection fluid **3**. The volume of collectate fluid can vary, depending upon the size of the absorbent and area wiped. The collection fluid **3** is selected to ensure transfer of the microbial contaminants from the test surface to the collection device and then to a disposable test device. Generally, the pH of the collection fluid **3** is between 5 and 8, but preferably between 6.0 and 7.0, The collection fluid preferably contains salts such as sodium chloride

between 0.1M and 0.3M, preferably about 0.25M NaCl to ensure survival of bacteria. The collection fluid **3** should contain a detergent such as 0.05% Tween 20 to ensure that the bacteria are easily removed from the test surface and collection apparatus.

Referring to **Figure 3-6**, a large volume concentrating apparatus **7**, concentrates a quantity of collectate fluid into a disposable test device. An appropriate sized Luer-tipped syringe is attached to the inlet **8** of the large volume concentrating apparatus **7** and then positive pressure is applied to the syringe plunger causing the collectate fluid to flow out of the outlet **9**.

The collectate fluid flows through the filter bottom **11** of the disposable test device **10**. "O" rings **14** and **15** provide a leakproof seal. After completing the concentration of the collectate, upper compartment **13** is separated from the lower compartment **16** to expose the lip **12** of the disposable test device **10**. The disposable test device is then manually removed from the lower compartment **16**.

The bottom portion of the disposable test device is inserted into holes **18**. Appropriate volume of wash or somatic cell lysing solutions can be added and a vacuum can be applied to outlet **19** to remove fluid from the disposable test device **10**.

The positive pressure apparatus **20** is comprised of a plunger **(19)** and a barrel **21**, a disposable test device **10**, and device holder **25** is comprised of an absorbent pad or disk **26** to absorb the fluid waste. The disposable test device is inserted into holder **24**. An aliquot of collectate fluid (i.e. 50 to 100 μl) is added and an appropriate volume of wash or somatic cell lysing solutions can be added. The rubber seal **23** of the positive pressure device is positioned on top of the disposable test device **10**. Applying pressure to plunger **19** forces air through barrel **20** and out through outlet **22**, displacing the fluid which passes into the absorbent

disk **26**. Additional wash solution can be added and the process repeated.

The disposable test device **10**, its respective positioning **28** into the draw slide **27**, and the relationship to the photosensor means **30** is shown in Figure 7. The body of the disposable test device **10** is comprised of optically clear molded plastic material, such as polystyrene, which is capable of nearly complete transmission of light within a 500-600 nm wavelength range. Fused to the lower surface of the device is a semi-permeable membrane **11** which is characterized by its strength and lack of deformation under pressure, and a pore size distribution which insures surface retention of bacterial cells, while facilitating complete passage of any associated liquid phase during pressurization. This membrane must also have sufficient surface tension to retain the measurement solution even after wetting.

The draw slide is an integral part of a luminometer instrument. The draw slide is pulled out and the disposable test device is positioned into hole **28** so that a window to the translucent wall of the disposable test device is exposed to the photosensor means when the draw slide is returned to a complementary dark chamber of the luminometer.

In a general bacterial screen based on bioluminescence, after a microbial sample has been concentrated in the disposable test device, a bacteriolytic reagent is added to lyse the bacteria and free the ATP. An appropriate volume of luminescent substrate (i.e. luciferin-luciferase) is added to the disposable test device and the draw slide is returned to the dark chamber of the luminometer. Measurement of light emission is made by digitalizing or converting the electrical signal from the photosensor means to a number of relative light units. If the method is to be used to detect specific bacteria, a specific antibody conjugated to a chemiluminescent or enzyme probe is added. In the

preferred embodiment, the antibody is placed in the disposable test device and allowed to react for 10 minutes. Additional wash steps may be performed by adding a wash solution and evacuating the wash solution. A luminescent substrate solution is then added. In the preferred embodiment such substrate consists of a mixture of hydrogen peroxide and luminol. The draw slide is returned to the dark chamber of the luminometer. Measurement of light emission is made by digitalizing or converting the electrical signal from the photosensor means to a number of relative light units.

In another embodiment of the invention, all of the chemicals and solutions may be in a disposable membrane device **100**. As with the systems described above, all systems and procedures described below involve the detection and quantification of bacteria in samples which may also contain somatic cells, free ATP, and constituents such as chloride ions which are known to inhibit the luciferin-luciferase enzyme reaction.

The membrane device **100** preferably comprises a hinged two sided plastic, cardboard, or paper support **101** having a top section **102** and bottom section **103**. An absorbent pad **104** is positioned on top of the inner side **105** of the top section **104**. The absorbent pad **104** is comprised of a material made of cellulose. The material may be cotton, corn silk, possibly fiberglass, or other absorbent material. On top of absorbent disk **104** is a glass filter membrane **106**, which may be held in place by a plastic or paper rigid layer **107**.

The bottom section **103** of the membrane device **100** preferably comprises a transparent window **108** on the outer side **109** of the bottom section, and a luciferin-luciferase solution immobilized on the membrane disk **111**. The membrane disk fits in a hole **113** in the bottom section **103** of the device **100**.

In one embodiment of the invention, somatic or bacterial cell releasing agent may be incorporated into

the glass membrane **106** in much the same manner as the luciferin-luciferase solution is incorporated in to the membrane disks **111**.

To use the membrane device **100**, a sample volume of 25 μl, collected by normal means, is applied through a hole **110** in the rigid layer **107** onto the surface of the glass filter membrane **106**. The glass filter membrane **106** retains bacterial and somatic cells on the surface of the glass filter membrane **106** while fluids pass into the absorbent disk **104**.

In one embodiment of the invention, somatic cell releasing agent is then added onto the surface of the glass filter membrane **106**. The somatic cell releasing agent is added dropwise onto the surface of the glass membrane **106** so as to avoid flooding the membrane device and washing the cells out of the glass filter membrane **106**.

After the addition of the somatic cell releasing agent, the somatic cells have lysed and the released ATP from the somatic cells, along with free ATP and inhibitory materials which could have contaminated the results, are trapped in the absorbent pad. At this stage only bacterial cells are left intact on the surface of the glass filter membrane disk **106**. In another alternative approach, somatic releasing reagent may be placed onto the swab used to streak the surface area being tested. In yet another embodiment of the invention, the need to add somatic cell releasing agent to the test sample is eliminated when the somatic cell releasing agent has already been bound to the glass membrane **106** prior to use of the membrane device.

Next, 10 μl of bacterial releasing agent are applied to the glass filter membrane **106**, or the surface of a membrane **111** positioned on the inner side **112** of the bottom section **103** of the membrane device **100**. The membrane **111** contains immobilized luciferin-luciferase. The luciferin-luciferase may be either saturated

throughout the membrane **111**, or found at the surface of the membrane **111**.

In another embodiment, bacterial releasing agent may be immobilized on the glass filter membrane **106** or on membrane **111**. The top section **102** and bottom section of **103** of the membrane device **100** are then compressed together, preferably upon the insertion of the disposable membrane device into the draw slide of the luminometer. As the top section **102** and bottom section **103** of the membrane device **100** make contact, the light producing reaction:

$$\text{Luciferase} + \text{Luciferin} + \text{ATP} \xrightarrow[O_2]{Mg^{++}} \text{oxy-Luciferin} + \text{AMP} + \text{light}$$

is initiated.

This results in RLU's over a ten second integration period, which corresponds with the bacterial content of the sample.

As shown in Figure 11, the membrane device **100** is preferably placed in the draw slide **200** of the luminometer with the luciferin-luciferase membrane face down, directly over a reading hole **201**. The photomultiplier tube **30** is positioned directly under the hole **201**.

In another embodiment of this invention, particles coated with a specific antigen are added with the sample fluid to the disposable test device, with the antigen binding a virus specific antibody.

The methods described above may be used not just for the testing of surfaces, but also for the testing of fluids of all kinds, including air and liquids. To test

the bacterial level of an air sample, the membrane device or disposable test device may be placed over an impactor or vacuum device of any kind, allowing air to be drawn through the collection or membrane device. The bacterial contaminants from the air will then be trapped on the surface of the membrane devices, ready for testing.

The invention is further illustrated by means of the following examples.

Example 1 - General Bacterial Screen on Hard Surfaces.

This example involves a procedure for testing a stainless steel surface for the presence of microbial contamination.

Escherichi coli were grown on tryptic soy agar for 18 hours at 30°C. A sample of the bacteria was introduced into 10 mls of peptic soy broth and incubated for an additional 18 hours. Bacteria were harvested by centrifugation and washed three times in 0.9% NaCl that had been sterile filtered. The optical density of the solution was measured at 650 nm and the concentration was adjusted so that the optical density was 0.300. Three serial 10-fold dilutions were prepared to arrive at a concentration of 10^5 microbes/ml. 100 μl of this solution was dribbled over an area of 10 X 10 cm demarcated on the surface of a stainless steel sheet that had been previously cleaned with bleach, alcohol and sterile distilled water. The solution containing the bacteria was allowed to dry for 5 hours at room temperature.

Control demarcated areas were prepared with no bacteria.

Individual sponges of 10 X 10 cm were premoistened with approximately 750 μl of a collection fluid comprised of 0.15M NaCl containing 0.05% Tween 20 in a bag. This solution was just sufficient to completely wet the sponge. Each sponge was removed from a bag and wiped over demarcated areas of the surface with 10 strokes in each direction. The sponge was then returned to the bag and squeezed manually ten times yielding a collectate. An aliquot (25 μl) of the collectate was removed from the bag and placed in a disposable test device. 25 μl of bacterial releasing agent was added and 50 μl of a luciferin/luciferase/magnesium mixture was added. The draw slide was closed and the relative light units determined.

In a second set of experiments, swabs were premoistened with approximately 300 μl of collection fluid in a bag as outlined above. The swabs were used to wipe similarly demarcated areas of a stainless steel surface as described above.

In each case, control areas which had not had bacteria seeded on the surface were also tested. In addition, the bacterial solution that had been seeded onto the surface was placed directly into the collection fluid as a positive control Each data point represents the average of three samplings. Referring to Table 1, approximately 80% of seeded bacteria could be detected using either a sponge or a swab as a collection means.

Table 1

Collection Device	Negative Control Surface (Relative Light Units)	Positive Control Direct Seeding (Relative Light Units)	Sample from Seeded Surface (Relative Light Units)	% Recovery of Seeded Bacteria
Sponge	0	115	88	79%
Swab	0	330	272	82%

Example 2: Chemiluminescent Salmonella Assays

This example involves a procedure for testing for the presence of salmonella.

Bacteria, either *Salmonella typhimurium*, ATCC 14028 or *Aeromonas hydrophila*, ATCC 7966, were streaked from frozen stocks onto tryptic soy agar plates and incubated for 18 hours at 26°C. Bacterial colonies were harvested into sterile 0.9% NaCl. The optical density of the solution was measured at 650 nm and the concentration was adjusted so that the optical density was 0.300 by diluting the bacteria in 0.05M Tris, 0.05M EDTA, 0.15M NaCl, pH 8.2.

An aliquot (10 µl) of a 0.5% solution of latex microspheres coated with antibody to salmonella was added to the disposable test device. An aliquot, 100 µl, of

the diluted bacteria were placed in a disposable test device with a filter on the bottom surface composed of 1.2 micron Biodyne C. After the aliquot of the bacteria was added, the solution was allowed to sit for 10 minutes.

Positive pressure was applied and the fluid was evacuated onto an absorbent pad. The trapped antigens were washed by adding 200 μl of wash solution consisting of 0.01M PBS, pH 7.2 containing 0.05% Tween 20. Positive pressure was again applied and the fluid was evacuated onto an absorbent disk. A horseradish peroxidase labeled antibody directed against Salmonella was added to the disposable test device and allowed to sit for 10 minutes at room temperature. Positive pressure was again applied and the fluid evacuated from the disposable test device. A wash solution was added and evacuated with positive pressure two more times. The disposable test device was placed in a luminometer. 100 μl of Lumiglo Chemiluminescent substrate (Kirkegaard and Perry Laboratory Gaithersburg, MD) was added, the drawer slide was immediately closed and the light emission determined.

The results shown in Table 2 indicate that the concentrations as low as 10^5 organisms could be easily distinguished from a negative control using this system.

Table 2: Results of a Test for Salmonella

Total Number of Organisms	Relative Light Units for *Salmonella typhimurium*	Relative Light Units for *Aeromonas hydrophila*	Signal to Noise Ratio
10^8	18,940	5,290	3.6
10^7	13,780		2.6
10^6	10,720		2.0
10^5	9,220		1.7

A second procedure was used similar to that detailed above, except that no latex beads were added to the disposable test device prior to the introduction of the aliquot of the bacteria. In this case, the signal to noise ratio for a solution of *S. typhimurium* (10^8 organisms): *A. hydrophila* (10^8 organisms) was 5.91.

A third procedure was also tested. In this method, 40 μl of sample, and 40 μl of horseradish peroxidase labeled anti-salmonella antibody were added to a disposable test device. The mixture was incubated for 20 minutes at room temperature. Positive pressure was used to evacuate the fluid from the test device. The trapped material was washed three times by introduction of 200 μl of .01M phosphate buffered saline pH 7.2 containing 0.05% Tween 20 followed by evacuation of the fluid from the disposable test device using positive pressure. The disposable test device was placed in the luminometer and 100 μl of Lumiglo Chemiluminescent substrate (Kirkegarrd and Perry Laboratories, Gaithersburg, MD) was added. The

drawslide was immediately closed, and the light emission determined. The signal to noise ratio for a solution of *S. typhimurium* (10^6 organisms): *A hydrophila* (10^6 organisms) was 1.83.

Example 3: Detection of Bacterial ATP from A Powdered Food Sample

A suspension of powdered food sample is prepared by mixing 25g of food sample in 225 ml of SRA solution in a beaker. 50 μl of the suspension is placed into the filtravette. Four (4) drops of SRA are added to the suspension. The Filtravette™ is placed into the plastic stand with blotter paper underneath. The suspension is filtered using the positive pressure device, thereby extracting all of the somatic cell ATP and allowing them to be absorbed by the blotter paper while retaining all bacterial cells on the filter paper. Six more drops of SRA are placed into the filtravette™ and the filtration step is repeated using the positive pressure device.

The filtravette™ is then placed into the microluminometer, and 50 μl of BRA are added to release the bacterial ATP. The pipette tip is used to mix the BRA and the suspension.

50 μl of reconstituted LL is added and mixed 2 to 3 times, using the pipette tip. The sample drawer is closed immediately to being a 10 second period. The reading of the relative light units (RLU's) are recorded from the microluminometer's digital readout.

Example 4: Method of Determining the Amount of ATP from Yeast Employing Various Membranes.

The strains of *Escherischia coli* and *Streptococcus pyogenes* were grown on Tryptic Soy Agar and *Saccharomyces cerevisiae* were grown on Rose Bengal Agar. Freshly grown colonies of each test organism were taken in 0.01M PBS. The suspension was adjusted to an OD^{650} value of 0.3, representing ca. 3×10^8 bacterial cells/ml and 3×10^6 yeast cells/ml. Two ten fold dilutions were made in 0.01M PBS from each suspension. A Filtravette™ was placed in the plastic stand with blotter paper stacked underneath. A 50 µl sample from each suspension (ca 10^6 bacterial cells/ml and 10^4 yeast cells/ml) was added in the Filtravette™. Four drops of SRA were added. The solution was filtered using the positive pressure device. This was repeated by adding 6 drops of SRA again. The filtravette was then placed in the microluminometer drawer. Fifty µl of BRA was added to the filtravette. Fifty µl of reconstituted LL was then added followed by mixing 3-4 times by pipetting up and down. The sample drawer was closed immediately to begin a ten second integration period, and the readings in RLU were recorded from the digital readout. These RLU values were considered absolute in order to obtain 100% retentate (control).

The results are summarized as follows:

TABLE: EFFICACY (%) OF VARIOUS MEMBRANES IN FILTRATION/RETENTION OF BACTERIA AND YEAST

Membrane Type	*S.pyogenes*		*E. coli*		*S.cerevisiae (Yeast)*	
	% retentate	% filtrate	% retentate	% filtrate	% retentate	% filtrate
Membrane 1	100	0	100	0	100	0
Membrane 2	ND	ND	94.1	5.9	99.8	0.2
Membrane 3	ND	ND	7.6	92.4	95.4	4.6
Membrane 4	81.1	18.9	ND	ND	99.9	0.1
Membrane 5	66.2	33.8	ND	ND	99.9	0.1
Membrane 6	79.5	20.5	ND	ND	99.9	0.1
Membrane 7	76.2	23.8	ND	ND	99.9	0.1
Membrane 8	0.1	99.9	ND	ND	69.9	30.1
Membrane 9	25.5	74.5	28.9	71.1	99.3	0.7

ND: Not Done

Example 5: Detection of Bacterial ATP from Gasoline Sludge Sample

Prior to taking the samples from a gasoline sludge, the gasoline sludge sample is left undisturbed until clear zones of separate phases appeared. A total of up to three phases with two interphases could appear in the gasoline sludge sample. The specimens are collected from each phase using a pasteur pipette and placed in an eppendorf tube.

50 µl of the sample from each eppendorf tube representing the respective phases of the gasoline sludge are placed into the Filtravette™. Four drops of SRA are added. The Filtravette™ is placed in the plastic stand with blotter paper underneath, and the solution is filtered using the positive pressure device. This step extracts all of the somatic cell ATP and subsequently allows them to be absorbed by blotter paper while retaining all bacterial cells on the filter paper. Six more drops of SRA are added into the Filtravette™ and the filtration step is repeated using a positive pressure device. The filtravette™ is placed into the microluminometer, and 50 µl of BRA is added to release the bacterial ATP, and the liquids are mixed thoroughly using the pipette tip.

50 µl of reconstituted luciferin-luciferase is added and mixed two to three times, using the pipette tip. The

sample draw is closed to begin the ten second integration period, and the readings in Relative Light Units (RLU's) are recorded from the microluminometer's digital readout.

Using the above described method, a gasoline sludge sample having three distinct phases (1. upper translucent oil phase; 2. middle brown phase; and 3. lower deep brown viscous phase) is tested. Each of the above listed phases was tested, including two additional "interphases" present between the three primary phases. The results are summarized as follows:

PHASES OF GASOLINE-	BACTERIAL ATP
Phase 1	401
Phase 2	480
Phase 3	462
Inter-phase 1	2890
Inter-phase 2	3060

A loopful of specimen from each phase was streaked on Trypticase Soy Agar (TSA; Difco). The plates were then incubated for 24-48 hours at 37° C. Bacterial colonies were observed on the plate representing a sample from phase 2 with a count of 5.5×10^3 colony forming units/ml.

The other phases did not yield any visible colonies on their respective plates.

Example 6: Competitive Testing of Tap Water

A tap water sample was tested using the conventional heterotrophic plate count (HPC) method widely used to monitor drinking water; and comparing the results with the invention's method of utilizing a large volume concentrating apparatus (FIG 3, #7) to collect and concentrate water obtained from two different sites. The water sample was collected in the plastic device. Four drops of SRA were then added to the plastic device and processed according to the Steps in Example 2.

Sample Site	Holding	HPC	ATP	Volume
I	0	1	10	80
	2	3	11	80
	3	387	140 143	80 80
II	0	3	4 7	80 160
	2	3.76×10^4	3120 1261 1111	80 40 40
	3	4.73×10^5	3630 3250 3880	40 40 40

The result of these two sampling periods show that the invention will be able to detect bacteria wll below 100 CFU/mL, probably also to below 10 CFU/mL.

Example 7: Determination of the Effectiveness of Immobilizing Luciferin-Luciferase on a Membrane

Utilizing a plastic device similar to Figure 10 with luciferin-luciferase on a glass membrane. A 10μl sample of an TP standard was added. The Relative Light Unit value of the ATP standard is referenced to a liquid phase LL system. Results show a correlation between the LL immobilized on a plastic device and a liquid phase Ll system.

Testing information on three lots of membrane devices to determine if there is a direct correlation between the amount of ATP present and the light released using a microluminumeter.

Mid ATP Control	Membrane Device I	Membrane Device II	Membrane Device III
Sample 1	10039	10117	12941
Sample 2	9007	10877	10331
Sample 3	9699	10686	13430

Table 1. Counts of three L.L. devices using a mid range ATP control solution. (ATP Solution Counts ~10000)

High ATP Control	Membrane Device I	Membrane Device II	Membrane Device III
Sample 1	20000	17856	20000
Sample 2	20000	20000	19353
Sample 3	20000	20000	20000

Counts of three Membrane devices using a high range ATP control solution. (ATP Solution Counts~20000)

Example 8: Detection of Bacterial Content of Water During Squential Purification Step

This example involves a procedure for testing for the bacterial content of ultrapure water samples used in the manufacturing process for silicon chips during various stages of purification. Samples were taken from collectates from each of the sequential purification steps. For each assay a membrane device was inserted into an assist apparatus which served to compress the glass filter membrane portion between two "o"-rings, allowing relatively large sample volumes to be drawn through the filter when negative pressure was applied. The membrane device was removed, 10μl of bacterial releasing agent was added to the luciferin-luciferase membrane, and the device was closed and inserted into the draw-slide of th luminometer. Results are tabulated in the following table and clearly indicate the progressive

depletion of bacterial content in each sequential purification stage.

Purification Stage No.	Site	Volume Tested (mL)	Total RLU's
1	"Multimedia" Mixed bed resin	10	132
2	Carbon Bed	10	100
3	Pre-Reverse Osmosis-1200	10	21
4	Reverse Osmosis-1200	10	15
5	Reverse Osmosis-1300	10	0
	Supply Water Supply Water Supply Water	1000 2000 3000	0 6 22

All of the above examples and tests can be performed using the membrane device version of the invention.

Many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood within the scope of the appended claims the invention may be protected otherwise than as specifically described.

What is claimed is:

1) A method for determining the presence and quantity of an analyte, comprising the steps of:

a) collecting a sample of said analyte;

b) placing said sample on a disposable test device with permeable filter means, said disposable test device being a membrane device, said membrane device comprising: a hinged two side support having a top section and a bottom section, an absorbent pad positioned on top of an inner side of said top section, a filter membrane, on top of said absorbent pad, a rigid layer holding said filter membrane in place; rigid layer positioned over the filter membrane; a hole in said rigid layer positioned over said filter membrane; a hole in the bottom section of the membrane device; a membrane disk fitted in said hole of said membrane device; an illumination solution immobilized on said membrane disk; a transparent window on an outer side of said bottom section under said membrane disk; wherein said sample is applied through a hole in the rigid layer onto the surface of the filter membrane;

c) applying a somatic cell releasing agent to the surface of the filter membrane;

d) applying a bacterial releasing agent to the surface of the membrane disk positioned on the inner side of the bottom section of the membrane device;

e) compressing the top section and bottom section of the membrane device;

f) sliding the membrane device in a photometer by means of a draw slide; and

g) measuring the light emission resulting from said luminescent reaction with a photometer comprising a photodetector means and a light tight chamber for said disposable test device and a means to measure light passing through said transparent wall of said membrane device; and

h) causing the said photometer means to output a signal indicative to the presence and amount of analyte.

2) A method according to Claim 1 wherein steps (c) through (g) are performed within 5 minutes.

3) A method according to claim 2, wherein steps (c) through (g) are performed within 2 minutes.

4) A method according to Claim 1 wherein disposable collection apparatus means is comprised of a soft absorbent.

5) A method according to Claim 1 wherein disposable collection apparatus means consists of a spongy absorbent.

6) A method according to Claim 1 wherein disposable collection apparatus means is comprised of a soft absorbent and a shaft.

7) A method according to Claim 1 wherein collection fluid contains a detergent.

8) A method according to Claim 1 wherein collection fluid contains a salt.

9) A method according to Claim 1 wherein said analyte

contains adenosine triphosphate (ATP) and said light emission correlates with the concentration of said ATP.

10) A method according to Claim 1 in which a large volume concentrating apparatus is used to concentrate the fluid in said disposable test device as part of step (c).

11) A method according to Claim 1 in which said filter means is a hydrophilic permeable membrane.

12) A method according to Claim 1 in which all light emitting substances are retained within disposable test device during the performance of step (f).

13) A method according to Claim 1, wherein said membrane device is comprised of a material selected from the group consisting of plastic, cardboard, or paper.

14) A method according to Claim 1, wherein said absorbent pad is comprised of cellulose.

15) A method according to Claim 1, wherein said absorbent pad is selected from the group consisting of cotton, corn silk, and fiberglass.

16) A method according to Claim 1, wherein said filter membrane is comprised of a glass filter.

17) A method according to Claim 1, wherein said illumination solution is a luciferin-luciferase solution.

18) A method according to Claim 17, wherein said luciferin-luciferase solution comprises luciferin, luciferase, and magnesium.

19) A method according to Claim 18, wherein said luciferin-luciferase solution further comprises the chemicals selected from the group consisting of

trehalose,di thiothreito, HEPES Buffer, and combinations thereof.

20) A method according to Claim 1, wherein said somatic cell releasing agent is added dropwise onto the surface of the filter membrane so as to avoid flooding the membrane device and washing the cells out of the filter membrane.

21) A method according to Claim 1, wherein said photodetector means is positioned directly under the membrane disk, said membrane disk being face down over said luminometer.

22) A method for determining the presence and quantity of an analyte, comprising the steps of:

a) collecting a sample of said analyte;

b) placing said sample on a disposable test device with permeable filter means, said disposable test device being a membrane device, said membrane device comprising: a hinged two side support having a top section and a bottom section, an absorbent pad positioned on top of an inner side of said top section, a filter membrane, on top of said absorbent pad, a somatic cell releasing agent attached to the surface of the filter membrane; a rigid layer holding said filter membrane in place; a hole in said rigid layer positioned over said filter membrane; a hole in the bottom section of the membrane device; a membrane disk fitted in said hole of said membrane device; an illumination solution immobilized on said membrane disk; a transparent window in said bottom

section under said membrane disk; wherein said sample is applied through a hole in the rigid layer onto the surface of the filter membrane;

c) applying a bacterial releasing agent to the surface of the membrane disk positioned on the inner side of the bottom section of the membrane device;

d) compressing the top section and bottom section of the membrane device;

e) sliding the membrane device in a photometer by means of a draw slide; and

f) measuring the light emission resulting from said luminescent reaction with a photometer comprising a photodetector means and a light tight chamber for said disposable test device and a means to measure light passing through said transparent wall of said membrane device; and

g) causing said photometer means to output a signal indicative to the presence and amount of analyte.

23) A membrane device for placing in a luminometer a sample for obtaining a bacterial count, said membrane device comprising: a hinged two side support having a top section and a bottom section, an absorbent pad positioned on top of an inner side of said top section, a filter membrane, on top of said absorbent pad, a rigid layer holding said filter membrane in place; a rigid layer positioned over the filter membrane; a hole in said rigid layer positioned over said filter membrane; a hole in the bottom section of the membrane device; a membrane

disk fitted in said hole of said membrane device; an illumination solution immobilized on said membrane disk; and a transparent window in said bottom section under said membrane disk; wherein said sample is applied through a hole in the rigid layer onto the surface of the filter membrane.

24) The membrane device according to claim 22, further comprising said somatic cell releasing agent is incorporated in the surface of the filter membrane.

25) A method for determining the presence and quantity of an analyte comprising the steps of:

a) collecting said analyte with a disposable collection apparatus means;

b) adding collection fluid to said collection apparatus means;

c) placing said collection fluid in a disposable test device with permeable filter means at its bottom end, an open top end, and transparent side walls;

d) applying pressure to said disposable test device to force said fluid to pass through said permeable filter means and retaining said analyte on said permeable filter means;

e) adding a reagent into said disposable test device thereby establishing a luminescent reaction in said test device;

f) measuring the light emission resulting from aid luminescent reaction with a photometer comprising a photodetector means and a light tight chamber for said

disposable test device and a means to measure light passing through said transparent side wall of said test device; and

g) causing the said photometer means to output a signal indicative to the presence and amount of analyte.

26) The method according to Claim 25 wherein steps (c) through (g) are performed within 5 minutes.

27) The method according to Claim 25 wherein said disposable collection apparatus means is comprised of a soft absorbent.

28) The method according to Claim 25 wherein said disposable collection apparatus means consists of a spongy absorbent.

29) The method according to Claim 25 wherein said disposable collection apparatus means is comprised of a soft absorbent and a shaft.

30) The method according to Claim 25 wherein said collection fluid contains a detergent.

31) The method according to Claim 25 wherein said collection fluid contains a salt.

32) The method according to Claim 31 wherein a washing fluid is added to said salt collection fluid in said disposable test device in step (c) and said wash solution is comprised of a salt solution containing detergents, and said wash solution lyses somatic cells and does not lyse microorganisms.

33) The method according to Claim 25, and after performing step (d), the additional steps of adding a

wash solution and applying pressure to said disposable test device, where said pressure forces said wash solution to pass through said filter means.

34) The method according to Claim 25 wherein a bacteriolytic reagent is added to said disposable test device in step (e).

35) The method according to Claim 25 wherein said analyte contains adenosine triphosphate (ATP) and said light emission correlates with the concentration of said ATP.

36) The method according to Claim 25 wherein said luminescent reaction of step (e) is established by adding luciferin-luciferase.

37) The method according to Claim 25 wherein the luminescent reaction is established by adding isoluminol.

38) The method according to Claim 25 in which a large volume concentrating apparatus is used to concentrate the fluid in said disposable test.

39) The method according to Claim 25 in which said filter means is a hydrophilic permeable membrane.

40) The method according to Claim 25 in which all light emitting substances are retained within said disposable test device during the performance of step (f).

41) The method for determining the presence and quantity of an analyte comprising:

a) collecting said analyte with a disposable collection apparatus means;

b) adding collection fluid to said collection apparatus means;

c) placing said collection fluid in a disposable test device with permeable filter means at its bottom end, an open top and, and transparent side walls;

d) applying pressure to said disposable test device to force said fluid to pass through said permeable filter means and retaining said analyte on said permeable filter means;

e) adding a detection reagent into said disposable test device, said detection reagent binding to said analyte;

f) adding a wash solution to said disposable test device and applying pressure to said disposable test device to force said fluid to pass through said permeable filter means;

g) establishing a luminescent reaction in said test device;

h) measuring the light emission resulting from said luminescent reaction with a photodetector means comprising a light tight chamber for said disposable test device and a means to measure light passing through said transparent side wall of said test device; and

i) causing the said photometer means to output a signal indicative to the presence and amount of analyte.

42) The method according to Claim 41 wherein steps (c) through (i) are performed in within 30 minutes.

43) The method according to Claim 41 wherein said disposable collection apparatus means is comprised of a soft absorbent.

44) The method according to Claim 41 wherein said disposable collection apparatus means consists of a spongy absorbent.

45) The method according to Claim 41 wherein said disposable collection apparatus means is comprised of a soft absorbent and a shaft.

46) The method according to Claim 41 wherein said collection fluid contains a detergent.

47) The method according to Claim 41 wherein said collection fluid contains a salt.

48) The method according to Claim 41 wherein a washing fluid is added to said collection fluid in said disposable test device in step (c), and said washing fluid is comprised of a salt solution containing detergents.

49) The method according to Claim 41 wherein particles coated with a specific antibody that binds said analyte are added to said disposable test device in step (c) with said collection fluid.

50) The method according to Claim 41 wherein particles coated with a specific antigen or antibody that binds said analyte are added to said disposable test device in step (c), with said collection fluid.

51) The method according to Claim 41 wherein after performing step (d), the additional steps of adding a

wash solution and applying pressure to said disposable test device, where said pressure forces said wash solution to pass through said filter means are performed.

52) The method according to Claim 41 further comprising adding capture particles coated with a binder, such that said binder binds to said analyte or analyte fragment, said binder being selected from the group consisting of antibodies, ATP enhanced antibodies, antigens, lectins, DNA fragments, viruses, and combinations thereof.

53) The method according to Claim 41 wherein said luminescent reaction of step (f) is established by addition of a chemiluminescent substrate.

54) The method according to Claim 41 wherein step (d) is eliminated so that the said collection fluid and the said detection reagent are placed together into said disposable test device.

55) The method according to Claim 41 in which a large volume concentrating apparatus is used to concentrate the fluid in said disposable test device as part of step (c).

56) The method according to Claim 41, wherein an air sample may be tested by for bacterial agents, by placing the collection device over an impactor or vacuum, allowing air to be drawn through the collection device.

57) The method according to Claim 1, wherein an air sample may be tested by for bacterial agents, by placing the collection device over an impactor or vacuum, allowing air to be drawn through the membrane device.

58) The method according to Claim 41 wherein particles coated with a specific antigen are added with said collection fluid to said disposable test device in step (c), said antigen being binding a virus specific antibody.

59) The method according to Claim 1 wherein particles coated with a specific antigen are added with said sample fluid to said disposable test device, said antigen binding a virus specific antibody.

60) The method according to Claim 1, further comprising said bacterial cell releasing agent incorporated in the surface of the filter membrane.

61) The method according to Claim 52, further comprising the addition of enzyme labeled antibodies to the capture particles and the collectate such that all are simultaneously reacted within the test device, and said enzymes of said enzyme labeled antibodies are selected from the group consisting of peroxidase, a phosphatase, an oxidase, a luciferase, and combinations thereof.

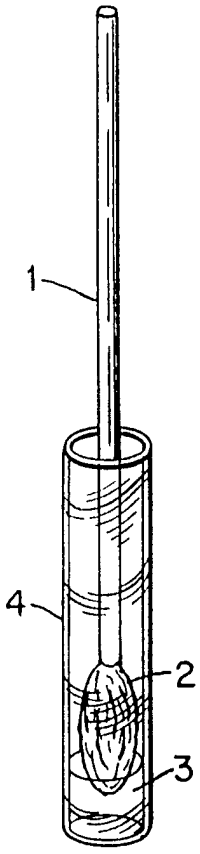


Fig.1

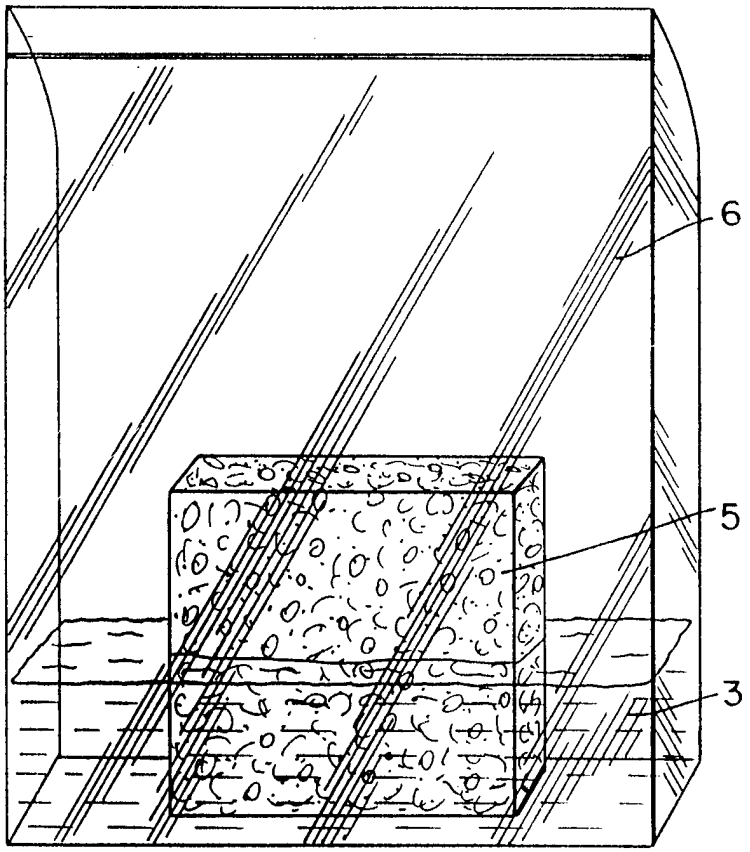


Fig.2

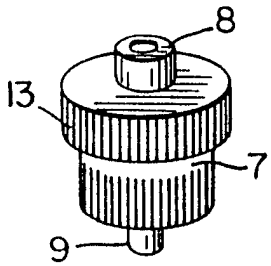


Fig.3

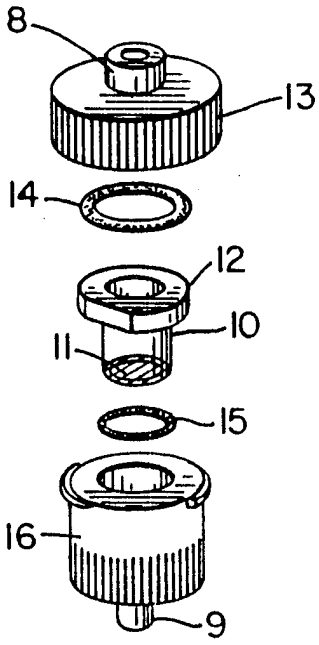


Fig.4

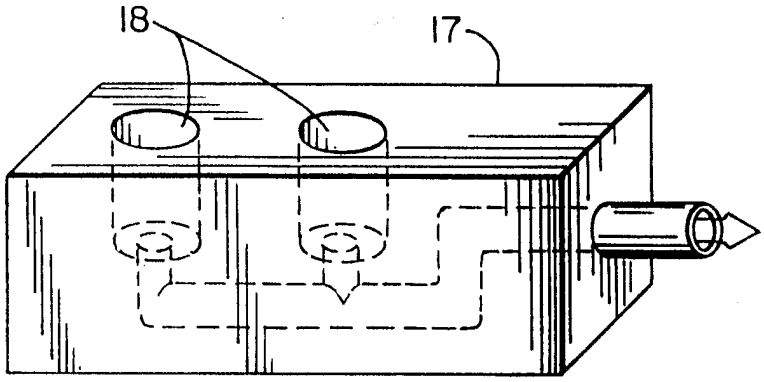


Fig.5

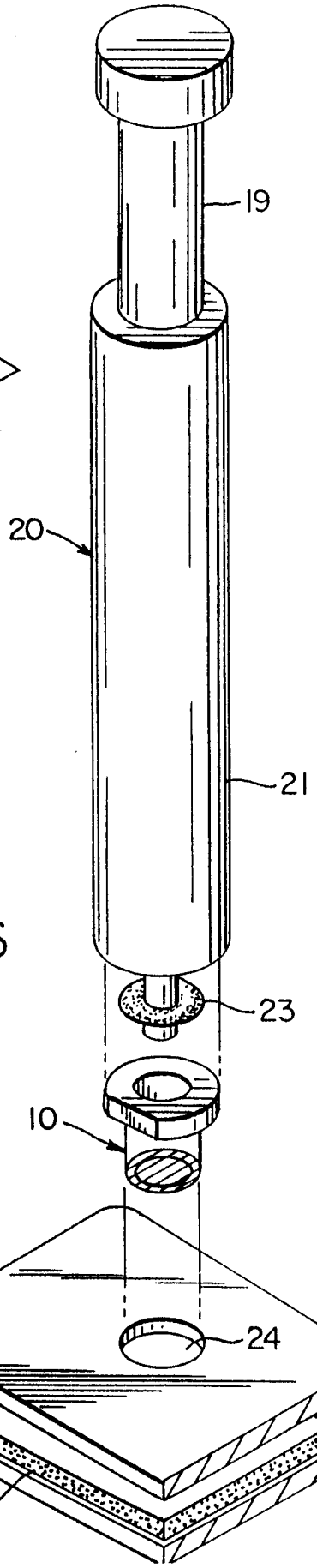
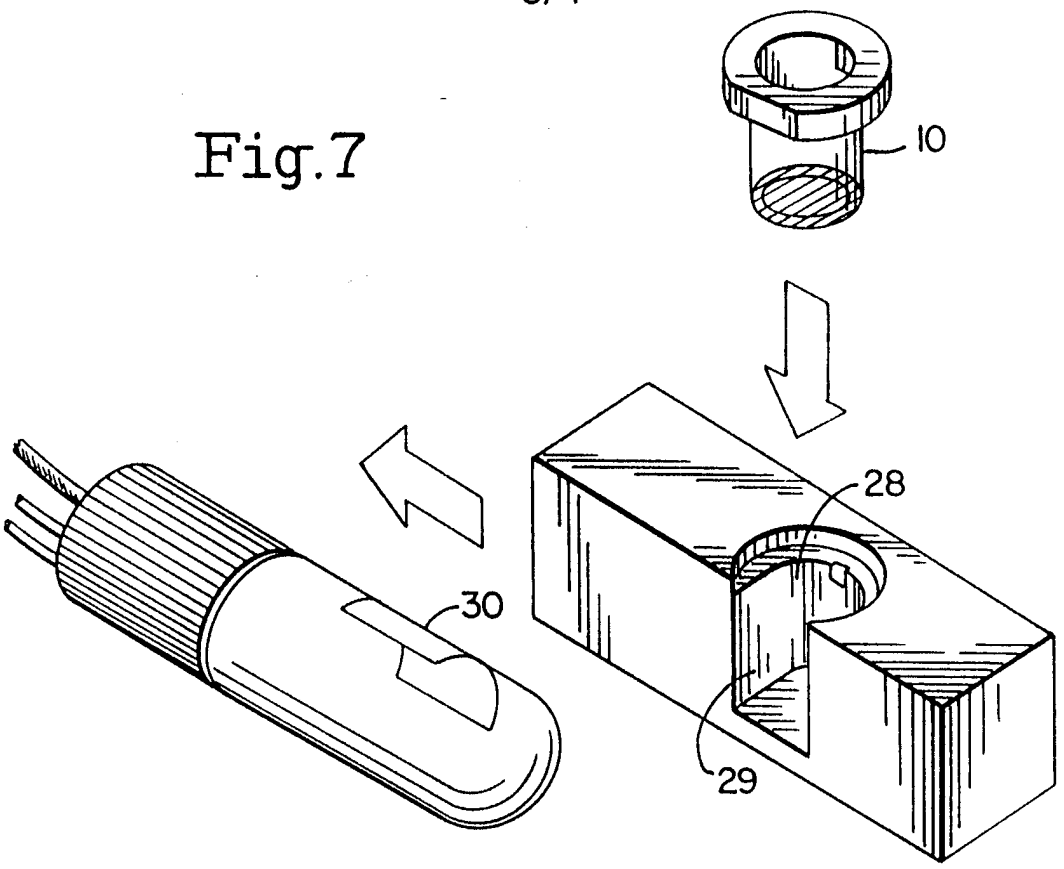


Fig.6

Fig.7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28
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Fig.8

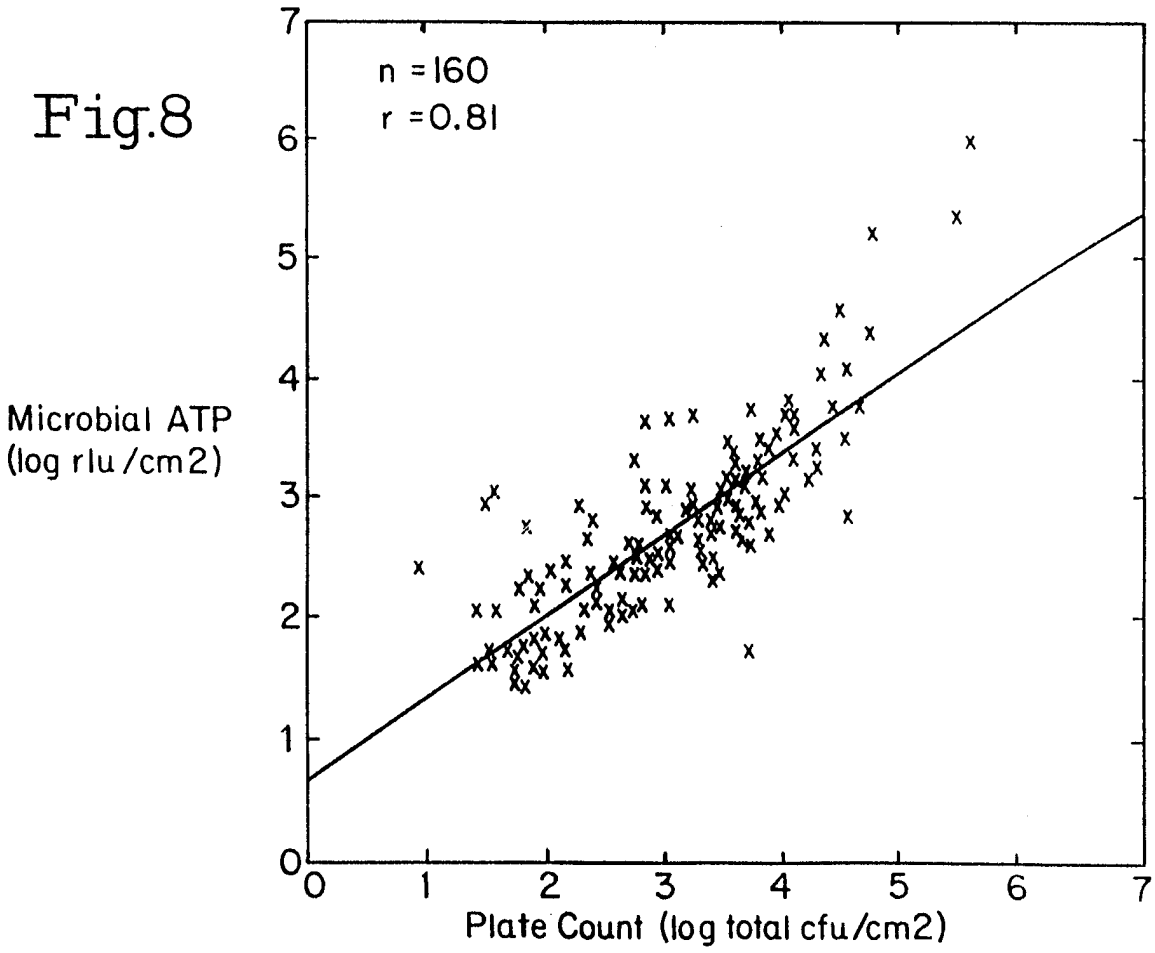

n = 160
r = 0.81
Microbial ATP
(log rlu/cm2)
Plate Count (log total cfu/cm2)
0
1
2
3
4
5
6
7

Fig.9

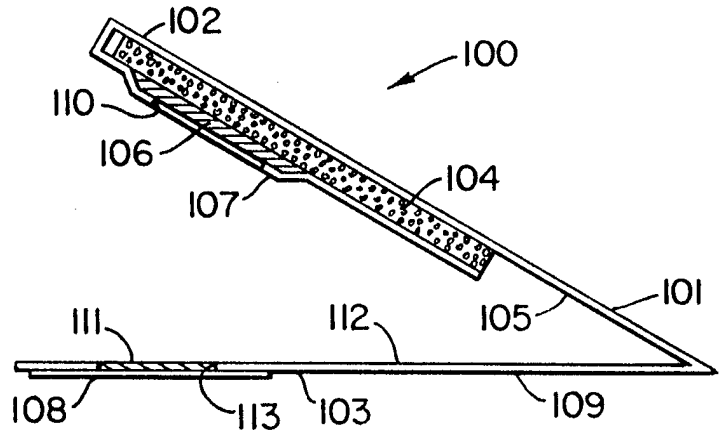


Fig.10

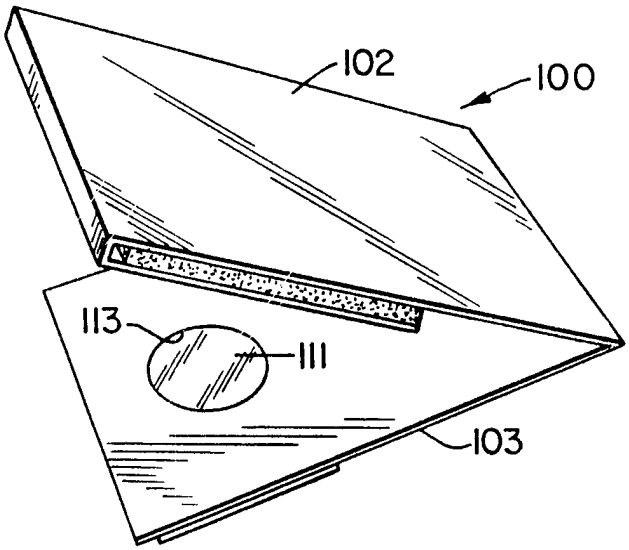
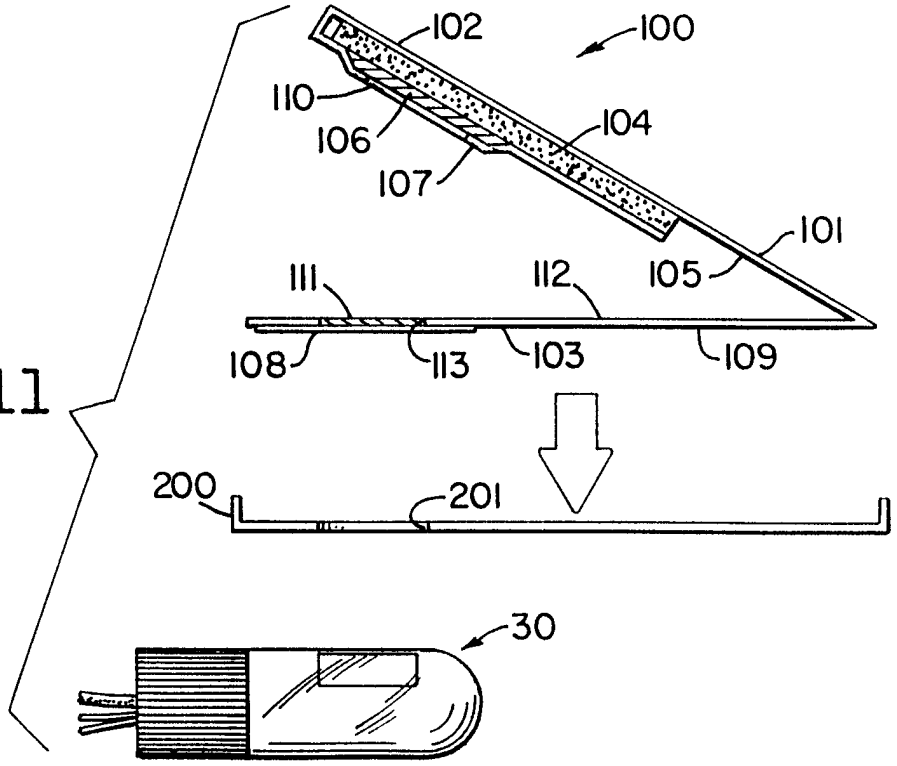


Fig.11



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/04289

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/569
US CL :435/7.4

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)

U.S. : Please See Extra Sheet.

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4,963,325 A (LENNON et al.) 16 October 1990, see entire document.	1-61

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"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
"A"	document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier document published on or after the international filing date	"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
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"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 30 JULY 1997	Date of mailing of the international search report 29 AUG 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CHRISTOPHER CHIN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)★

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

422/55, 56, 58, 61, 101;
435/7.2, 7.21, 7.32, 7.4, 7.9, 7.92, 8, 34, 287.1, 287.2, 287.7, 287.9, 810;
436/169, 172, 518, 528, 805, 807, 810