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D. R. WARREN

3,817,839

BI-MEDIA DIP PLATE

Filed March 29, 1971

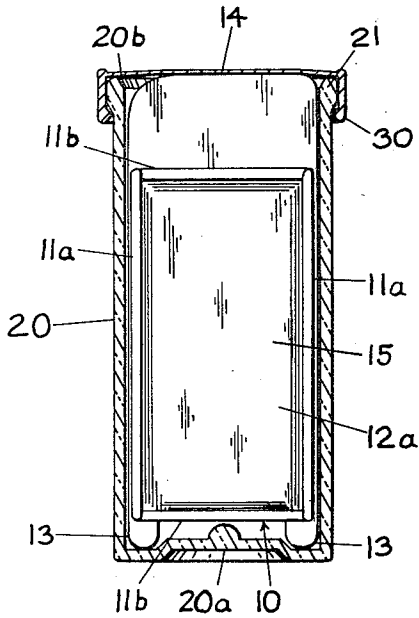


Fig. 3

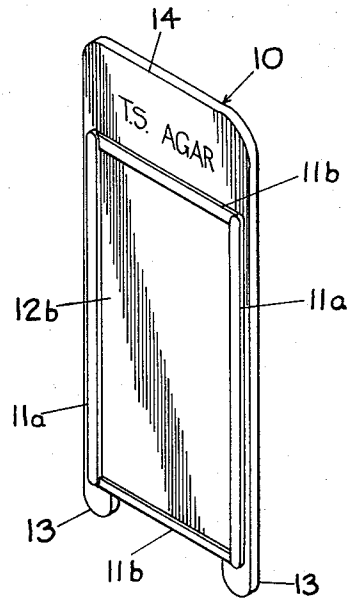


Fig. 1

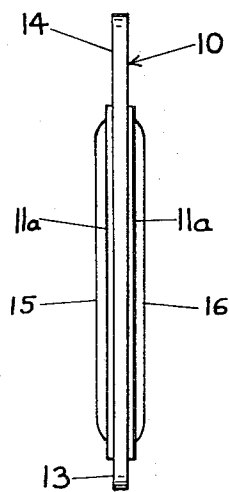


Fig. 2

INVENTOR.
DON R. WARREN

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BI-MEDIA DIP PLATE

Don R. Warren, 715 S. Few St.,
Madison, Wis. 53703

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1 Claim

ABSTRACT OF THE DISCLOSURE

A bi-media dip plate for use in performing a method of bacterial quantitation and differentiation. The dip plate has a raised border on each opposed face for separating finite areas of deposit for culture media from potential contaminants in contact with the edges of the dip plate and has legs for spacing the area of deposit above a supporting surface. The dip plate is used in combination with a vial which maintains the dip plate in a stable, erect position.

BACKGROUND OF THE INVENTION

(1) Field of the invention

This invention pertains generally to devices for supporting culture media for use in performing methods of screening for the presence of bacteria, and more particularly to a bi-media dip plate for use in performing a method of bacterial quantitation and differentiation.

(2) Description of the prior art

Chemical methods of detecting bacteriuria and the like have been preferred by doctors and hospitals because of the speed with which the test may be completed. However, such methods suffer from a high percentage of false-negative results which seriously impair their usefulness for screening patients.

Other methods such as the pour plate colony count or the use of calibrated loops have not been accepted because of the additional time and labor required by the older methods. Quantitative methods require prompt shipment of samples to the laboratory in order to obtain an accurate reading.

A method of diagnosing micro-organisms is disclosed in U.S. Pat. No. 3,368,549. A diagnostic swab coated with a culture medium is brought in contact with an area of the anatomy of suspected infection. The innoculated swab is transferred to a test tube and incubated in an atmosphere conducive to the growth of the suspected micro-organism. The culture medium contains inhibitors to prevent the growth of any other organisms which might be mistaken for the specific micro-organism being tested for.

A similar method has been adopted for use in testing for bacteriuria. One such diagnostic swab is coated with a culture medium such as Eosin-Methylene Blue (EMB) agar which inhibits the growth of contaminating bacteria. A second swab is coated with a culture medium such as nutrient agar which will support the growth of contaminating bacteria as well as urinary tract supported organisms.

Both swabs are inoculated with urine and placed in separate vials for incubation. A comparison of the two swabs will indicate whether any growths on the EMB agar are a result of the presence of bacteriuria, or may be due to contaminating bacteria. A comparison of the EMB agar swab to various photographic reference standards indicates the quantitative bacteria growth.

Unfortunately the cost of performing the test and the time required to complete it are quite substantial because two separate swabs, two incubating vials, and two separate inoculations must be performed to complete one test.

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A slide culture method of bacteriuria has been devised using ordinary glass microscopic slides. The method is basically the same as the dual swab method except that the two differentiating culture media are placed on either side of a single glass slide. Only one inoculation is required to inoculate both media and incubation is carried out in a single vial. Thus there is a saving in time and expense.

Placing both culture media on a single slide has produced many new problems. Condensation and excess test specimen which collects in the bottom of the incubating vial may reinoculate the culture media to adversely affect the test result. Also condensation collecting at the edges of the glass slide can dissolve the water soluble dyes in the EMB agar. These dyes may then diffuse to contaminate the nutrient agar, or other culture medium, on the opposing face of the slide. This results in no differentiation between the bacteria growth on the test medium and that on the control medium. A final problem is that it is difficult to visually distinguish the bacteria growth on one side of the glass slide from the growth on the other side. This of course is because both the glass slide and the culture media are transparent. As a result it is difficult to make a quantitative determination of the bacteria growth on the test medium.

A spacer is often included in the bottom of the incubating vial to prevent reinoculation of the test media. However, the spacer may adhere to the bottom of the glass slide during incubation. Care must be exercised when removing the slide so that the spacer does not flip up as the slide is retracted to contaminate the media and spoil the test just before it is completed.

SUMMARY OF THE INVENTION

I have invented an improved bi-media dip plate for use with the slide culture method of bacteria quantitation.

My dip plate is fabricated from plastic and has a raised border on each face encompassing a finite area thereon for the deposit of a culture medium. The raised border spaces and isolates the culture media from the juncture of the edge of the dip plate and the wall of the incubating vial. Such spacing effectively separates the culture media from contact with any condensate forming on the abutting wall of the vial. This in turn prevents solubilizing and subsequent contamination of the test media by the condensate.

The dip plate also has a pair of legs extending downwardly from the lower edge of the plate. When the innoculated dip plate is placed in the incubating vial the legs space the culture media above any condensation or excess test specimen that may drain into and accumulate at the bottom of the vial. The media are safe from reinoculation and there is no cumbersome or awkward spacer needed.

The dip plate can be transparent or opaque. It can also be manufactured in any color. This makes it possible to employ numerous combinations of different culture media, selecting a dip plate in a color appropriate to provide a proper optical contrast between the dip plate and the bacteria growth. Also the problem of confusing the bacteria growth on one side of the dip plate with that on the other side is eliminated by use of a non-transparent dip plate in almost all cases.

Further objects and advantages will become apparent from the following detailed description together with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a perspective view of a dip plate exemplifying my invention.

FIG. 2 is a side elevation of the dip plate of FIG. 1 with culture media applied thereon.

FIG. 3 is a front elevation of the dip plate of FIG. 2 enclosed within a capped cylindrical incubating vial which is shown in section.

DESCRIPTION OF A PREFERRED EMBODIMENT

Referring now more particularly to the drawings my dip plate is shown generally at 10.

The dip plate 10 is an elongated sheet of high pressure molded high impact polystyrene. It is produced in any color depending on the optical contrast required between the culture media and the bacterial colonization thereon. For urine cultures, for example, a black colored dip plate 10 is preferred to prevent the reading of colony counts on one side upon observation from the other side. The colony counts may be read easily with reflected light. For throat cultures, on the other hand, the dip plate 10 is made of transparent polystyrene because hemolysis of the red blood cells may be observed more easily with transmitted light.

A margin is allowed at the top of the dip plate 10 as a handle 14 for grasping the plate during inoculation and evaluation. The handle 14 also provides a space on each face of the dip plate 10 for labeling or imprinting selected indicia for promotional or informational purposes. For example one face might be labeled "T.S. Agar" to advise the user that the culture medium coated thereon is tryptic soy agar, as illustrated in FIG. 1.

Raised borders 11a and 11b on opposite faces of the dip plate 10 encompass finite areas 12a and 12b for the deposit of culture medium. The raised borders 11a and 11b have a width of approximately .047 inches. Side borders 11a have a height of .015 inches, whereas end borders 11b have a slightly lower height of .012 inches to facilitate handling of the dip plate during application of the media. Finite areas 12a and 12b are one inch wide and two inches long.

The function of the raised borders 11a and 11b is to prevent contamination of the culture media on the finite areas 12a and 12b. The raised borders 11a which extend downwardly along the sides of the dip plate are spaced inwardly a uniform distance along their length from the side edges of the dip plate and thus uniformly space the finite areas 12a and 12b away from the inside wall of the incubating vial 20 to separate the culture media from condensate which may form on the wall. The separation prevents such condensate from dissolving and contaminating the culture media. The raised side borders 11a should provide a minimum spacing between the finite areas 12a and 12b and the adjacent edges of the dip plate of approximately .075 inches. In addition, the raised borders confine the culture media to the finite areas 12a and 12b during application of the media to the dip plate 10, and during transportation and storage of the coated plate.

A pair of legs 13 extend downwardly from the bottom edge of the dip plate 10. The legs 13 space the dip plate 10 above any contaminants on the surface supporting the dip plate 10. Typically this surface will be the lower end 20a of the cylindrical vial 20 in which the dip plate 10 is stored during the incubation period of the bacteria. Excess test specimen and condensate will drain off the dip plate and onto the supporting surface where such materials will not contaminate the inoculated media on the dip plate. The legs 13 should be of sufficient length to provide a space of approximately .125 inches between the bottom edge of the dip plate 10 and the principal surface of the vial lower end 20a.

It should be noted that incorporation of a spacing means on the dip plate 10 eliminates the need for a separate spacer element in the bottom of the incubating vial 20. Elimination of the spacer reduces the cost of manufacture and eliminates any hazard of contamination caused by a tipping of the spacer element.

During the incubation period the dip plate 10 is stored

in the cylindrical incubating vial 20 shown in section in FIG. 3. The dip plate 10 is in close contact with the walls of the incubating vial 20 and it is secured snugly in position by contact with the lid 21 which closes the open upper end 20b of the vial 20. Together the vial 20 and the lid 21 hold the dip plate 10 in a fixed, substantially vertical position during incubation. If the dip plate 10 were tipped, movement of liquid from one media to the other would be facilitated, with resulting contamination.

The culture media to be applied to the dip plate 10 will be determined by the particular bacterial test to be performed. In this respect any two culture media which will differentiate between the bacteria being tested for and bacteria present because of contamination will do. As mentioned above the dip plate 10 can be manufactured of transparent polystyrene or of opaque polystyrene in any of a multitude of colors. By proper choice the best optical contrast between the dip plate 10 and the bacteria growth can be determined. As a result determining the quantitative bacteria colony count on the test medium is greatly facilitated.

To provide a bi-media dip plate for urine analysis, for example, it is preferred to coat the finite area 12a of the dip plate 10 with Eosin-Methylene Blue (EMB) agar 15, shown in FIGS. 2 and 3. The dyes present in the EMB agar inhibit the growth of bacteria other than infection-causing gram negative rods. Bacteria growth on the EMB, therefore, will indicate the presence of the infectious pathogens being tested for.

The finite area 12b on the other face of the dip plate 10 is coated with Tryptic Soy (T.S.) agar 16, shown in FIG. 2. The T.S. agar provides a medium upon which the common contaminants such as diptheroids, staphylococci and streptococci, as well as the infectious pathogens will grow.

It is preferred that the dip plate 10 be black for use in urine analysis. The bacteria colonies that grow on the media in urine analysis will be easily observable by reflected light against the black dip plate 10. Also there is no possibility of confusion due to observation of bacteria colonies on the opposite side of the plate as may occur with a transparent glass slide.

The dip plate 10 may be of transparent manufacture, however, if the test conditions warrant it. For example a bi-media dip plate for throat culture testing would be prepared with T.S. Agar as the control medium on the finite area 12b and Tryptose blood agar as the test medium on the finite area 12a. A positive reaction on the Tryptose blood agar is hemolysis of the red blood cells which can more easily be observed by transmitted light. Thus the dip plate 10 should be transparent for throat culture testing.

In use inoculation and incubation of the culture media will be substantially similar for most bacterial tests. For example the dip plate 10 discussed above for use in a urine analysis would be innoculated by dipping it into the urine specimen. When only a small volume of sample is available, the culture media may be inoculated by pouring the specimen directly over the entire surface of each medium. The medium may be looped or streaked with specimen if conditions so require.

After inoculation excess sample is drained off and the dip plate 10 is placed upright in the incubation vial 20. The lid 21 is placed on the vial 20 to secure the dip plate 10 snugly in place. The sample is incubated at least overnight (18 hours) at 37° C. or for 24 hours at room temperature.

After incubation the opposing sides of the dip plate 10 are compared to test for contamination. A difference between the bacteria counts on the T.S. agar and the EMB agar suggests that the specimen was contaminated during the collection process and that a high colony count on the EMB, therefore, may not be meaningful. However, abundant growth on nutrient agar with scant or imperceptible growth on EMB suggests infection with an organism other than gram-negative rods, particularly if

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the growth seems to be primarily of one colony type. A reliable test is indicated by substantial uniformity between the colony counts on both sides of the dip plate 10. This indicates that little or no contaminating bacteria are present.

It should be noted that although the dyes in the EMB agar suppress or inhibit the growth of bacteria that might be mistaken for the presence of infectious bacteria, the EMB supports growth of gram-negative organisms. These other organisms however have distinctive features which the technician in this field will recognize and easily identify. These organisms therefore need not be discussed here.

Quantitation of the bacteria growth on the EMB in a test determined to be reliable is performed by comparing the colony density on the EMB with standardized full scale photographs of serially diluted bacterial suspensions. These suspensions of standardized counts are confirmed by the accurate but slower pour plate culture method.

The colony count per millimeter of urine can be quickly calculated from the colony count on the slide. For example, if the dimensions of the raised borders 11a and 12a are one inch by two inches, the agar surfaces coated therein upon the finite areas 12a and 12b will each total two square inches. Such an area will absorb between .01 and .02 millimeters of urine. The colonization per millimeter of specimen is therefore calculated to be the colonization per finite area 12a or 12b, times a constant of 75, which constant is an average between

$$\frac{1}{.01} (100) \text{ and } \frac{1}{.02} (50).$$

Since it is the surface area of the agar that determines specimen retention, it is unnecessary to apply precisely

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the same quantity of agar to all dip plates. For example, a normal media thickness of .125 inches may be varied by as much as 10% with only an insignificant variance in surface area. The quantitation method is accurate up to about 10^7 colonies per millimeter.

Although the foregoing description of a preferred embodiment is necessarily of a detailed character in order that the invention may be completely set forth, it is understood that my invention embraces all such modified forms thereof as come within the scope of the following claim.

I claim:

1. A bi-media dip plate for use in performing a method of bacterial quantitation and differentiation, said dip plate having a pair of opposed surfaces and a pair of side edges, a raised border on each said surface, each of said raised borders comprising a pair of side borders and a pair of end borders enclosing a finite area on one of said surfaces for the deposit of a culture medium, said side borders being spaced inwardly from the side edges of said dip plate, said side borders being raised at least .015 inch high and spacing said finite areas at least .070 inch from the side edges of said dip plate, and said end borders being raised approximately .003 inch less than said side borders.

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ALVIN E. TANENHOLTZ, Primary Examiner

U.S. Cl. X.R.

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