United States Patent

[11] **3,632,478**

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[45] Patented **Jan. 4, 1972**
Continuation-in-part of application Ser. No. 579,581, Sept. 15, 1966, now abandoned. This application Nov. 25, 1968, Ser. No. 778,678

[54] **DISPOSABLE CULTURE ASSEMBLY**
3 Claims, 7 Drawing Figs.

[52] **U.S. Cl.** **195/139,** 195/103.5, 195/126, 195/120
[51] **Int. Cl.** **C12k 1/06,** C12b 1/16
[50] **Field of Search** ..... 195/139 LE, 139, 126

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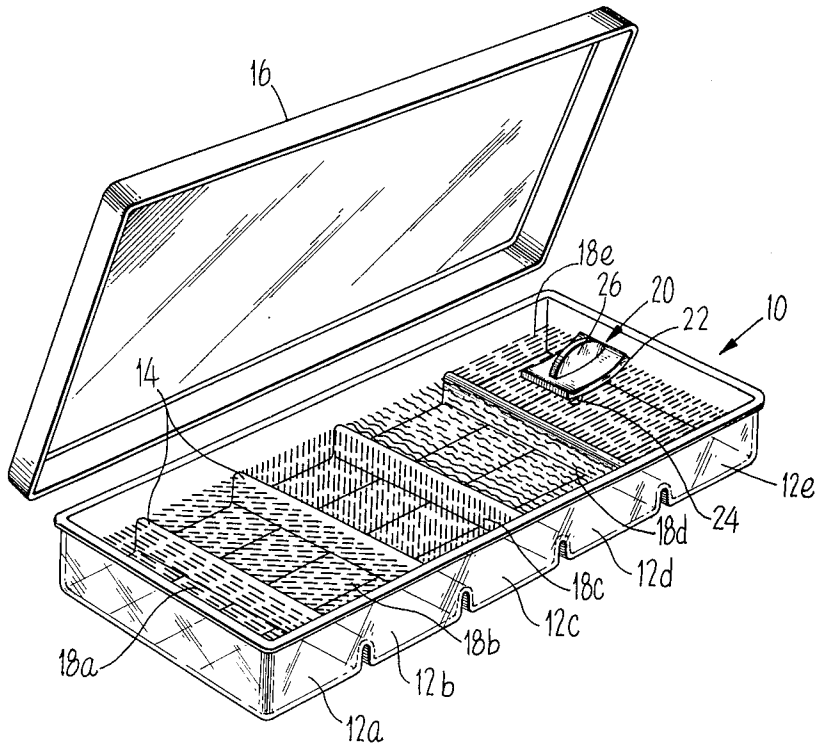
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ABSTRACT: A disposable culture assembly incorporates a uniformly compartmented rectangular culture plate containing solid media, a probe device that affords an anaerobic bacterial analysis. Sterile test conditions are realized by providing a sealed container including a sterilized anaerobic probe device, angled specimen spreader having one dimension coextensive with one dimension of each plate compartment, and a dropper to achieve control of the amount of specimen to be applied to the surfaces of the media.



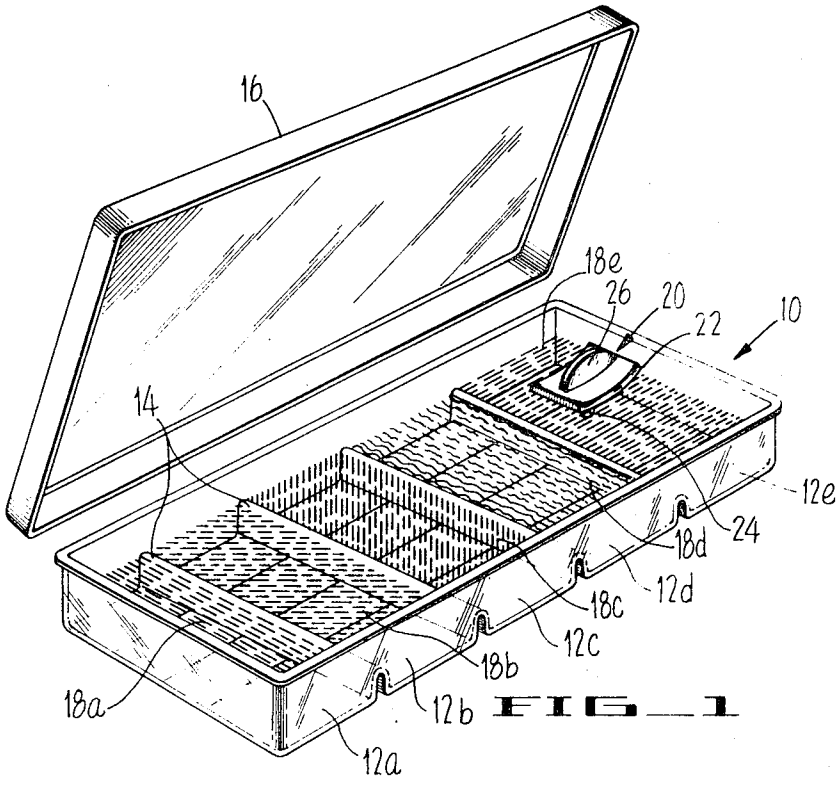


FIG_1

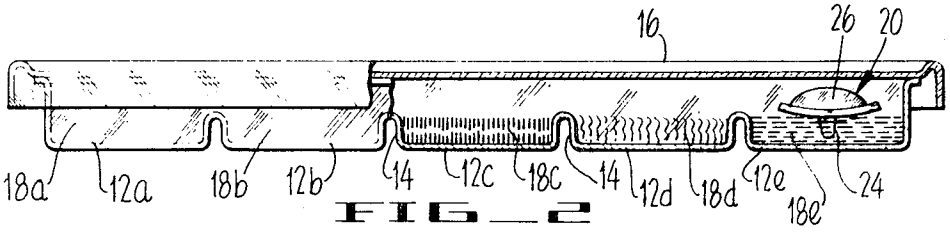


FIG_2

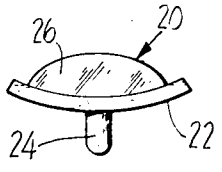


FIG_3

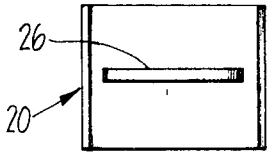


FIG_4

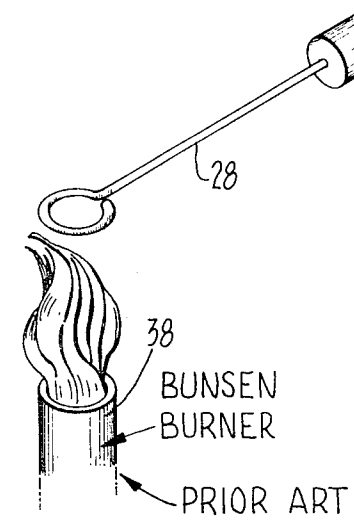


FIG_5A

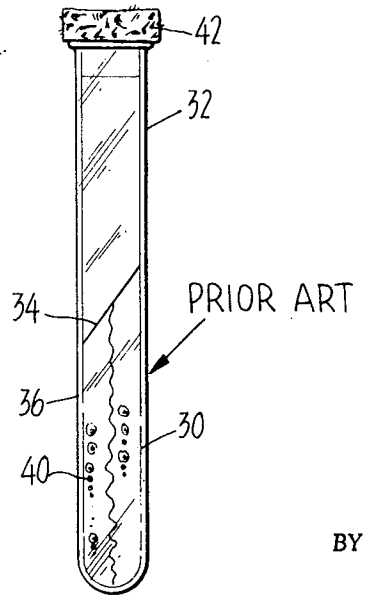


FIG_5B

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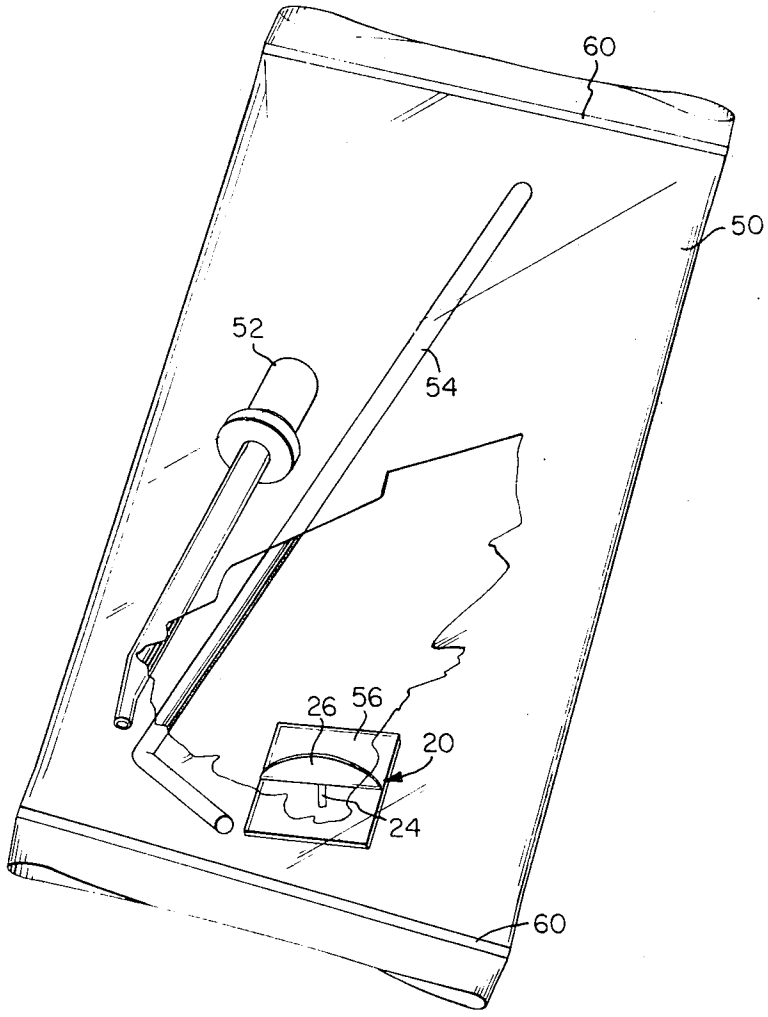


FIG. 6

DISPOSABLE CULTURE ASSEMBLY

CROSS-REFERENCE TO RELATED APPLICATION

In copending U.S. Pat. application Ser. No. 556,484 filed June 9, 1966 and now abandoned and assigned to the same assignee, there is described a novel and improved disposable culture device which affords expeditious and convenient bacterial analysis in a less expensive and less time-consuming manner. It would be advantageous to employ such an integral culture plate containing a multiplicity of media, and to include means for achieving aerobic and anaerobic conditions simultaneously in these media.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to a disposable culture assembly, and in particular to a bacterial analysis kit that enables both aerobic and anaerobic analyses.

2. Description of the Prior Art

The present practice of bacterial analysis comprises a primary isolation phase to establish the existence of bacterial infection, followed by a differential evaluation stage to determine the kind or species of bacteria. These steps are usually accomplished in sequence, and require separate laboratory tools and aids, as well as separate procedures. These sequential stages or phases each take from 18–24 hours, such that almost 2 days elapse before a final determination is made of the presence of infection and the type of bacteria. Thus, a substantial time is expended before the practitioner can prescribe the correct type and dosage of antibiotic.

Furthermore, at present, culture analysis of a specimen taken from a subject or patient requires a plurality of containers or receptacles, such as petri dishes and test tubes having different media. For example, in one well known analytical technique, specifically for differential analysis, a single culture medium such as triple Sugar Iron (TSI) agar is disposed separately and independently in a test tube in a configuration providing a solid "butt" and an angled or "slant" surface. A sterile loop wire is inserted into the suspect specimen and then the loop is stabbed into the butt and followed by streaking along the slant to inoculate the specimen onto the surface of the TSI agar. In this manner, an aerobic condition is established at the slant for the bacteria being cultured, whereas an anaerobic condition is set up within the mass of the butt.

Triple Sugar Iron (TSI) agar is routinely employed test tube differential medium. TSI and also Urea agar are utilized as the initial differential analytical media after the primary plating phase has established the presence of a bacterial infection. Inoculation of the tubed TSI agar is generally accomplished by means of a pure culture specimen to effect differential identification. In its uninoculated state, TSI contains three sugars, glucose, lactose, and sucrose; and, in addition, phenol red indicator, and ferrous sulfate. The glucose concentration is about one-tenth of the concentration of lactose and sucrose, whereby the fermentation of glucose alone may be detected. The small amount of acid produced by fermentation of glucose is oxidized rapidly in the slant portion, when freely exposed to atmospheric (aerobic) oxygen and accordingly the slant will revert rapidly from a yellow (acid pH) to the uninoculated alkaline red color or remain red (alkaline pH). In contrast, under lower oxygen tension (anaerobic) in the butt, the acid yellow reaction is maintained. To promote the aerobic condition in the slant, free exchange of air is essential, achieved by replacing screw caps with loose cotton plugs.

A common urine infecting organism, for example, *Proteus Mirabilis* is a glucose fermenter but a nonlactose fermenter and would display an acid (yellow) butt and alkaline (red) slant. Such an organism, by forming hydrogen sulfide gas, can affect the ferrous sulfate compound, so that a black coloring develops in the anaerobic butt portion of the test tube allowing further type specificity. Still another property of this TSI agar can be the development of gas bubbles trapped in the anaerobic butt portion of the tube, typical of *E. coli* bacteria for example. It is apparent that establishing an anaerobic condition and expediting bacterial growth under such condition are highly desirable.

SUMMARY OF THE INVENTION

An object of this invention is to provide a novel and improved disposable culture assembly.

Another object of this invention is to provide a means that enables an anaerobic bacterial analysis as well as aerobic analysis.

Another object is to provide suitable initial reference conditions, including a sterile condition, so that error is minimized for bacterial culture analysis.

In an embodiment of this invention, a disposable culture apparatus comprises an integral plate of substantially rectangular geometry, having a multiplicity of substantially rectangular compartments. Selected media are disposed respectively in such compartments to afford simultaneous culturing of bacterial colonies, such that visual analysis, by color change and bacterial growth or count for example, may be effectuated simply and expediently. Furthermore, the culture apparatus comprises a probe device having an inoculating surface configuration that allows establishing an effective anaerobic condition, and which affords an environment that is conductive to more rapid bacterial metabolism, thereby providing quicker analysis and results.

An additional feature is the provision of a sealed, airtight sterile package for containing the specimen processing tools, that are used to apply and distribute the specimen uniformly, and to achieve an anaerobic effect.

BRIEF DESCRIPTION OF THE DRAWING

The invention will be described with reference to the drawing in which:

FIG. **1** is a perspective view of the culture apparatus, in accordance with the invention;

FIG. **2** is a side elevational view of the novel culture apparatus;

FIG. **3** is a side view of the inventive anaerobic probe device, used in combination with a culture plate, in keeping with this invention;

FIG. **4** is a top view of the anaerobic probe device;

FIGS. **5A** and **5B** are views of apparatus used in the prior art for culturing bacteria in a single medium, and

FIG. **6** is a plan view of a transparent sealed plastic package containing liquid specimen handling tools and anaerobic disk, for cooperating with the media of the culture plate, in accordance with this invention.

Similar numerals refer to similar elements throughout the drawing.

DESCRIPTION OF THE PREFERRED EMBODIMENT

With reference to FIGS. **1** and **2**, a disposable culture plate or dish **10** is formed with a multiplicity of right angle sections or compartments **12***a–e*, which are delineated by ribs **14** that extend from side to side across the width of the dish **10**. A cover **16** is provided for capping and enclosing the culture dish or plate **10**, and provides a substantially close-fit cover while still allowing requisite air transfer when positioned thereon. The plate **10** and cover **16** are made preferably from a transparent plastic, such as polystyrene, which is inexpensive and therefore disposable after a single use, with a minimum of economic sacrifice.

Each compartment **12** contains a medium **18**, each of which is adapted for culturing certain types of bacteria. For example, the partitioned rectangular plate may contain Blood agar in compartment **12***a*, EMB agar in compartment **12***b*, MacConkey agar in compartment **12***c*, Urea agar in compartment **12***d*, and TSI agar in compartment **12***e*.

Each compartment is approximately the same in dimension, and contains a similar quantity of agar. Thus, the substantially

simultaneous introduction of a foreign agent or analytical sample in each compartment establishes a "zero reference" condition against which subsequent bacterial growth may be measured at the same time in the several compartments. The measurements may be made visually or otherwise, in a facile and expedient manner, since all the media and cultures are positioned side by side in an integral plate providing easily measureable geometric areas.

Furthermore, calibration marks or indicator lines may be inscribed across the bottom of selected compartments, whereby the compartment areas are subdivided into equal zones, or quick count zones. These zones may represent, when totally occupied by bacterial colonies, a predetermined bacterial count. In this manner, the medical practitioner or laboratory analyst may quickly determine the degree of infection of the subject under study, after allowing a given time for bacterial growth in a culture oven, by way of example.

In keeping with this invention, a probe device **20** is employed in conjunction with differential media, such as TSI agar **18***e*, for establishing an anaerobic condition whereby differential analysis is accomplished. The probe device **20** (see FIGS. **3** and **4**) has a rectangular convex surface **22**, with a protuberance or tine **24** projecting from the center thereof. On the opposite surface, an arcuate handle **26** is provided for facile grasping or holding by the fingers of the practitioner. The handle **26** serves to avoid contamination of the surface **22** and tine **24** to which the specimen bacteria are introduced. The probe device **20** is formed preferably from an inexpensive clear plastic that is feasibly disposable after each use.

In practice, predetermined amounts or calibrated drops of specimen are aseptically introduced by means of a dropper **52** (see FIG. **6**) to each of the media **18** substantially simultaneously. In particular, two measured drops are placed at spaced portions of the differential medium, TSI agar **18***e*. Within the area of one of the drops, the probe device **20** is inserted with the tine **24** breaking the surface of the medium **18***e*. The device **20** is positioned by means of the handle **26** onto the TSI agar until a major portion of the convex surface **22** is in close contact with the surface of the agar, thus displacing any trapped air bubbles. The convexity of the surface **22** serves to force air out of the area between the surface of the medium **18***e* and the surface **22** of the probe device **20**, thus producing a true anaerobic condition. The tine **24** carries some of the specimen and its bacteria into the subsurface mass of the medium **18***e* thus inoculating the depths of the medium. By means of the inserted tine **24** and the contiguous relation of the convex surface **22** and the surface of the medium achieved by tine fixation and surface tension cohesion, a substantially broad region for anaerobic development is established. Furthermore, it should be noted that when the media plate is turned over, i.e., with the cover downward, to eliminate moisture, this surface tension cohesion and tine fixation prevent the probe device **20** from falling out of the medium, which could occur as a result of gravitation or other applied forces. After positioning the probe device, the remaining portion of the TSI agar is streaked with a right angle rod **54** (FIG. **6**) to simulate the tube "slant" while the tine device simulates the tube "butt".

The culture is placed in a culture oven wherein the bacterial development proceeds under both aerobic and anaerobic conditions. By providing both primary isolation media and differential media in the same integral container for simultaneous bacterial development and analysis of both aerobic and anaerobic conditions, the practitioner can now realize a substantial savings of time in the laboratory. The use of the inoculating probe device **20** affords a much more rapid growth of bacteria under anaerobic conditions than achieved heretofore. Furthermore, the inventive probe device is useful for transferring bacteria from one medium to another in the same container (or to other containers of media) by simple penetration of the tine into a colony, and then inoculation into the second selected medium.

FIGS. **5A** and **5B** illustrate the devices and techniques followed by prior art practitioners. A looped wire **28** containing a sample of the specimen to be analyzed is used to inoculate a differential medium **30**, such as TSI agar, in a test tube **32**. The TSI agar **30** is so disposed within the tube **32** to provide a slant surface **34** and a butt area **36**, as explained supra. The looped wire **28** is first sterilized by heating over a burner **38** and introduced to the sample or specimen, and then stabbed into the butt **36** and streaked across the slant surface **34** of the differential medium **30**. The stabbing or penetration of the butt **36** creates the desired anaerobic condition for bacteria growth, which is subsequently evidenced by change in color and production of gas bubbles **40**. At the same time, aerobic culturing of bacteria occurs along the slant surface. To enable aerobic slant growth without undue accumulation of moisture or dust contaminants, a porous cotton cap **42** is used to close the test tube **32**. With this technique, anaerobic growth is relatively slow since the region affected initially by the loop wire is limited, when compared to the broader inoculating surface provided by the probe device of the instant invention.

To ensure that the probe **20**, dropper **52** and spreader rod **54** are all sterile at initiation of the analysis, these parts which are to contact the media surfaces are stored in an airtight enclosure **50**, which may be a plastic bag closed by a heat-sealing process along ribs **60**. The dropper **52** has a predetermined volume and configuration, so that a measured amount of specimen is dispensed to be spread over the known surface area of each medium.

The invention is not limited to the use of the particular assembly or media container design or specific probe device disclosed therein. The culture plate and the probe device need not be rectangular, but may be elliptical, trapezoidal or the like. A multiplicity of tines, in lieu of a single tine, may be used with the probe device. Also, a thin plastic or glass film or slide or other flat planar surface may be used for simulating the anaerobic condition by contiguous contact with the surface of the differential culture medium. A concave plate or cap may alternatively be utilized to establish the desired condition. However, applicant has discovered that the combination set forth in this application affords a most expedient and optimum analysis of bacterial development. Furthermore, since both the integral media container, the probe device, spreader and dropper are made from an inexpensive plastic, and thus are disposable, it is possible to eliminate repeated cleansing, sterilization, incubations, and separate handling of test tubes, Petri dishes, wire loops, inoculating needles and the like, as is necessary in prior art practice. Also, the possibility of error in multiple labeling and identification of the tubes and plates of specimens from different subjects and patients is precluded. Since the media container and probe device are made preferably of clear plastic, the practitioner can visually observe color change and bacterial colony growth to make a rapid, expedient determination.

By means of this invention, it is now possible to conduct simultaneously a primary isolation determination for existence of bacterial infection, and a differential analysis to ascertain the type of kind of bacteria present.

What is claimed is:

1. A disposable culture assembly comprising:

a transparent plate having a plurality of serially disposed rectangular compartments of like geometry and dimensions;

a cover for providing an airtight sealed container in combination with said plate;

solid culture media in each of said compartments, each medium being of the same volume and disposed to the same height in each compartment; and

inoculating means formed from a transparent plastic body having a convex surface and a tine projecting from the center of said surface, and a handle projecting from the surface opposite to said convex surface, so that upon inoculation of a specimen adhering to said tine into said media air is displaced by said tine and said convex surface

to form an anaerobic condition for said specimen at the surface and below the surface of the inoculated media.

2. A disposable culture assembly as in claim 1, wherein each compartment has a different culture medium.

3. A disposable culture assembly as in claim 2, wherein the different media are of the primary and differential analysis type.

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