July 15, 1969 J. C. CURRY ET AL 3,455,788

MULTIPLE INOCULATION DEVICE

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FIG. 1

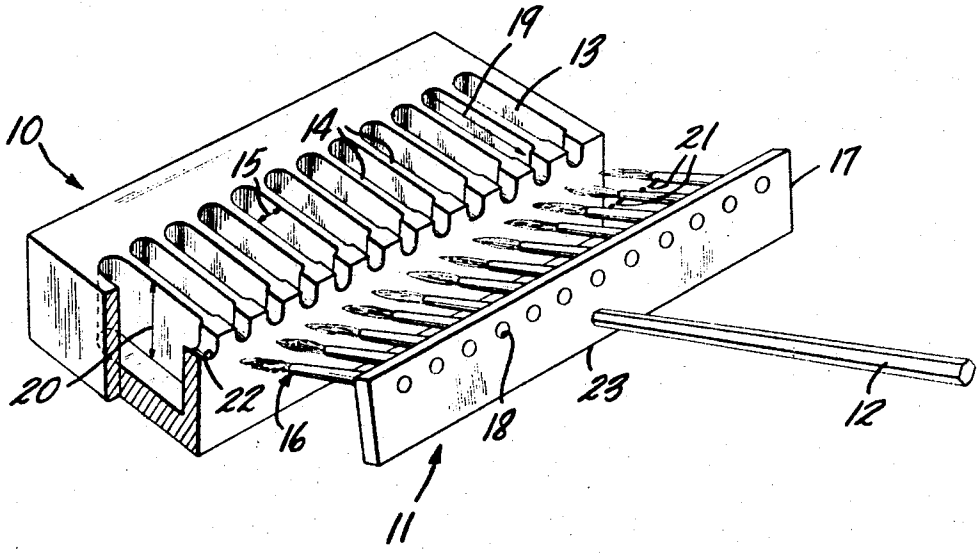
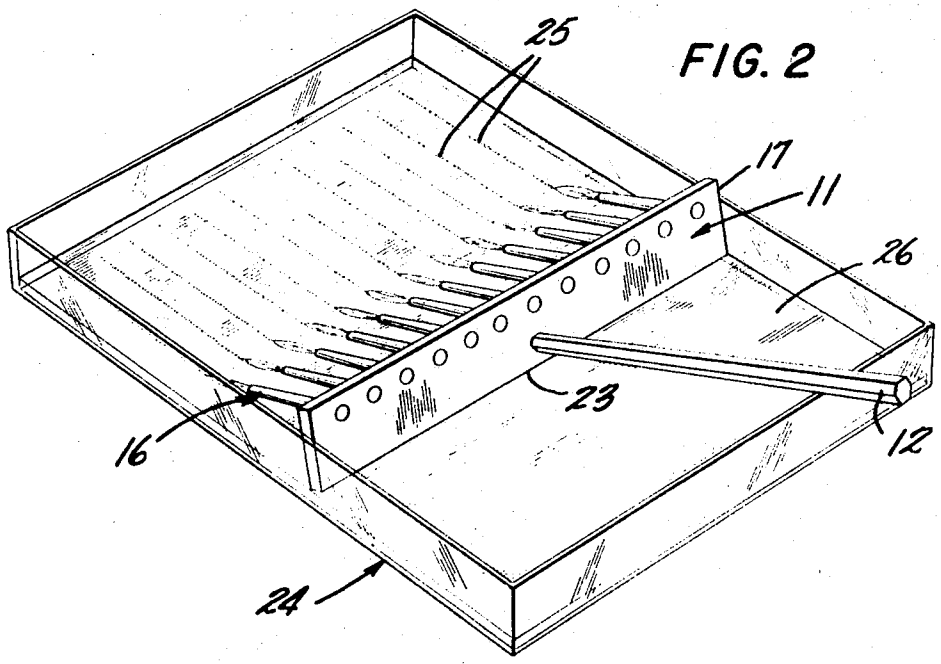


FIG. 2



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MULTIPLE INOCULATION DEVICE

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U.S. Cl. 195—139 6 Claims

ABSTRACT OF THE DISCLOSURE

This invention relates to improvements in an apparatus for simultaneously streak inoculating a substrate with a plurality of microbial cultures. The apparatus consists essentially of an inoculum block containing a plurality of small receptacles adapted to hold suspensions of microbial cultures, and an applicator unit composed of a plurality of applicator elements attached to a holder with a handle and insertable in the receptacles in the inoculum block.

This invention relates to improvements in an apparatus for simultaneously streak inoculating a substrate, such as an agar gradient plate, with a plurality of microbial cultures.

The utilization of traditional techniques in screening antimicrobial compounds to determine their effectiveness has demonstrated how difficult it is to attain an adequate screening standard. The reasons for this difficulty are thought to be the considerable amount of equipment, time and space which are necessary to test the response of each microbial organism to the anti-microbial compound being tested. In small laboratories, screening may necessarily be confined to one or two well-known test organisms, such as *Staphylococcus aureus* and *Escherichia coli*. The limited amount of information derived from these tests is inadequate in many instances to predict the realistic activity of an antimicrobial compound under normal conditions of use.

Considerably more screening information can be acquired by using a gradient plate screening procedure based on a method reported by W. Szybolski in Science 116: 46–48 (1952). A square phage-typing dish is placed on an inclined platform so that a base layer of agar can harden on it in the form of a wedge. A second layer of agar containing the germicide is hardened with the plate in a level position. A concentration gradient is formed as the base layer is diluted by diffusion from the second layer. The phage-typing dish with the two diffused layers of agar constitutes the gradient plate. Streak inoculation of the surface of the two layers results in the development of a growth front which is a function of the MEC (minimum effective concentration). Assuming linearity, the length of the area showing heavy growth is measured with MEC values calculated by direct proportion.

An object of the present invention is to provide an apparatus with which multiple streak inoculations of microbial culture suspensions can be applied simultaneously and rapidly to a substrate to increase the spectrum of culture growths available for comparison.

Referring to FIGURE 1 of the single sheet of drawings, the apparatus of the present invention consists essentially of an inoculum block **10** and an applicator unit **11** with a handle **12**. The inoculum block **10** may be constructed of metal, glass or some similar material. Aluminum is preferred because of its light weight and ease of sterilization after each use. The block **10** contains a plurality of small receptacles or wells **13** adapted to hold suspensions of the microbial cultures to be applied to a substrate, such as an agar gradient plate or some other surface suitable for screening antimicrobial compounds.

The applicator unit **11** is composed of a plurality of applicator elements **16**, such as small brushes, with the same number of elements **16** as there are receptacles **13** in the inoculum block **10**. The applicator elements **16** are arranged linearly and fastened to a holder **17**, preferably by inserting them into small holes **18** in the holder **17**. They may be fastened permanently, or be detachable for ease of sterilization. The holder **17** is preferably constructed of the same material as the inoculum block **10**, but any suitable material can be used. The holder **17** is fitted with a handle **12** with which the applicator unit **11** is manipulated. The applicator elements **16** are so positioned on the holder **17** that they can be inserted simultaneously into the receptacles **13** to adsorb specimens of the microbial cultures contained in the receptacles **13**.

FIGURE 2 illustrates the manner of using the apparatus of this invention to inoculate a gradient plate substrate **26**. In FIGURE 2, the applicator elements **16** are drawn across a substrate **26** contained in a phage-typing dish **24** to inoculate the substrate **26** by depositing thereon specimens of individual microbial cultures as streaks **25**.

In the preferred embodiment, the applicator elements are positioned near one edge of the holder **17**. The opposite edge **23** may then rest upon a flat surface and the holder **17** will act as a support for the elements **16** when not in use by holding them away from contact with contaminating objects. When a square phage-typing dish with raised sides **24** is used to contain a gradient plate substrate **26**, the applicator elements are moved along the substrate **26** without edge **23** touching the same, in a linear direction and deposit the desired amount of inoculum in streaks **25** along the substrate **26**. Manipulation of the holder **17** permits the application of controlled, equalized pressure on the applicator elements **16**, resulting in a plurality of straight streak inoculations **25** of approximately the same width.

In the preferred embodiment, the inoculum block **10** contains a plurality of small guide channels **22** that are equal in number to the receptacles **13**. The channels **22** are positioned on the upward face of the block **10** and lead from one end of the receptacles **13** to the near edge of the block **10**. The depth of the channels **22** will depend on the size of the applicator elements **16** being used. Channels of approximately ⅛ inch depth are preferred for use with small brushes.

The guide channels **22** are particularly helpful in preventing contamination of the applicator elements **16** and the inocula contained in the receptacles **13**, when the elements **16** are inserted into or withdrawn from the receptacles **13**. The channels **22** also serve as an obvious guide to the elements **16** when inserting them into the receptacles **13**, and assist in removing excess microbial culture suspension from the elements **16** when the elements **16** are withdrawn along the channels **22** from the receptacles **13**. The excess of each microbial culture suspension remains either in its receptacle **13** or channel **22**, and cannot contaminate the other cultures. The channels **22** also act as rest supports for the elements **16** when not in use.

The spacing **14** of the receptacles **13** in the inoculum block **10** and the spacing **21** of the applicator elements **16** on the holder **17** depend upon several important considerations. If the streak inoculations **25** are placed too closely to each other on the gradient plate, the individual microbial growth fronts tend to merge into each other, making them indistinguishable and difficult to measure. If the applicator elements **16** are so positioned on the holder **17** that they can at any time come in contact with each other, the possibility exists that an element **16** may be-

come contaminated with microbial cultures from an adjacent element **16**. It is therefore important that the distance **21** between the applicator elements **16** and the distance **14** between the individual receptacles **13** be sufficiently large that no likelihood of contamination exists and that no merging of parallel microbial colony strips on the substrate **26** occurs.

The number of receptacles **13** and applicator elements **16** can be varied according to the width of the substrate and the distance desired between each parallel inoculation. The width of the receptacles **15** can be varied according to the size and spacing of the applicator elements **16**, so that the applicator elements **16** can be inserted simultaneously with ease. The length **19** and depth **20** of the receptacles **13** can also be varied according to the amount of microbial culture suspension to be held by each receptacle **13**.

If the standard, square phage-typing dish is used to contain a gradient plate substrate, the width of the dish is appropriate for a maximum of 12 streak inoculations using 12 applicator elements of equal size. Under these conditions the likelihood of contamination of the microbial cultures is very low.

In the preferred apparatus for inoculating a substrate in a phage-typing dish, the inoculum block contains 12 receptacles, each of approximately $\frac{1}{16}$ inch width, $\frac{13}{16}$ inch length, and $\frac{3}{4}$ inch depth. They are evenly spaced and approximately $\frac{1}{16}$ inch apart on the upward face of the inoculum block. The applicator unit contains 12 applicator elements, evenly spaced on the holder to permit their simultaneous passage into the receptacles on the inoculum block. As applicator elements, red sable watercolor brushes of No. 1 size are preferred, although other suitable elements may be used.

The invention makes it possible to apply rapidly and efficiently a series of microbial culture specimens to a substrate, producing a plurality of microbial growth fronts in close proximity to each other for ease of comparison and measurement. A single operator, for example, can streak inoculate approximately 100 gradient plates within 15 minutes, each plate having 12 distinct micro-organisms. Screening procedures are accelerated through the use of the invention, and more information is available for a broadly-based prediction of the in-product activity of antimicrobial compounds.

The apparatus herein disclosed may also be used with the gradient plate procedure to measure other responses to compounds. For example, stimulation, pigment production changes, and mold sporulation effects can be measured. Synergism, antagonism and inactivation can also be studied using the disclosed apparatus with the gradient plate process.

It will be understood by those skilled in the art that the embodiments of the invention described above are meant to be exemplary and are susceptible of considerable variation and modification without departing from the spirit and scope of the invention. Therefore, the scope of the invention will not be deemed limited except as defined in the appended claims.

We claim:

1. Apparatus for simultaneously applying a plurality of microorganisms in streaks to a substrate, comprising an inoculum block containing a plurality of linearly arranged receptacles for receiving and holding inocula, a plurality of guide channels which are equal in number to the receptacles and which lead along the upward face of the block from the edge of the receptacles to the edge of the block, and an applicator unit containing a plurality of applicator elements linearly attached to a holder and having a handle, the elements on the applicator unit being equal in number to the receptacles in the inoculum block, and being spaced at intervals equal to the spacing of the receptacles, and insertable in the receptacles to withdraw inocula therefrom.

2. Apparatus as described in claim **1**, wherein the applicator elements are attached near one edge of the element holder.

3. Apparatus as described in claim **1**, wherein the inoculum block and applicator element holder are constructed of aluminum.

4. Apparatus as described in claim **1**, wherein the applicator elements on the applicator unit are brushes.

5. Apparatus as described in claim **1**, wherein the applicator elements are fastened permanently to the element holder.

6. Apparatus as described in claim **1**, wherein the applicator elements are detachable from the element holder.

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U.S. Cl. X.R.

15—166; 195—103.5, 120, 127; 220—21