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DEVICE FOR DETERMINING THE SENSITIVITY OF BACTERIA TO ANTIBIOTICS

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Fig. 1.

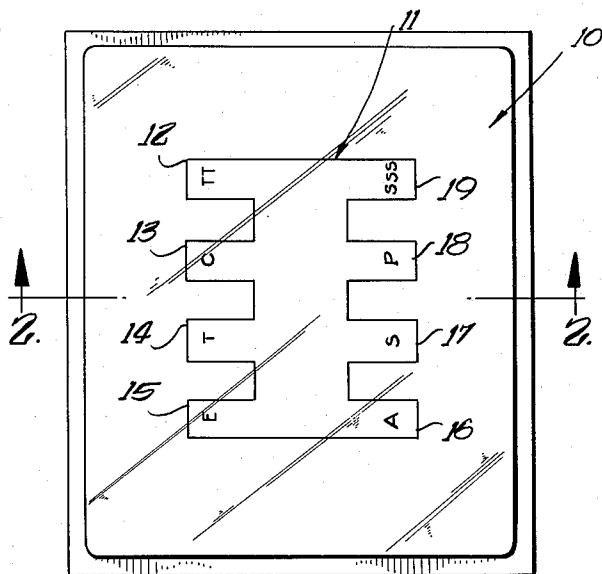


Fig. 2.

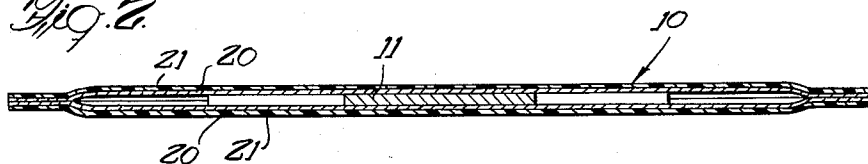
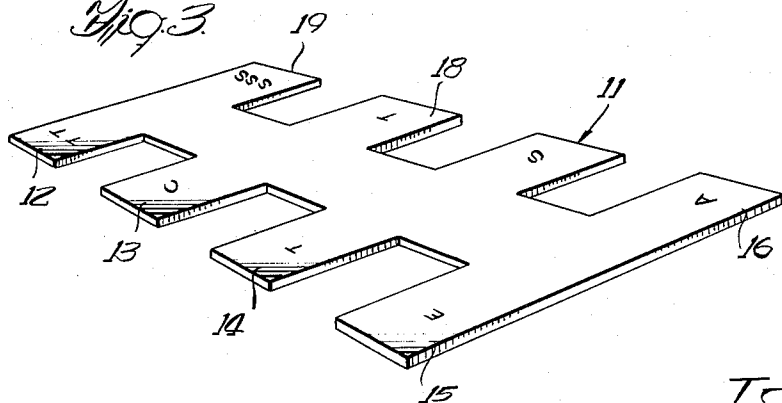


Fig. 3.



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DEVICE FOR DETERMINING THE SENSITIVITY OF BACTERIA TO ANTIBIOTICS
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This invention relates to a device for determining the sensitivity of bacteria to antibiotics, and is of particular usefulness when employed in doctor's offices, a small hospital, or clinic.

This application is a continuation-in-part of my co-pending application, Serial No. 656,644, filed May 2, 1957, now abandoned.

The problem of determining the sensitivity of bacteria to various antibiotics has become more frequent in recent years because of the increased use of antibiotics and the increasing tolerance or immunity of bacteria to antibiotics.

It is to be appreciated that the use of an antibiotic in the treatment of a disease caused by a bacteria which is resistant to that particular antibiotic, is often worse than no treatment at all. Both the physician and patient are expecting improvement through the antibiotic therapy, and none results. Because of the time and space limitations, small hospitals and clinics find it inconvenient to prepare cultures and perform the various bacteriological work that is usually considered necessary in determining the sensitivity to various antibiotics. It would, therefore, be desirable if a means were provided whereby the sensitivity of bacteria to antibiotics could be readily ascertained with a minimum of preparatory work.

A principal object of this invention is to provide a device for determining the sensitivity of bacteria to antibiotics and one which fills a long-felt want in that the procedure can be carried out with a minimum of preparatory work. Another object is to provide a novel device for determining the sensitivity of bacteria to antibiotics in which the problems heretofore associated with providing a stable useful nutrient medium are avoided. Still another object is to provide a stable, inexpensive and rapid device for determining the sensitivity of bacteria to antibiotics and one that can be readily and conveniently employed by unskilled technicians. Other objects and advantages of my invention can be seen as this specification proceeds.

This invention will be explained in conjunction with an illustrative embodiment, in the accompanying drawing, in which—

- FIG. 1 is a top plan view of the device;
- FIG. 2 is a cross-sectional view, taken along the line 2—2 of FIG. 1; and
- FIG. 3 is a perspective view of the sheet portion of the device.

In the illustration given, the numeral 10 designates a resealable container which houses an absorbent sheet 11. The sheet 11 is impregnated at spaced areas 12—19 with different antibiotics, the projections of the sheet 11 being suitably printed with a symbol indicative of the particular antibiotic involved. The sheet is also impregnated with an indicator responsive to bacterial growth, a nutrient and with a gel material.

For the best results, the resealable container 10 should be air-tight and moistureproof. Contact of the bacteria with air may prevent the reduction of the indicator. It is believed that with all bacteria, oxygen competes with the indicator for the electrons from the bacterial enzyme systems. I have found this competition most marked with streptococci. If the container 10 is constructed of a flexible plastic material such as that seen in the accompanying drawing, the material should be such as to

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prevent the passage of air therethrough. Also, the reduction procedure is implemented, where flexible plastic materials are used in the construction of the container 10, where the container face is in direct contact with the inoculated side of the sheet 11, as by laying flush there-against.

Suitable as a material of construction for the container 10 when a flexible, transparent, disposable unit is desired, is cellophane. Cellophane is substantially im-pervious to penetration by air, and will thereby avoid the problem of electron-competition mentioned hereto-fore.

When cellophane is employed as a material of con-struction for container 10, I have found that the best results are obtained when the cellophane (designated by the numeral 20 in FIG. 2) is laminated to a second flexible, transparent sheet 21. The sheet 21 may, for example, be polyethylene, which is advantageous in that it is impermeable to penetration by water. Where the container is subjected to the possibility of water pene-tration, there is a possibility that the antibiotics may deteriorate, since many antibiotics are stable only if maintained in a dry state.

For the absorbent sheet, excellent results have been obtained in using filter paper. Satisfactory results can be obtained through the use of ordinary blotting paper. However, filter paper apparently contains a lesser pro-portion of inhibitory substances which, in certain in-stances, may tend to mask the results of the testing. A number of commercially available filter papers may be employed, and exemplary of these in Schleicher and Schuell No. 470.

An example of an agent productive of discoloration upon bacterial growth is 2,3,5-triphenyl-2H-tetrazolium chloride. This oxidation-reduction indicator turns to a bright red color when in contact with growing bacteria. Optimum results are obtained when the concentration of the tetrazolium compound is in the order of 0.05% or less. Concentrations in excess of this percentage may tend to inhibit the bacterial growth. I also find it de-sirable to employ concentrations of the tetrazolium com-pound in excess of 0.005% in order to produce adequate color on the paper. In order to produce a concentra-tion of antibiotic in a zone corresponding to 10 micro-grams per liter on a disc plate, I employ 1 microliter of a 10 kilogram per milliliter solution of the antibiotic. The precise concentration may vary slightly with the paper used because of the various degrees of absorption. Thus, a suitable range may be that of from 1 to 10 mi-croliters of the solvent. The use of larger volumes of fluid may result in the antibiotic being spread over a larger area, which results in larger zones of inhibition which are not discrete.

A number of bacterial growth media or nutrients may be employed. Representative of these is the formulation seen in the following table:

Table		
Ingredients:		Amount
Peptone	grams	25.0
Glucose	do	2.0
Sodium chloride	do	5.0
Disodium phosphate	do	2.5
Yeast extract	do	1.0
Water	ml	1000

The sheet 11 is also impregnated with a small amount of gel such as a methylcellulose, carboxy methylcellu-lose, casein, or gelatin. The gel material employed in minor quantities, i.e., 1-10 grams, improves the rehy-drating ability of the sheet and the sheet rigidity. The gel material is also effective in improving the chromatog-

raphy of the antibiotics and improves the growth rate of most organisms.

The following example illustrates a procedure for the production of the device seen in the accompanying drawing:

EXAMPLE

First, I prepared a bacterial growth medium having the formulation set forth in the table above. To the foregoing medium was added 0.5 gram of 2,3,5-triphenyl-2H-tetrazolium chloride. Also added was 10 grams of 15 centipoise methyl cellulose. I have found that an impregnation solution containing about 1% of a gel yields beneficial results in imparting suitable rehydration properties for the paper, growth benefits to the bacteria, and desirable chromatographic characteristics to the antibiotic. The nutrient medium itself constitutes from about 2 to 5% of the impregnating solution. For most purposes, a weight ratio of the nutrient medium to the gel of about 3:1 is satisfactory. A filter paper, Schleicher and Schuell No. 470, was employed for the sheet 11 of a size about 3" x 2½" and notched as seen in the accompanying drawing. Sheets of paper of this character were dipped in the above solution. The paper was then dried at about 60° C. Drying at temperatures about 70° C. for longer than 15 minutes causes the indicator in some cases to be reduced. Thereafter, proper concentrations of various antibiotics were placed on the spaced projections 12—19. For example on the projection 16, Aureomycin (as indicated by the letter "A") was placed. Penicillin was placed on the projection 18, triple sulphur on the projection 19, chloromycetin on the projection 13, etc.

To perform a test determining the sensitivity of bacteria to antibiotics using the above device, the dehydrated sheet 11 is removed from the container 10. The rehydration of the paper can be most conveniently performed by floating the sheet 11 on water. The end-point, that is, when the proper amount of moisture has been absorbed, can be easily visualized by watching the surface of the paper. By virtue of providing a gel material in the paper, the gel-containing paper floats for about 15 to 30 seconds before becoming rehydrated. I have found that gel-less papers float only a second or two, which makes it difficult to determine the proper rehydration point. The importance of rehydration lies in the fact that if the paper is not sufficiently hydrated, bacteria will either not grow or will grow poorly. On the other hand, if the paper contains too much water, the antibiotics will "run" and if the paper is "dunked," the antibiotics will also spread over the surface of the paper. A further advantage accrues from a gel-impregnated paper in that the paper is somewhat rigidified, which facilitates the insertion of the sheet into the container 10 after it has been swabbed with the infectious material on the various projections.

After the sheet 11 has been reinserted into the container 10, the container opening can be suitably resealed in order to provide an airtight compartment for the sheet.

In the foregoing, I have set forth a detailed description of a means for determining the sensitivity of bacteria to antibiotics which can be accomplished in a rel-

atively short time (about two to eight hours), without the need of resorting to extraordinary preparations. The precise time involved will vary depending upon the type and concentration of bacteria. However, the foregoing detailed description has been given for clearness of understanding. No unnecessary limitations are to be inferred therefrom, and it will be understood that the details of the invention may be varied widely by those skilled in the art through the use of equivalents, or the like, without departing from the spirit of my invention.

I claim:

1. In a device for determining the sensitivity of bacteria to antibiotics, a container adapted to be sealed against entrance of air thereinto and a dehydrated, absorbent sheet in said container, said sheet being floatable on a water surface for rehydration, said sheet being impregnated with a bacterial nutrient medium, a gel, said gel being present in an amount sufficient to increase the rehydration time of said sheet to about 15–30 seconds, and a tetrazolium chloride over substantially all of its surface, said sheet also being impregnated with different antibiotics at spaced-apart areas.

2. The structure of claim 1 in which the said container is a flat flexible transparent envelope adapted to lie flush against a surface of said sheet.

3. In a device for determining the sensitivity of bacteria to antibiotics, a nonrigid plastic bag adapted to be sealed against entrance of air thereinto, and a dehydrated absorbent sheet in said bag, said sheet being floatable on a water surface for rehydration, said sheet being impregnated with a bacterial nutrient medium, a gel, said gel being present in an amount sufficient to increase the rehydration time of said sheet to about 15–30 seconds, and a tetrazolium chloride over substantially all of its surface, said sheet also being impregnated with different antibiotics at spaced-apart areas.

4. The structure of claim 3 in which the said tetrazolium chloride is 2,3,5-triphenyl-2H-tetrazolium chloride and the said gel is a methyl cellulose.

5. In a device for determining the sensitivity of bacteria to antibiotics, a unitary dehydrated absorbent sheet impregnated over substantially all of its surface with 2,3,5-triphenyl-2H-tetrazolium chloride said sheet being floatable on a water surface for rehydration, a gel, said gel being present in an amount sufficient to increase the rehydration time of said sheet to about 15–30 seconds, and a bacterial nutrient medium, said sheet also being impregnated with different antibiotics at spaced-apart areas.

6. The structure of claim 5 in which the gel is present in an amount of about one-third the weight of the said bacterial nutrient medium.

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