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A Multipoint Inoculator for Petri Dishes

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In recent years, rapid testing of pure cultures of bacteria for biochemical and physiological characters has been a point of great interest to clinicians and to microbial geneticists and taxonomists. The replicate plate technique (J. Lederberg and E. M. Lederberg, J. Bacteriol. **63**:399, 1952), although extremely useful and widely employed, has certain disadvantages, particularly with cultures having a tendency to spread or otherwise overgrow other inoculated cultures on a plate.

Various devices for inoculating simultaneously large numbers of pure cultures of bacteria have been described (M. Goodfellow and T. R. G. Gray, p. 117, *in* B. M. Gibbs and F. A. Skinner [ed.], *Identification Methods for Microbiologists*, Part A; Academic Press, Inc., New York 1966; P. Watt, L. Jeffries, and S. A. Price, p. 125, *Identification Methods for Microbiologists*). Most of the multipoint inoculation methods employ the Tarr inoculator (H. A. Tarr, Mon. Bull. Min. Health Lab. Serv. **17**:64, 1958) with vials of liquid cultures. Capillary-action replicators have been described for inoculating agar plates (R. L. Massey and R. H. T. Mattoni, Appl. Microbiol. **13**:798, 1965; P. A. Hartman and P. A. Pattee, Appl. Microbiol. **16**:151, 1968).

With multipoint inoculators, one can control to some degree the size of microdrop inocula; in addition, there are substantial savings in time and materials when this inoculator is used for transferring large numbers of cultures. However, each of the various inoculators which have been described has certain drawbacks, mainly because of the specificity of the design for a given application.

A method for introducing bacteria into separate test tubes was suggested by Quadling and Colwell (C. Quadling and R. R. Colwell, Can. J. Microbiol. **10**:87, 1964). A modification of that inoculator has been constructed for simultaneous multiple inoculation of agar plates. The device consists of a 4-inch (10.2 cm) square brass or stainless steel plate $\frac{1}{8}$ inch (3.2 mm) thick, with 25 stainless-steel prongs 1 inch (2.54 cm) long and $\frac{1}{16}$ inch (1.6 mm) in diameter evenly spaced on the plate (Fig. 1). A handle affixed to the back of the plate permits easy manipulation of the inoculator (Fig. 2). Although the inoculator was originally designed for use with the divided petri dish produced by Dyos Plastics Ltd., Surbiton, Surrey, England (P. H. A. Sneath and M. Stevens, J. Appl. Bacteriol. **30**:495, 1967), appropriate spacing of the prongs will permit use of the multipoint inoculator with petri dishes of any shape or size. The inoculator is noncorrosive, easily cleaned, and autoclavable. The prongs of the inoculator are beveled at the tips, thus minimizing accidental spattering or cross-contamination of inoculum during transfer.

The volume of the drop size delivered by needles 2 mm in diameter has been estimated to be ca. 0.0006 ml, with a standard deviation of 13% (P. R. Watt et al., p. 125, *Identification Methods for Microbiologists*, Academic Press, Inc., New York, 1966).

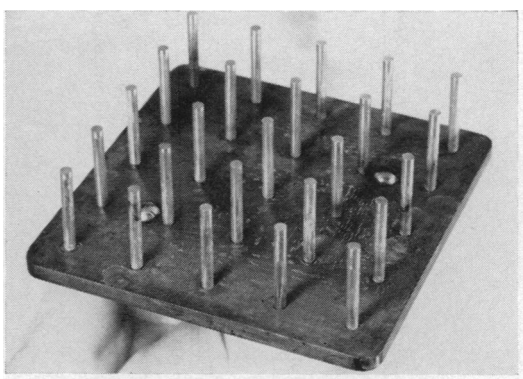

FIG. 1. *Bottom and side angle view of multipoint inoculator.*

Because the multipoint inoculator is small in size, easy to manipulate, and very inexpensive to manufacture, several of the units can be constructed, permitting inoculation of several sets of cultures without the necessity of autoclaving. In our practice, after the inoculation of three test plates, the multipoint inoculator was recharged for inoculation of a second series of three test media. Several hundred plates can be inoculated in 1 hr and, if several inoculators are available, more than one set of 25 cultures can be inoculated

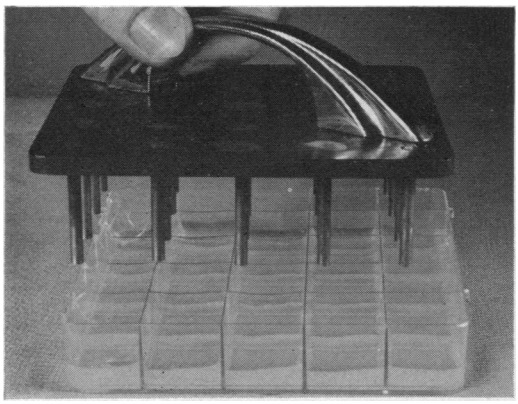

FIG. 2. *Operation of multipoint inoculator with the divided petri dish. Dish contains the sterile test medium (2 ml per compartment).*

into a number of different test media with minimal expenditure of time and effort.

Although automatic inoculators have the advantage of precision movement, the manual unit described here is decidedly inexpensive and more versatile in application.

The advantages of the divided petri dish have been cited elsewhere (P. H. A. Sneath and M. Stevens, J. Appl. Bacteriol. **30**:495, 1967). We have found the inoculator and divided petri dish ensemble extremely useful and economical. The multipoint inoculator-divided petri dish testing method is most efficient with test media in which overgrowth, spreading, or mutual interference in test reactions is a serious problem, viz., detection of lipase (G. Sierra, Antonie van Leeuwenhoek J. Microbiol. Serol. **23**:15, 1957), levan production (A. Fuchs, Nature **178**:921, 1956), and amylase (Society of American Bacteriologists, *Manual of Microbiological Methods*, p. 162, McGraw-Hill Book Co., Inc., New York, 1957).

The multipoint inoculator-divided petri dish technique has been used in our laboratory for the past few months. The efficiency and ease of operation have been sufficiently impressive that the bulk of taxonomic data for pure cultures of bacteria are now obtained by this method.

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