

[54] **COMBINED SAMPLING AND BACTERIOLOGICAL CULTURING DEVICE** 3,205,151 9/1965 Landau et al. 195/139
 3,589,983 6/1971 Holderith et al. 195/139
 3,702,806 11/1972 Oliva 195/139

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Related U.S. Application Data

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[52] U.S. Cl. **195/127; 195/139**

[51] Int. Cl. **C12b 1/02**

[58] Field of Search **195/127, 139, 142, 143**

References Cited

UNITED STATES PATENTS

2,992,974 7/1961 Belcove et al. 195/139

[57]

ABSTRACT

Improved devices are described for combined sampling and culturing to enable a measure of the micro-organism content of a liquid sample. The preferred structure is a long thin tube (e.g., a disposable sterile, transparent, pre-plugged serological pipette) having internal area thereof coated with a culture medium.

2 Claims, 4 Drawing Figures

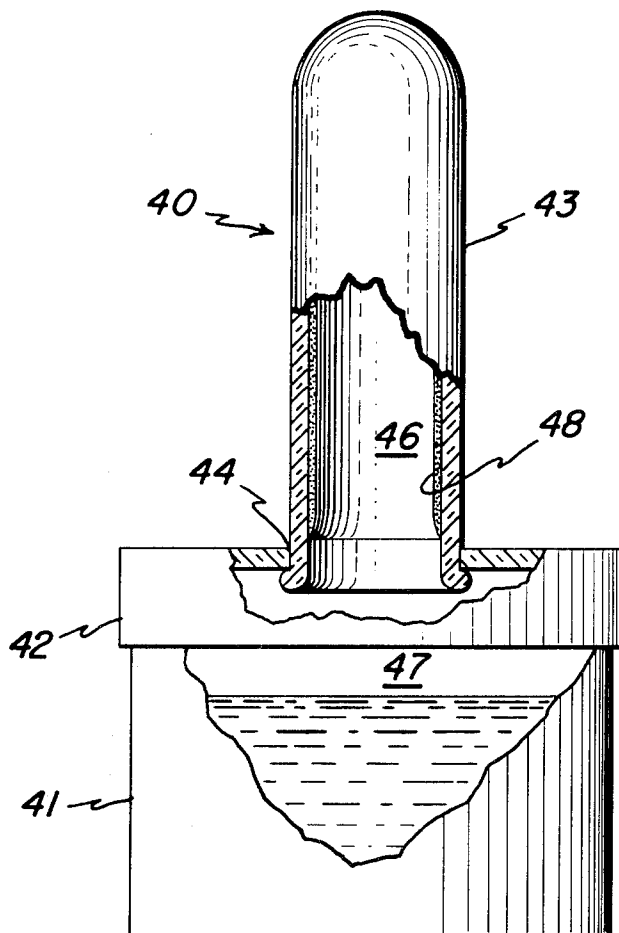


FIG. 1

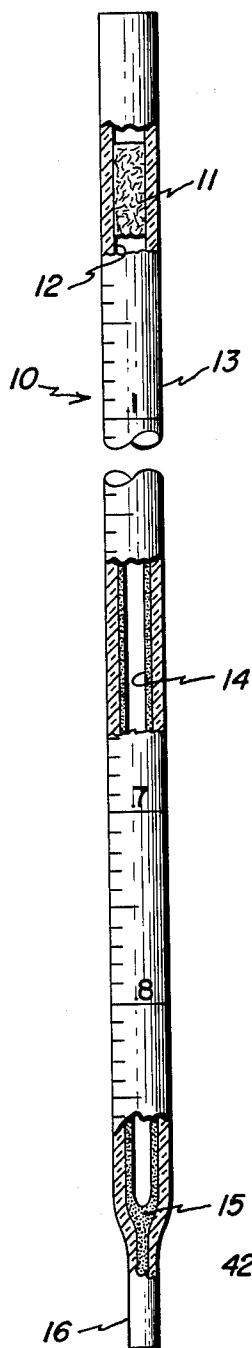


FIG. 3

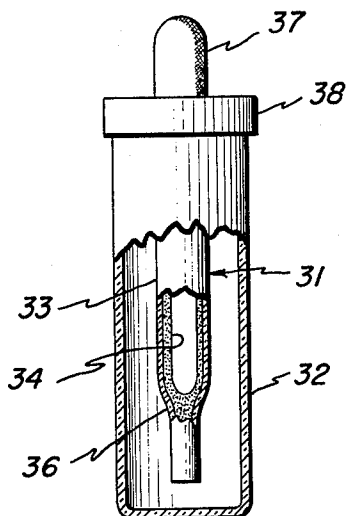


FIG. 2

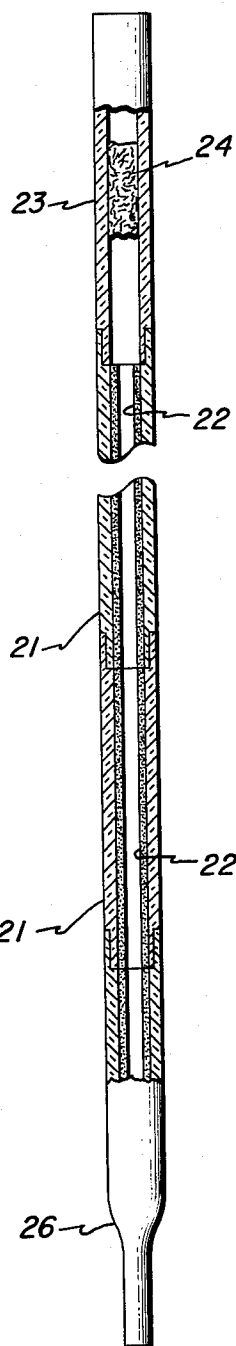
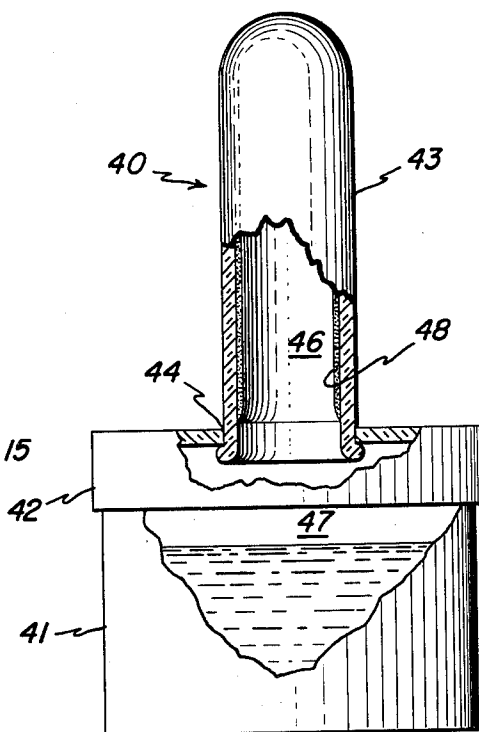


FIG. 4



COMBINED SAMPLING AND BACTERIOLOGICAL CULTURING DEVICE

This is a division of application Ser. No. 130,254, filed Apr. 1, 1971, now U.S. Pat. No. 3,838,013.

BACKGROUND OF THE INVENTION

The generally accepted clinical method for obtaining a quantitative measure of the microorganism content of a liquid sample is the pour-plate technique. According to this method a series (about six) of dilutions of the liquid sample are prepared. A known volume from each dilution is transferred to separate Petri dishes containing agar (not set) containing added nutrient at a temperature of about 45°C. Purified agar is the preferred suspending medium for the nutrient, because it is inert, non-toxic and has no nutrient value for most microorganisms. In each case the volume of sample dilution is stirred into the nutrient/agar mixture which is allowed to gel. Quantitative readout is obtained after incubation by counting the colonies per unit area in one or more dishes having a suitable colony density. This technique is too tedious and time-consuming for routine use. Further, the pour-plate technique poses relatively high volume requirements during incubation.

A semi-quantitative culture procedure developed to improve the economics of mass screening for bacteriuria consists of using a dropper pipette to dispense a single drop of urine when the tip is held near a plate coated with agar (containing added nutrient). The procedure is repeated with different patient samples of urine until one drop has been deposited in each of ten discrete areas of the plate. The plate is then placed in the incubator for 12-18 hour incubation. After incubation, the appearance of colonies within each drop as well as the appearance of the rim of that drop are compared to a series of standard pictures of known bacterial counts.

One other attempt to simplify this process is the "dip slide" technique in which a nutrient-coated surface (e.g., glass slide or a spoon coated with nutrient/agar) is removed from a sterile container, dipped into the liquid sample to immerse all the nutrient coating, drained, replaced and enclosed within the sterile container for incubation. In still another development, clear sterile polystyrene containers are lined with nutrient agar medium. Liquid sample is poured into the container, poured out again and the container is closed up to isolate the cultured medium and subjected to incubation. The latter development is described in "A Simple Quantitative and Qualitative Microbiological Screening Test for Bacteriuria" - Mackay-Scollay (J.clin.-Path.[1969], 22,651-653).

An instrumental method for counting viable bacteria and determining their antibiotic susceptibilities is described in "Capillary - Tube Scanner for Mechanized Microbiology" by Bowman, et al., [Science, Vol. 158, pp 78-83]. The suspension of bacteria (and antibiotics, when used) is added to melted nutrient/agar mixture. These materials are mixed and the new mixture is used to fill the desired number of capillary tubes. The filled tubes are then sealed at the ends with red sealing wax or plastic plugs. The prepared capillaries are scanned both before and after incubation. In a variant, the capillaries (to be filled later with a mixture of sample and nutrient) are said to be precoated "with a uniform standardized amount of antibiotic." The antibiotic is dis-

persed in a suspending medium, e.g., purified agar, which is inert, for introduction into the capillaries as a filament, later dried.

SUMMARY OF THE INVENTION

The devices of the instant invention greatly improve the simplicity of the sampling procedure and the efficiency of the entire technique by combining the functions of sampling and culturing. The preferred device is a disposable internally-sterile, transparent tube (e.g., a pre-plugged pipette of the type used in bacteriology or serology or a dropper pipette) that has been previously coated over a known area of the interior surface thereof with a culture medium for colony-producing microorganisms (i.e. bacteria, yeasts or molds). In addition, a combined sample container/culture tube is described wherein the culture tube (the inner surface of which is lined with nutrient medium) is detachably mounted on a wall of the sample container, the interior of the culture tube being in flow communication with the interior of the sample container. In this way the interior of the culture tube may be exposed to the liquid sample upon demand without the necessity of removing liquid sample from the sample container itself.

The term "solid" employed herein to describe the state of nutrient/agar deposits indicates that this material is present as a stiff gel capable of supporting itself as a layer along a wall.

BRIEF DESCRIPTION OF THE DRAWING

The exact nature of this invention as well as objects and advantages thereof will be readily apparent from consideration of the following specification relating to the annexed drawing in which:

FIG. 1 is an elevational view of an internally sterile pipette partly cut away to show the presence of a lining of nutrient medium and the plugs at both ends that maintain the sterile conditions;

FIG. 2 shows a modification of the pipette of FIG. 1 wherein joinable segments of tubing having the interior surfaces thereof covered with different preparations of nutrient media are connected to facilitate diagnostic use of and/or antibiotic susceptibility tests with the device of this invention;

FIG. 3 is a dropper pipette (internally coated and plugged at the lower end thereof according to this invention) and housed in a sterile container and

FIG. 4 is an elevational view (partly cut away) to show the combined sample container/culture tube combination.

DESCRIPTION OF THE PREFERRED EMBODIMENT

In FIG. 1 pipette 10 is shown made of a transparent material, preferably a plastic. Pipette 10 has plug 11 of cotton or similar fibrous material disposed in passage 12 at the mouthpiece end of barrel 13. The inner surface of passage 12 is covered at least in part by a layer 14 of a suitable solid culture medium. This same material forms tip plug 15 at the lower end thereof. After removal of plug 15 as described hereinbelow, the liquid to be assayed is drawn up into the bore by applying suction to the mouthpiece end thereof, e.g., with the mouth in the usual manner or by use of a flexible bulb (not shown).

The presence of the sterile barrier (plug 11) insures protection against the passage of microorganisms

therethrough in either direction. At the same time, if a suitable ambient is provided for culturing (i.e., oxygen-containing for aerobic bacteria; non- or low-oxygen content for anaerobic bacteria), plug 11 removes the need for hermetically closing off the mouthpiece end of pipette 10. This aspect is important, because of the automatic prevention or minimization thereby of fogging of the pipette wall, an objectionable condition frequently encountered in fully closed culturing devices.

Layer 14 and plug 15 of nutrient are introduced by drawing the nutrient into the sterile pipette in the warm, free-flowing state to some desired height and then permitting the nutrient to drain. A thin coating of nutrient remains on and uniformly coats the interior surface of the barrel 13. The nutrient material gels and the last portion of the draining nutrient, being more viscous as it begins to gel, accumulates in dropper tip 16 forming plug 15. The presence of plug 15 is a positive indication that the pipette has been coated and has not been used. In this manner both the nutrient layer 14 and plug 15 (to maintain sterile conditions within the pipette together with plug 11) are formed in the same process. Immediately before use, it is merely necessary to apply pressure within the bore (as by blowing through the mouthpiece) sufficient to dislodge plug 15. Removal of this plug serves to clear the tip 16 for the sampling/culturing and, as well, to leave plastic surface exposed in passage 12 at tip 16 for bonding thereto of the later applied clay plug (not shown) described hereinbelow. In addition, this returns the tip to the original dimensions and surface conditions that determine flow and drainage characteristics.

In use the liquid is drawn into the pipette to some predetermined height below the upper limit of the layer 14 and then permitted to drain therefrom. Liquid-to-nutrient contact is, therefore, made at least two times with pre-plugged pipettes. If unplugged (without plug 11) tubes are used, a single liquid-to-nutrient contact may be made, if preferred, by raising liquid in the tube, quickly turning the tube end-for-end and permitting drainage to occur.

In the case of pipette 10 the open end of dropper tip 16 is then replugged, e.g., by pushing tip 16 into a thin sheet of clay; the external surface of pipette 10 is disinfected, and the unit is ready for incubation in the vertical position at room temperature or in a thermostatically controlled oven (not shown). Incubation is usually carried out at a temperature of about 37°C for a period of about 18–24 hours. A very large number of these culture pipettes can be stored or incubated in a small volume.

After incubation, the pipette may be readily examined for microorganism growth (e.g., colonies of bacteria) using reflected light in the background by disposing the pipette with the longitudinal axis thereof at a small angle with the rays emanating from some light source. Visual readouts for bacteria concentrations ranging from 10^3 – 10^9 bacteria/milliliter may be readily obtained. By way of illustration, urine specimens containing more than 10^5 viable bacteria per milliliter are generally considered to be from patients with an active infection of the urinary tract. At the lower concentration (up to and including 10^3 bacteria/cc) the pipette will appear substantially free of colonies. Similarly, at very high concentrations (10^7 – 10^9 bacteria/cc) the barrel of the pipette may appear (to the naked eye) to be free of colonies except that at the margin between the

inoculated and uninoculated nutrient a clearly defined ring occurs. Seeing this ring, the observer will recognize that individual colonies are not visible, because the colonies are microscopic or because the nutrient has become completely overgrown indicating, thereby, a very high bacteria concentration. At the concentration in the 10^4 – 10^6 bacteria/cc range proportional densities of colonies per unit length of pipette will be seen.

Various suspending media (other than purified agar) may be used, e.g., agarose, cellulose derivatives, gums, pectins and gelatin. Nutrients added to the suspending medium selected may be, for example, tripicasesoy broth, veal infusion or brain-heart infusion media.

Cultures from samples (e.g., urine) taken from specific patients may be easily identified by the use of color coding (e.g., layers of different colors of clay forced into the dropper tip or colored markings at the mouthpiece end of the pipette) or of dual number tape of which one number is separated and attached at the mouthpiece end.

After use, plastic pipettes may be easily disposed of by heating to sterilizing temperatures above the softening point thereof further reducing the volume occupied thereby.

The pipette configuration per se (as contrasted to a simple small diameter tube coated as described herein) is particularly useful, because the restricted orifice at the tip end imparts important flow characteristics to the device. Thus, this restriction provides the technician with control capabilities over the intake of a sample of liquid in a cohesive mass and control over the drainage rate to promote uniform distribution of the liquid sample over the nutrient area. The former capability enables the intake of, and inoculation with a slug of less than 0.2 cc volume with a nominal 1 cc pipette, a feature of considerable importance in culturing urine samples from babies, old people or people being catheterized, for example. This low sample volume requirement makes more of a given sample available for microscopic and chemical tests. Any culture tube such as is described in the Mackay-Scollay article into which liquid sample may be readily poured would require at least 2–3 cc of liquid sample for effective inoculation.

Considering any cross-section transverse to the central axis of the longitudinally-extending nutrient-lined tube of this invention, it is preferred that the open area (the nutrient-lined bore) should have a perimeter between a maximum of about 0.50 inch (to reduce the requisite volume of sample to acceptable levels) and a minimum of about 0.15 inch (to reduce confusion between diametrically located colonies during viewing). However, bore perimeters ranging from about 0.06 inch to about 0.90 inch may be used.

Although the tubes employed are ordinarily prepared as right circular cylinders, (except for the dropper tip) the structure (and passage 12) need not be in this form, but may take other geometric shapes in cross-section, e.g., triangular, square. The pipette configuration is also useful as a tool for mixing a liquid sample (e.g., urine) to render it homogeneous before culturing.

In the case of transparent tubes internally uniformly coated with nutrient and not equipped with plug 11 or dropper tip 16 containing plug 15, such tubes may be sterilized after manufacture by being sealed in a plastic container and then irradiated. After inoculation they may be sealed on both ends.

If desired, passage 12 may be lined with a series of successive layers of different nutrient materials overlapping in part the preceding layers so as to expose a bore of a plurality of different nutrient media in sequence to the inoculation process and providing a useful diagnostic mechanism. Thus, a nominal 1 milliliter pipette was coated internally with Trypticase Soy Agar (TSA), that was in turn provided with a partial overlay (about half-way up the barrel) with MacConkey agar. A gram positive organism was cultured therein. The gram positive bacteria grew on the TSA, but did not grow on the MacConkey agar as anticipated.

In addition, one of these nutrient materials in the overlapping relationship may contain antibiotics mixed therein in order to simultaneously determine antibiotic susceptibility of the cultured organisms. Such thin layers of nutrient/agar may be applied within these pipettes that even layers using expensive nutrient/agar can be used economically.

An alternate construction for achieving such a multimedia exposure is shown in FIG. 2 wherein a tubular construction is approximated by connecting together a series of interconnectable modules 21. These modules can be prepared so that each has a lining 22 of a different nutrient thereby providing for a selection of nutrient media for inoculation from the same sample. One of the modules 23, useful as the mouthpiece, contains plug 24 of cotton or suitable cellular material. Module 26 may be used to provide a dropper tip. Once assembled as by snapping the modules together, a plurality of these modules 21 provides a longitudinally-extending tubular nutrient-lined pre-plugged bore for use in substantially the same manner as has been described hereinabove.

The modular construction of FIG. 2 provides the opportunity not only of easily testing for antibiotic susceptibility with a number of antibiotics, but also facilitates the use of different concentrations of antibiotics to enable a determination of both the correct antibiotic for use and the optimum concentration thereof. Further, this testing device remains inexpensive, because of the thin layers of nutrient/suspending medium required.

Also, as is shown in FIG. 3, a dropping pipette 31, e.g., a nose dropper, removably housed in a sterile container 32 and modified according to this invention may be used. Barrel 33, thereof is uniformly internally coated with nutrient/suspending medium layer 34 (as described for the pipette construction hereinabove) and this will result, as well, in the formation of tip plug 36. Air pressure is utilized (as in the previous embodiments) to accomplish transport of sample liquid from the sample container to the nutrient layer. This is done in dropping pipette 31 by the application of suction by squeezing and then releasing flexible (e.g. rubber) bulb 37.

The culture is prepared by removing pipette 31 (with bulb 37 and cap 38 affixed thereto) from container 32, dislodging plug 36 by forcefully squeezing bulb 37, inserting the tip of pipette 31 into the sample liquid and releasing the squeezing force thereby permitting bulb 37 to return to its normal shape. The suction so generated will draw sample liquid up into pipette 31 to coat a given portion of the layer 34. The liquid is then expelled and pipette 31 is returned to container 32 and cap 38 is re-connected to container 32. The entire unit

39 (inoculated pipette 31 and container 32) is then incubated.

Another device for combining the function of sampling and culturing is shown in FIG. 4. This unit 40 is of particular value in those instances in which the donor deposits the liquid to be assayed, e.g., urine, into cup 41 provided with top 42 that may be hermetically joined thereto (e.g., by snap-on fit or screw threads) after the sample has been contributed. In the construction shown a culture tube 43 is removably connected to top 42 by means of a hermetic juncture at collar 44 that places volume 46 in flow communication with volume 47 (the interior of cup 41).

The inner wall of culture tube 43 is covered uniformly over a known area with a suitable solid culture medium. Thus, it is merely necessary to invert unit 40 to cause liquid sample to enter culture tube 43 without nuisance or unsanitary exposure and thereby be brought into contact with layer 48 of the culture medium. After inoculation, unit 40 is returned to the upright position for storage or transport.

The inoculation can be made very shortly after the sample is obtained and, thereafter (as, for example, during the time of transit of the specimen between the clinic and the laboratory), culture tube 43 will drain. After draining, it is simply necessary to separate tube 43, from top 42, affix a top (not shown) thereto and subject tube 43 to incubation in the vertical position. The balance of the liquid sample in cup 41 may be properly covered to await the conduct of other tests. Thus, the structural combination provided in unit 40 obviates the need to remove liquid sample from the sample container, as by pouring, to obtain a culture.

The devices of this invention are particularly useful for obtaining counts of the microorganism content of urine specimens, as for screening purposes. In addition to clinical applications any liquid with microorganism content in the range of concentration suitable to the device may be examined in the same manner.

In culturing anaerobes, the pipette must be maintained in a low-oxygen, or oxygen-free, environment during incubation or the pipette (after inoculation) can be charged with such environment and sealed.

With those nutrient materials which undergo a color change as bacterial growth develops thereover, it may be necessary to deposit a relatively thick layer of nutrient material within the barrel of the pipette or culture tube. Application of the teachings set forth herein should be easy for the technician to prepare the necessary structure. A large enough diameter pipette is selected so that the nutrient-lined passage remaining after introduction of the nutrient will have a cross-section area within the limits set forth hereinabove and claimed hereinbelow.

It is recognized that many bacteriological tests depend upon the identification of specific attributes of colony growth, i.e., size and shape of colonies, surface characteristics, color and sheen, and upon color changes within the specific agar(s) used. Thus, the tube or pipette should be made of a material permitting easy sectioning through the wall thereof in the region of any colony of interest so that the colony can be transferred for study by standard methods.

What I claim as new and desire to secure by Letters Patent of the United States is:

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1. A device for the dual purposes of sampling a liquid and culturing microorganisms present therein comprising in combination:

- a. a container cup defining a first volume for receiving and containing a liquid and having closure means therefor and
- b. a transparent tube having an open end and a closed end removably and sealably connected at its open end to said closure means and projecting outwardly therefrom,

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- c. at least one layer of sterile solid nutrient material for the growth of microorganisms covering the inside surface area of said tube and defining a second volume, said second volume being in flow communication with said first volume.

2. The device of claim 1 for the dual purpose of sampling and culturing wherein the connection between said closure means and the nutrient-lined tube is a snap-on connection.

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