April 6, 1971 R. L. BOWMAN 3,574,063

METHOD FOR BACTERIA COUNTING AND ANTIBIOTIC SENSITIVITY ASSAY

Filed June 26, 1967 2 Sheets-Sheet 1

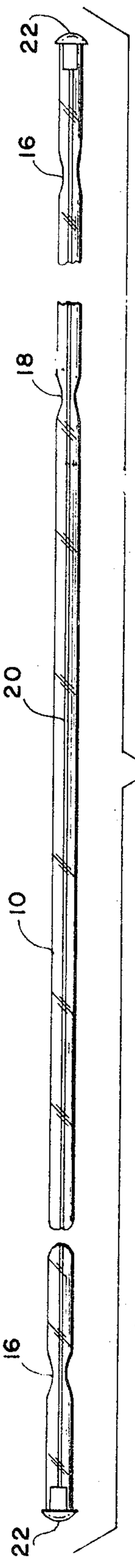

FIG. 1

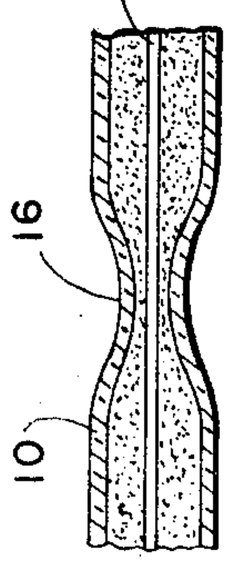

FIG. 2

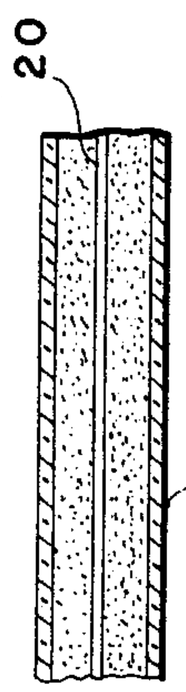

FIG. 3

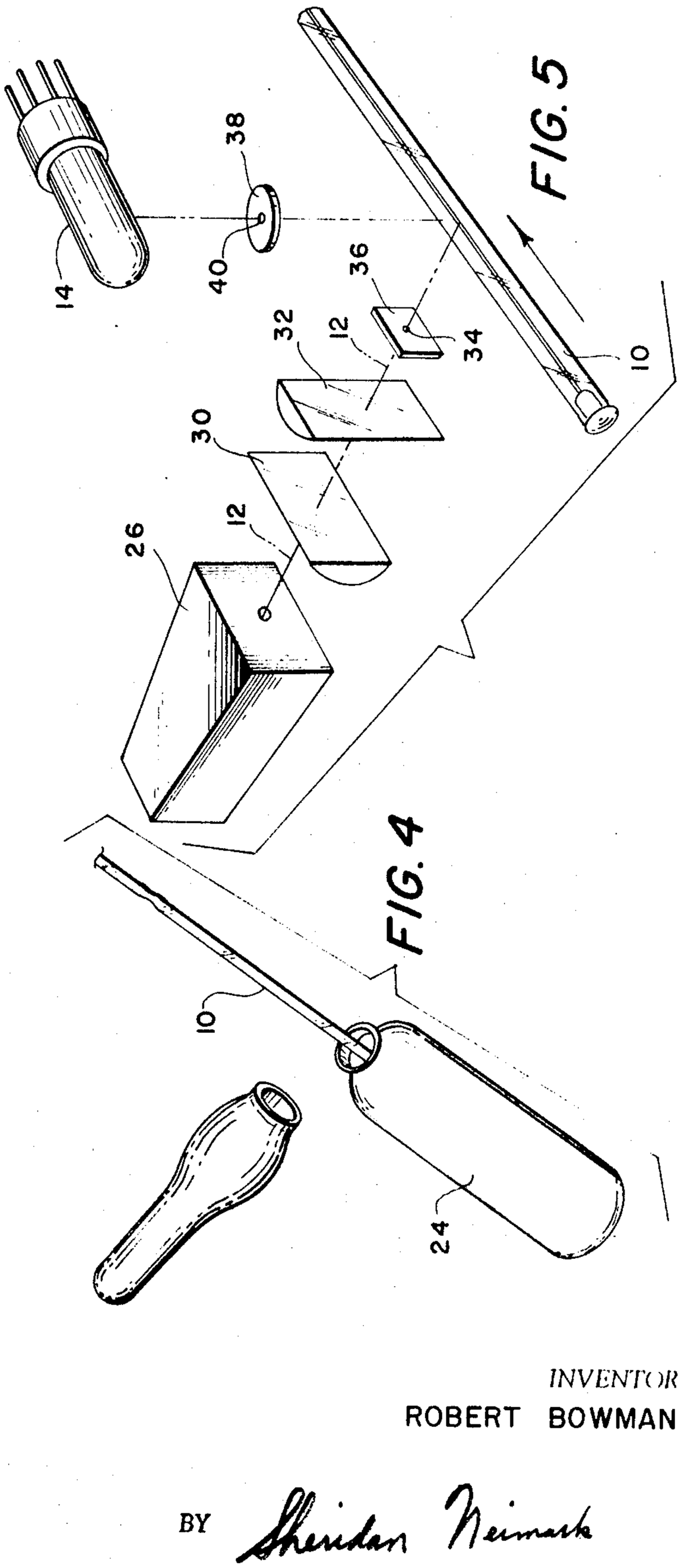

FIG. 4

FIG. 5

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METHOD FOR BACTERIA COUNTING AND ANTIBIOTIC SENSITIVITY ASSAY

Filed June 26, 1967 2 Sheets-Sheet 2

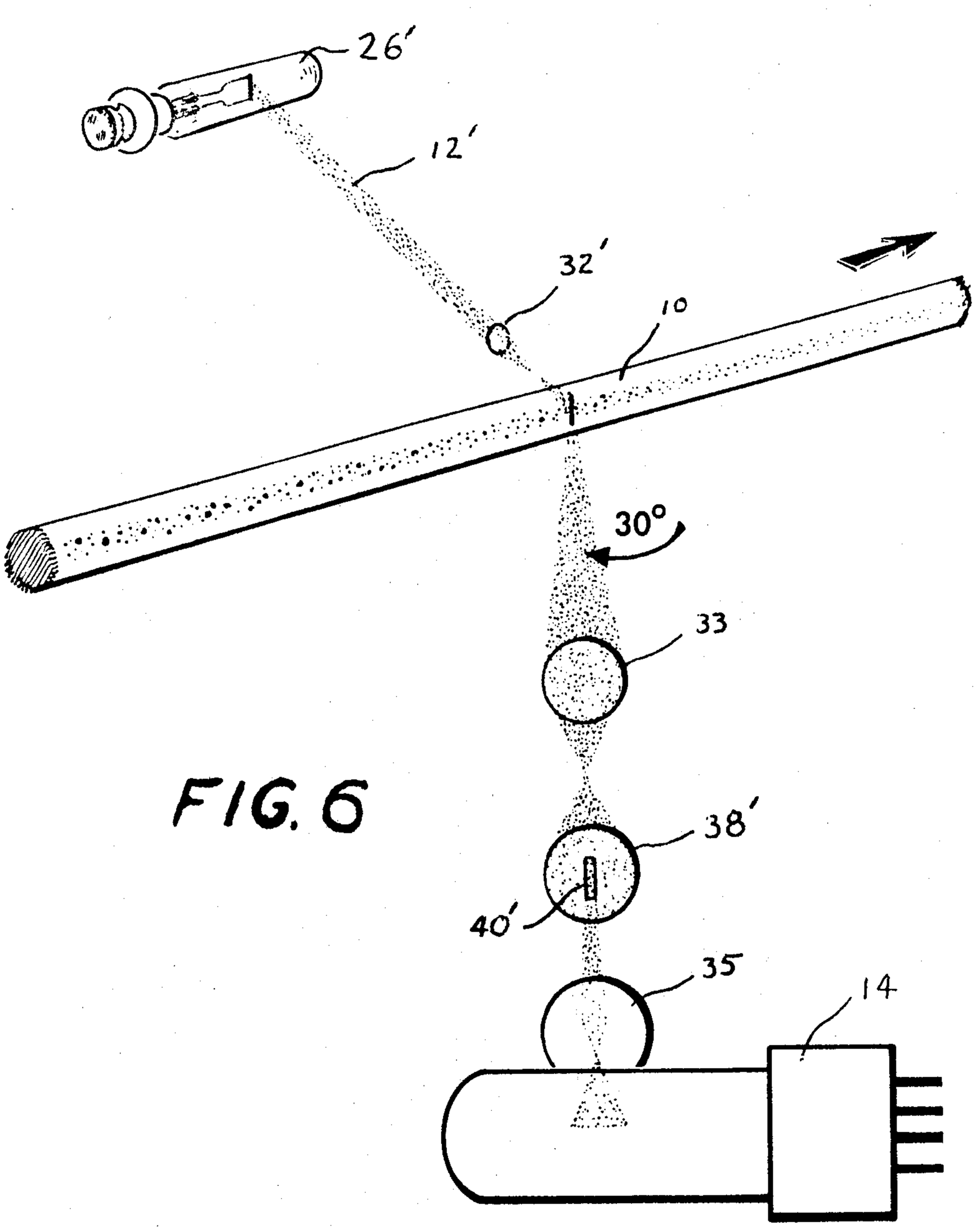

FIG. 6

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METHOD FOR BACTERIA COUNTING AND ANTIBIOTIC SENSITIVITY ASSAY

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U.S. Cl. 195—103.5 6 Claims

ABSTRACT OF THE DISCLOSURE

The disclosure relates to a method for counting bacterial colonies quickly and simply and for assaying the antibiotic sensitivity of such bacteria. Organisms are cultured in an extremely narrow channel and counting of the colonies is based on the scattering from a light beam focused into the narrow channel.

The present invention relates to a method of counting minute particles of an organic nature and, more particularly, to a method and device for counting viable bacteria and assaying the antibiotic sensitivity of such bacteria.

In the diagnosis and treatment of bacterial infections, the preferred initial approach almost always involves culturing a specimen obtained from the infected area in order to identify the etiologic agent, to determine its antibiotic sensitivity and, frequently, to determine the number of viable organisms present in the specimen. As currently performed, these procedures require highly trained personnel and are tedious and time-comsuming. In addition, they are seldom performed in the physician's office; that is, usually either the patient is sent to a separate laboratory specializing in these procedures, or a specimen obtained from the patient is sent to the laboratory. Thus, there is a substantial delay, usually at least 24 hours. before a physician has the information necessary to complete his diagnosis for the treatment of his patient.

The art has recognized the above problems and the undesirability of present procedures and many attempts have been made to solve these problems. Included among these are various indicating tests for specific bacteria and also improved methods of incubating and counting live bacteria. However, for one reason or another, these various prior art procedures have not attained the desired results and, hence, have not been widely adopted.

It is therefore an object of the present invention to obviate the deficiencies of the prior art, such as indicated above.

It is another object of the present invention to provide a method for bacteria counting and obtaining an antibiotic sensitivity assay.

It is another object of the present in vention to simply, efficiently and quicky count viable bacteria.

It is another object of the present invention to quickly, simply and easily culture bacteria for subsequent simplified counting.

It is another object of the present invention to count minute particles disposed in a very narrow channel by the use of a sharply focused beam of light emanating from a suitably focused line source such as the linear filament of an incandescent lamp, or a laser beam.

These and other objects and the nature and advantage of the present invention will be more apparent from the following description taken in conjunction with the drawings wherein:

FIG. 1 is a plan view of an embodiment of a narrow channel in which the minute particles to be counted may be maintained and/or cultured;

FIG. 2 is a sectional view of a portion of a channel of FIG. 1;

FIG. 3 is another sectional view of another portion of a channel of FIG. 1;

FIG. 4 is a schematic representation of a method of filling a channel in accordance with FIG. 1 with a bacterial culture;

FIG. 5 is a schematic representation of a system of counting minute particles in accordance with the present invention; and

FIG. 6 is a schematic representation of another counting system.

In accordance with the general procedure for counting bacteria and/or obtaining an antibiotic assay in accordance with the present invention, the bacteria are immobilized in solid nutrient placed in narrow channels or tubes, such as the capillary tube **10**. The initial presence of the bacteria, as well as their subsequent proliferation, is then detected by moving each tube **10** through a sharply focused beam of light **12** and measuring the light scattered at an angle by means of a photomultiplier tube **14**. Organisms immobilized by agar or other gel containing nutrient can go through several divisions without exhausting their supply of oxygen and nutrient and repeated observation of the light scattered from the micro colonies so formed will indicate proliferation and thus differentiate the scattering due to growing colonies from scattering non-proliferating material. These first few divisions produce micro colonies that are conveniently scanned or measured when they are distributed within the narrow channel. The present invention utilizes a narrow light beam that enters the narrow channel at right angles thereto and which is sharply focused to define the resolution of the system to individual colonies, thereby obviating the need for producing an image of the colony to count it. The light, scattered by the colonies at some angle that maximizes the light scattered and minimizes the collection of illuminating light, is simply collected to provide a best signal to noise ratio.

An essential feature of the present invention is that the illuminating beam be focused to a very narrow intense sheet so that it will be scattered by the bacterial colonies in the narrow channel in a pattern that can be presented to the photo-multiplier tube. Resolution must be high to count the smallest colonies and recognize growth to obtain the results as soon as possible.

The method permits a number of automatic procedures to be carried out and thereby reduces the time, effort and expense in obtaining a bacterial culture. This, in turn, provides that the culture results for diagnostic and therapeutic use by the physician are available in a period of a few hours, thereby allowing specific antibiotic therapy to be initiated earlier in the course of the disease. In counting bacterial colonies, each of which is the result of a proliferation of a single organism, the present method gives a more reliable bacterial count than those methods which involve the measurement of the overall metabolic activity of a fluid culture in which cells other than bacterial, or rapidly growing but non-pathogenic bacteria, will lead to falsely high results. Furthermore, antibiotic assay by incorporation of specific antibiotics in the nutrient medium can be simply and easily obtained with attendant savings of space, time, nutrient and antibiotics. Such relatively crude procedures as watching the organisms under a microscope for the first sign of division can thereby be eliminated.

In more detail, the narrow channel (e.g. capillary tube) **10** is provided, preferably having constrictions **16** and **18** at both ends thereof. These constrictions provide a reduced inner diameter to support a glass fiber **20** which has approximately the same outer diameter as the inner

diameter of the constrictions **16** and **18**. The glass fiber **20** is thereby supported in the capillary tube **10** at the axis thereof.

The tube **10**, with the supported glass fiber **20**, is prepared for use by first effecting sterilization thereof and then filling the tube, except for the volume occupied by the fiber, with a suitable transparent and solidifiable bacterial nutrient. Thus, for example, the tube may be first sterilized and then filled with melted nutrient agar by applying suction at one end of the tube. Noting FIG. 3, it may be seen that the constriction **16** provides an inner diameter slightly greater than the outer diameter of the fiber **20** and hence vacuum may be applied to draw the melted agar into the capillary tubing **10**. When the agar has jelled, the tube is ready for use. In this condition, the tubes may then be capped, such as with sterile caps **22**, and then stored for later use. Such agar filled tubes may be directly supplied to physicians or to laboratories specializing in such culturing procedures, or they may be prepared at their situs of use.

When it is desired to test an unknown bacterial specimen, such specimen is preferably diluted with a measured amount of melted agar or other solidifiable nutrient material and placed in a suitably sterile container, such as the container **24**. The capillary tube **10** is then broken at one of the constrictions **16** or **18** and is then dipped into the bacteria and agar mixture. The fiber **20** is withdrawn from the other end of the capillary tube **10** and, acting as an aspirator, the fiber **20** draws the melted agar and bacteria mixture into the center of the tube **10** which it previously occupied. Thus, when the specimen mixture has jelled, the capillary tube contains an annular body of agar and a central core of agar which contains the bacteria culture. The use of the fiber tract is, however, not essential and the capillary may be filled throughout with the nutrient and bacterial mixture.

Upon being filled with the bacterial culture, the sample tube **10** is preferably scanned as schematically illustrated in FIG. 6 for an initial reading, and after a suitable incubation period, it is again scanned and the results compared.

In the embodiment of FIG. 6, a line source of light **12'** is used, e.g. a tungsten straight wire incandescent lamp **26'**. Here the light beam **12'** is first passed as a sheet through an objective lens **32'** and thereby focussed into the core of the capillary tube **10** as a thin transverse slice of illumination. The light scattered at an angle of 30° is collected by an objective lens **33**, then passed through a screen **38'** defining an aperture **40'** therein and finally through an ocular lens **35** and into the photo-multiplier tube **14**.

If a helium-neon laser beam **12** is used as the high intensity, well defined light beam as shown in FIG. 5, it is first passed through a red filter **30** for removal of its blue light and then through a cylindrical lens **32**, the axis of which is aligned parallel to the intended line of reflection, and perpendicular to the direction of movement of the sample tube **10** passed through the laser beam; the cylindrical lens **32** causes the initially rod-like beam **12** to be focused to the requisite narrow intense sheet that defines a thin transverse slice of illumination. The light finally passes through a small aperture **34** in a screen **36** before encountering the sample tube **10**.

As indicated above, the sample tube **10** is passed as shown by the arrow in FIG. 5, through the path of the sheet-like beam in a direction perpendicular to the axis of the cylindrical lens **32**. At the point of focus in the center of the tube **10**, the beam is reduced to an extremely fine line, the height of which is limited by the aperture **34**. The light beam thereupon enters the side of the sample tube **10** and transects the central portion of the nutrient where the bacteria have been deposited. Because of the dimensions of the light beam and its entry only into the central portion of a capillary, it will not reach and reflect off the top and bottom surfaces of the capillary tube **10**.

Placed along a line parallel to the axis of the cylindrical lens **32**, and at an angle 90 degrees from the plane defined by the beam and the capillary tube **10** and directly above (or below) the capillary tube **10**, is a second screen **38** defining an aperture **40** therein. Laser light reflected from the bacteria passes through the aperture **40**, and such scattered light reaches the photo-multiplier **14** placed directly above the screen **38**. If desired, the scattered light may be collected by a suitable lens as in the FIG. 6 embodiment before being passed to the photo-multiplier **14**.

As stated above, it is particularly preferable and desirable to first scan the tube **10** immediately after filling thereof. Pulses obtained at this time may be due to bacteria, viable or not, or to particulate contaminants all of which are immobilized in the solid nutrient. However, all of the pulses are recorded and stored. After a suitable period of incubation, the tube is then rescanned. At this time, if bacterial proliferation has occurred, a larger colony will exist in the same location where initially there had been only a single bacterium. Consequently, the second reading will result in a decrease in the number of smaller pulses and a corresponding increase in the number of larger ones. From this comparison the number of viable bacteria in the original specimen is easily determined by subtracting the non-increased pulses caused by inert particles.

To determine the sensitivity of the originally unknown bacteria to various antibiotics, various sample tubes may be filled as described above, each with a mixture of the unknown bacteria and a different antibiotic. Several of such tubes, each containing a different antibiotic, are then scanned after a given and equal incubation period.

Rather than directly mixing the antibiotic with the bacteria and agar for aspiration into the agar containing capillary tubes, the originally produced sample tubes **10** containing the glass fiber **20** may be provided with different nutrient compositions; in other words, certain of such tubes may be manufactured and supplied containing only the nutrient as described above, and other such tubes may be supplied containing the nutrient and any one of a number of particular antibiotics mixed therewith.

The use of the narrow channel, i.e. the capillary tube, which is an essentially linear configuration in which to count the micro-organisms in place of the usual planar configuration, i.e. the surface of a Petri dish, is permitted by the use of the light beam which is capable of being focused to such a fine line that it will not be reflected off the inner surfaces of the capillary tube.

Devices capable of providing a laser beam or suitable incandescent lamps are known and are available at a reasonable cost. These beams can be focused finely enough so that the optical resolution is quite independent of the position of the organism in the capillary tube.

The present system is useful not only in counting bacteria and determining antibiotic sensitivity, but it may also be used in counting other minute particles, such as blood cells.

The following examples are illustrative and not limitative of the present system in use.

EXAMPLE I

A sample tube **10** was prepared from an 8 cm. length of 1.4 mm. outer diameter thin-walled glass capillary tubing. The tubing was constricted at points 1 cm. and 2 cm. from both ends to provide the constrictions **16** and **18**, so that the inner diameter at these points was reduced to about 0.1 mm. A glass fiber **20** with a diameter of about 0.1 mm. was then inserted into the tube **10** so that it was supported in a generally concentric position by the constrictions **16** and **18**. The tube, after sterilization, was then filled with melted nutrient agar by applying vacuum to one end thereof. When the agar had jelled, the tube **10** was ready for use. Similar tubes **10** were prepared as

described except that a different antibiotic was added to the melted nutrient agar which was sucked into each tube.

The bacterial specimen, in this case *E. coli,* was then diluted with a measured amount of melted agar. Each of the sample tubes was broken at one of the constrictions and was dipped into the bacterial suspension. The fiber **20** in each tube was then withdrawn and the melted agar rose to fill the tract left by the fiber **20**. When the specimen mixture had jelled in each tube, the tube contained agar, the central portion of which included a number of bacteria as well as other particulate material.

The scanning apparatus in this case consisted of a CW helium-neon laser operating with hemispherical mirrors at 6328 A. The beam **12** was passed through the red filter **30** and then through the cylindrical lens **32**, the axis of which was disposed vertically, and then through the aperture **34** of the screen **36**, such aperture having a diameter of 0.6 mm. Any suitable device may be provided to move the sample tube **10** horizontally through the path of the beam **12** downstream from the aperture **34**. For example, the tube could be clamped at both ends and moved axially through the operation of a pneumatic or hydraulic piston.

The laser beam entered the center of the capillary tube **10** and was reflected off the particles therein at 90° through the aperture **40**, having a diameter of 0.3 mm. and to the photo-multiplier tube **14** where the reflections were counted.

Each tube **10** was so scanned immediately after filling and again after a suitable incubation period. It has been found that readily countable bacterial colonies can be obtained in two to four hours of incubation and in this case each tube **10** was permitted to incubate for four hours.

From a comparison of the various runs, the growth rate of the organism was calculated and its sensitivity to each antibiotic determined.

EXAMPLE II

The procedure of Example I was followed except that the apparatus of FIG. 6 was utilized for scanning. A sheet of light from the incandescent tungsten lamp **26'** was passed through a 3.2 power objective lens **32'** and then into the core of the tube **10** which was moved axially. Scatterings at 30° were collected by a 3.5 power objective lens **33** and passed through the opening **40'**, through the ocular **35** and then into the photo-multiplier tube **14**. Each tube **10** was scanned immediately after filling and again after two hours of incubation; the results were compared. The opening **40'** defines the area of the cross section of the capillary that is desired to be counted.

It will be obvious to those skilled in the art that various changes may be made without departing from the scope of the invention and the invention is not to be considered limited to what is shown in the drawings and described in the specification.

What is claimed is:

1. A method of counting microorganisms comprising:

providing a relatively long carrier of very small cross-section containing the microorganisms to be counted, said microorgansims being immobilized in a solid transparent medium;

placing said carrier and contents in the path of a narrow beam of light;

focusing said light beam to a thickness less than the inner diameter of said carrier and passing said focused beam as a narrow sheet of light into the interior of said carrier, said beam being reflected as scatterings off of particles in the carrier, said particles including microorganisms and inert particles;

passing said reflected scatterings into a photo-multiplier device and recording the number and size of the pulses produced by said scatterings;

culturing said microorganisms in said carrier; and repeating the sensing and recording of the number and size of the pulses produced within said carrier;

and determining the number of microorganisms from the number of increased pulses.

2. A method in accordance with claim **1**, wherein said carrier comprises a capillary tube and wherein said microorganisms are placed in said capillary tube and said capillary tube is moved axially past said focused beam.

3. A method in accordance with claim **2** wherein said microorganisms are bacteria.

4. A method of obtaining a bacterial assay comprising filling a plurality of capillary tubes with bacteria and a transparent solid nutrient, some of said tubes also being filled with an antibiotic, and, in each tube in accordance with claim **2**, sensing and recording said pulses, culturing, and repeating said sensing and recording.

5. A method in accordance with claim **4** comprising the additional preliminary steps of providing said plurality of capillary tubes, each having a pair of constrictions adjacent each end thereof, placing a glass fiber in each said capillary tube, said fiber being supported in approximately the center of each said capillary by said constrictions, pre-filling each said capillary with bacterial nutrient by disposing one end of each said tube in said nutrient and applying vacuum to the other end thereof, and then effecting said filling of said capillaries with a mixture of bacteria and nutrient by disposing one end of each said capillary in a mixture of said bacteria and nutrient and drawing said mixture into said capillary by withdrawing said glass fiber from the other end of said capillary.

6. Bacteria culturing means for carrying out the process of claim **5** comprising an elongated capillary tube of very small internal cross-sectional area, a pair of constrictions adjacent each end of said tube, a fiber plunger disposed longitudinally along the center of said capillary and generally supported by said constrictions, and a solid bacterial nutrient disposed within said capillary and surrounding said fiber plunger.

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