Inventors
James R. Brown
John T. Adams, Jr.
William G. Whitney

Attorneys
This invention relates to a microbiological testing method and to the structure used therein, and more specifically, to a method and article particularly suitable for determining bacterial susceptibility to antibiotics.

The increasingly large number of strains of highly pathogenic bacteria and the varying degree of susceptibility of such strains to the numerous anti-microbial agents currently available have given rise to the demand for a rapid and simple method for selecting the most effective antibiotic for use in each individual case. While the conventional disc-agar method is commonly used in hospitals and large clinics, it is far too time-consuming as a preliminary step in the chemotherapy required to arrest the rapid growth of microorganisms, check the spread of infection and prevent the rapid destruction of tissue of which some pathogenic organisms are capable. Furthermore, the conventional disc-plate technique is generally considered too slow and cumbersome for ordinary office practice. Efforts have been made to develop more satisfactory sensitivity or susceptibility testing techniques, particularly colorimetric methods, but the results of these efforts have been generally unsatisfactory, principally because of the time and manipulative steps needed for such tests.

Accordingly, it is an object to overcome the aforementioned defects and disadvantages of prior practices and to provide a method and structure particularly suited for making a rapid determination of bacterial susceptibility. Another object is to provide a microbiological assay method and associated structure wherein the growth characteristics of bacteria in the presence of different anti-bacterial agents may be easily and quickly determined. Another object is to provide means for supporting a plurality of test cultures in separated but closely-spaced condition so that comparative colorimetric determinations may be made as to the susceptibility of the organisms of each culture to one or more antibiotics. Another object is to provide a multiple-disc tray and cover provided with means for preventing the dislodging of the discs during handling and shipping of the package. It is a further object to provide a method and structure which eliminates or greatly reduces the possibilities of contamination and drying of the test specimens. A still further object is to provide a disc and testing tray combination for determining the susceptibility of bacteria to antibiotics, the entire combination being readily incinerable following use thereof.

Other objects will appear from the specification and drawings in which:

FIGURE 1 is a perspective view of a covered tray embodying the present invention;
FIGURE 2 is a broken top plan view of the tray with the cover removed therefrom;
FIGURE 3 is an enlarged broken sectional view of the covered tray taken along line 3-3 of FIGURE 1;
FIGURE 4 is an enlarged broken sectional view of a tray with the top removed therefrom and illustrating a preliminary step in the method of the present invention;
FIGURE 5 is an enlarged cross sectional view similar to FIGURE 4 but illustrating a further step in the method.

In the structure illustrated in the drawings, the tray is generally designated by the numeral 10 and the cover is designated by the numeral 11. Both of these parts may be formed from a plastic material such as polystyrene or from any other suitable material having the desired properties of strength, impermeability and combustibility. The tray and cover are generally rectangular in shape, each having a large rectangular central depression defined in part by the generally vertical and sealingly encircling side walls 12 and 13 respectively (FIGURE 3). The outwardly extending flange 14 of the cover is provided with a peripheral groove or channel 15 and the curved under surface of the flange along this channel is adapted to seat within the channel 16 of the tray's flange 17 to assist in the formation of a tight wedge seal between the parts for preventing evaporation of the tray's contents. In addition, the interlocking channel portions rigidify the cover and tray and thereby prevent inadvertent breaking of the seal between side walls 12 and 13 during normal handling and use of the combination.

Looking to FIGURES 1 and 2, it will be observed that the flange of the tray is provided with recesses 18 and that similar recesses 19 are provided by the flange of the cover. The recesses of the tray and cover are not superimposed with the result that the portion of the flange of one part exposed by the recess of the other part permits the flanges to be gripped and separated from each other, thereby permitting removal of the cover from the tray.

Formed in the bottom wall 20 of the tray are a plurality of circular pockets or wells 21. Similar but smaller depressions 22 are formed in cover 11. As shown most clearly in FIGURE 3, the depressions in the upper surface of the cover produce corresponding bulges 23 on the under surface thereof. Projections 23 are substantially shorter than the depth of the wells so that when the imperforate cover and tray are nested together a plurality of closed spaces or chambers 24 are provided.

Each of the chambers 24 is adapted to contain a fibrous testing disc 25, as shown most clearly in FIGURE 5. The discs may be formed from a porous paper or other suitable fibrous material and contain dehydrated bacteriological media, a dye capable of changing color in accordance with bacterial growth or activity, and any one of various chemotherapeutic agents. Preferably, the discs are arranged in pairs, the discs of each pair containing high and low concentrations of the same antibacterial agent. Thus, where the tray has a total of 28 pockets or wells, as shown in the drawings, the maximum number of different chemotherapeutics in the discs is 14. It will be understood, of course, that trays with larger or smaller number of pockets may be provided depending upon the number of agents to be tested for their antibacterial properties.

Triphenyltetrazolium chloride, which has been found particularly effective as the color indicator, is colorless in its oxidized form but is reduced to red-colored triphenylformazan by actively metabolizing cultures of microorganisms. If the microorganism is in a medium containing an antibiotic to which it is sensitive, this reduction fails to take place and the indicator remains colorless. While triphenyltetrazolium chloride is preferred, other tetrazolium dyes such as tetrazolium blue, tetrazolium violet, netrotetrazolium, lodo nitro tetrazolium and nitro blue tetrazolium might also be used. Use may also be made of other oxidation reducing dyes and pH indicators such as Rassulgin, Janis Green B, Phenol Red and Bromo Thymol Blue.

The test discs 25 may be prepared by saturating those discs with a broth medium containing the color indicator, impregnating the disc with the desired antibacterial agent, and thereafter removing the water from the disc by evaporation. A suitable broth medium may be prepared from
3,107,204

a combination of 1.0 percent glucose, 0.5 percent peptone and 0.075 percent 2,3,5-triphenyltetrazolium chloride.

After addition of the antibacterial agent, the disc may be dried at a temperature below 60 degrees C. Any suitable antibacterial agent may be used to impregnate the disc, such as penicillin, streptomycin, erythromycin, tetracycline, chlorotetacycline, triple sulfonamide, chloramphenicol, and the like.

An important aspect of the invention lies in the fact that the testing is performed entirely within the pockets or wells of the tray and the pockets are sealed immediately after the discs contained therein have been moistened with a small quantity of the bacterial suspension, thereby greatly reducing the possibilities of contamination of the specimens by other agents.

As shown in FIGURE 4, the bacterial suspension may be applied by means of a pipette directly to the dry impregnated discs. The discs may be moistened solely by the fluid of the bacterial suspension and will be maintained in a moist condition because of the tight seal occurring between the side walls and the cover and tray. The fluid tight closure thereby prevents the evaporation of moisture which would otherwise interfere with the microbiological tests.

It has been found that the depending projections of the cover not only act as individual stoppers or closures for the pockets but, in addition, do not allow capillary action of the liquid which might otherwise result in cross-contamination of the materials in adjacent pockets or wells. Therefore, despite the close proximity of the wells or pockets, an important advantage in comparative colorimetric evaluation of the test results, the materials in the several wells remain separated from each other.

Following the addition of the bacterial suspension to the discs and the replacement of the lid to seal the several wells, the entire tray and cover assembly may be placed within an incubator and maintained at approximately 37 degrees C. until a sufficient color response has occurred to permit an evaluation of bacterial susceptibility. As brought out above, the discs are preferably arranged in pairs with the discs of each pair containing different concentrations of the same antibiotic. When pairs of the antibacterial discs have color changes equal to or in excess of a control disc, resistance of the organisms to that chemotherapeutic is definitively indicated. On the other hand, when only the discs having low concentration of chemotherapeutics change color, the indication is that the bacteria are susceptible to high dosages of the drug. When both types of discs show no color change, the indication is that the organism is highly susceptible to the drug.

As noted above, the perforate tray and cover are preferably formed of plastic. The cover, and preferably both the cover and tray, are transparent so that color changes arising from the reduction of the triphenyltetrazolium may be readily observed without removing the cover and exposing the contents of the pockets to evaporation and contamination. As used herein, the word "transparent" refers to any material capable of permitting the passage of light therethrough so that color changes may be observed and, therefore, refers to translucent as well as clear materials.

While in the foregoing we have disclosed the present invention in considerable detail for purposes of illustration, it will be understood that many of these details may be varied without departing from the spirit and scope of the invention.

We claim:
1. In a microbiological testing method, the steps of individually supporting a plurality of dry absorbent elements in a plurality of separate open-topped pockets of a moisture-impermeable sheet, said elements all being impregnated with the same dried culture medium and colorimetric indicator and each being impregnated with different concentrations and types of dried antibacterial agents, each of said elements containing only a single colorimetric indicator and a single antibacterial agent, moistening absorbent elements in separate pockets of a rigid tray, said elements all containing the same dried culture medium and the same colorimetric indicator and each being impregnated with different concentrations and types of dried antibacterial agents, each of said elements containing only a single colorimetric indicator and a single antibacterial agent, individually moistening each of said elements with a solution containing bacteria, and thereafter simultaneously and individually sealing all of the pockets of said rigid tray with a transparent cover to prevent evaporation and contamination of the contents thereof.

2. A microbiological testing method comprising the steps of individually supporting all plurality of dry moisture-absorbent elements in separate pockets of a rigid tray, said elements all containing the same dried culture medium and the same colorimetric indicator and each being impregnated with different concentrations and types of dried antibacterial agents, each of said elements containing only a single colorimetric indicator and a single antibacterial agent, individually moistening each of said elements with a solution containing bacteria, and thereafter simultaneously and individually sealing all of the pockets of said rigid tray with a transparent cover to prevent evaporation and contamination of the contents thereof.

3. A device for use in performing comparative microbiological tests comprising an perforate tray having a plurality of shallow pockets therein, a dry moisture-absorbent element in each of said pockets, said elements all containing the same culture medium and the same color indicator and each being impregnated with different concentrations and types of dried antibacterial agents, each of said elements containing only a single colorimetric indicator and a single antibacterial agent, and a removable transparent cover extending over said tray and sealingly engaging the same, said cover being provided with projections along the undersurface thereof extending into each of said pockets for individually closing the same and for preventing cross-contamination of the contents thereof.

4. The structure of claim 3 in which said tray and cover are formed from a readily combustible material.

5. The structure of claim 3 in which said tray and cover are each provided with peripheral flanges, said tray having a peripheral channel extending about the flange thereof and said cover having a sealing element seated within said channel.

6. A device for use in performing comparative microbiological tests comprising a rigid plastic tray having a plurality of spaced and separated shallow pockets therein, a plurality of dry and moisture-absorbent elements individually disposed in said pockets, and a stiff transparent cover extending over said tray and equipped with means for sealingly engaging said tray to prevent evaporation and contamination of the contents of said pockets, each of said elements containing a culture medium, an antibacterial agent and a color indicator capable of changing color in the presence of enzymes produced by growing bacteria, said means including a plurality of integral projections along the undersurface of said cover, said projections fitting into each of said pockets for individually closing the same and preventing cross-contamination of the contents thereof.

7. The structure of claim 6 in which said color indicator is triphenyltetrazolium chloride.

8. In a microbiological testing method, the steps of individually supporting a group of discrete, dry absorbent elements in a series of shallow separate open-topped pockets in a moisture-impermeable sheet, said elements all being impregnated with the same dried culture medium and colorimetric indicator and being impregnated with different concentrations and types of antibacterial agents, each of said elements containing a single antibacterial agent and a single colorimetric indicator, moistening a plurality of said dry elements with a bacteria-containing liquid, and immediately thereafter individually sealing all of the open-topped pockets with a transparent cover to individually confine said elements and thereby prevent evaporation and cross-contamination of the contents of said pockets.
A structure for use in comparing the sensitivity of bacteria to different antibacterial agents comprising an imperforate tray formed from a rigid, transparent plastic material and equipped with a plurality of discrete pockets therein, said pockets containing a plurality of dry moisture-absorbent elements impregnated with the same culture medium and the same color indicator and each being impregnated with different concentrations and types of dried antibacterial agents, each of said pockets containing only a single moisture-absorbent element, and an imperforate cover formed of a rigid, transparent plastic material extending over said tray and sealingly engaging the same for preventing evaporation and contamination of the contents of said pockets following moistening of the same, said cover being provided with a plurality of projections along the underside thereof, said projections fitting into said pockets to provide individual closures for the same.

The structure of claim 9 in which said tray and cover are each provided with laterally-projecting peripheral flanges, one of the flanges of said tray and cover being provided with a peripheral channel and the other of said flanges being provided with a peripheral sealing element adapted to seat within said channel for sealing said cover and tray together.

Claims:

9. A structure for use in comparing the sensitivity of bacteria to different antibacterial agents comprising an imperforate tray formed from a rigid, transparent plastic material and equipped with a plurality of discrete pockets therein, said pockets containing a plurality of dry moisture-absorbent elements impregnated with the same culture medium and the same color indicator and each being impregnated with different concentrations and types of dried antibacterial agents, each of said pockets containing only a single moisture-absorbent element, and an imperforate cover formed of a rigid, transparent plastic material extending over said tray and sealingly engaging the same for preventing evaporation and contamination of the contents of said pockets following moistening of the same, said cover being provided with a plurality of projections along the underside thereof, said projections fitting into said pockets to provide individual closures for the same.

10. The structure of claim 9 in which said tray and cover are each provided with laterally-projecting peripheral flanges, one of the flanges of said tray and cover being provided with a peripheral channel and the other of said flanges being provided with a peripheral sealing element adapted to seat within said channel for sealing said cover and tray together.

References Cited in the file of this patent

UNITED STATES PATENTS

2,657,998 Peters ------------------ Nov. 3, 1953
2,787,581 Scherr ------------------ Apr. 2, 1957
2,874,091 Fisk ------------------ Feb. 17, 1959
2,894,844 Shakman ------------------ July 14, 1959
2,904,474 Forg ------------------ Sept. 15, 1959
2,955,044 Tupper ------------------ Oct. 4, 1960
2,956,931 Goldberg ------------------ Oct. 18, 1960