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Multipoint Inoculator System

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The use of numerical taxonomic techniques in microbial ecology requires treating a large number of bacterial isolates to an extensive characterizing test battery. To minimize the time and materials expended during the testing operation, multipoint devices have been innovated which simultaneously inoculate many cultures either onto solid media in petri dishes (5, 8) or into liquid media contained in several kinds of culture tubes (1, 10). Gibbs and Hartman describe or reference other types of multipoint instruments (2, 3).

This report gives the design and some details of the efficiency of a system that uses a multipoint device to inoculate both solid media contained in petri dishes and semisolid or liquid media contained in serological tubes (Pyrex, Corning Glass Works, Corning, N.Y.; 12 by 75 mm) or in 0.5-dr vials, termed minitubes (Kimble Products, Owens, Ill.), held in specially designed racks, or in both.

The multipoint inoculator and tube racks were constructed so that each of sixty-two spikes (or needles) in the inoculator were coincident with the center of each tube-positioning hole in the racks. The arrangement and dimensions for the spike and tube hole center points are given in Fig. 1a. The multipoint inoculator is a $\frac{1}{8}$ inch thick aluminum disc, $5\frac{5}{16}$ inches in diameter, with holes drilled into its surface for insertion of inoculating spikes, $\frac{1}{8}$ inch in diameter by 1 inch long (Fig. 1b). A multipoint needle (or loop) has also been constructed by threading cut-off inoculating loops (Scientific Products Co., Evanston, Ill.) and screwing them into drilled and tapped holes in a similar aluminum disc.

The minitube racks were constructed from aluminum plates, 0.5 inch thick, machined to a disc, $5\frac{5}{16}$ inches in diameter, perforated with holes, $1\frac{5}{32}$ inch in diameter, drilled in the aforementioned pattern (Fig. 1a). The serological tube rack was constructed of three plates, $5\frac{5}{16}$ inches in diameter by $\frac{1}{16}$ inch in thickness, separated by 1-inch tubular spacers placed at 120 deg intervals near the circumference of the plates (Fig. 1c). The upper and middle plates were perforated with holes arranged in the usual pattern (Fig. 1a), drilled ($1\frac{5}{32}$ inch diameter drill) slightly larger than the outside diameter of the tubes. The lower plate was left imperforated as a bottom support for the tubes. The spacers were secured between the plates with bolts extending through both.

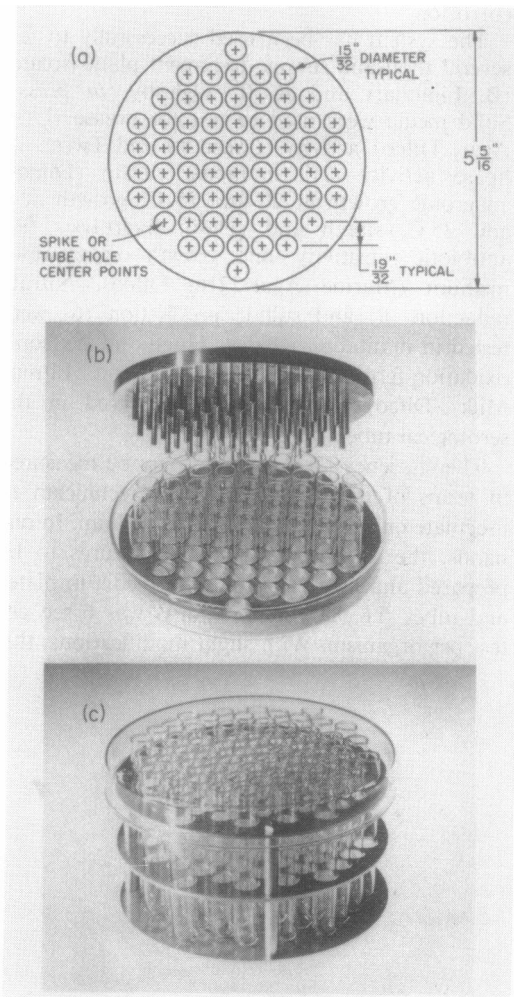

FIG. 1. *Center point pattern for multipoint inoculator, and minitube and serological tube racks (a), multipoint inoculator with minitubes in rack (b), and serological tubes in rack (c).*

To operate the system, the multipoint inoculator was first charged by dipping it into washed cultures previously dispensed into a master rack of sterile minitubes. One of the asymmetric minitubes contained 0.5% aqueous methylene-blue for culture orientation purposes. The inoculator spikes were then carefully touched to the surface of various solid media in petri dishes or dipped into tubed media. The multipoint needle was used to stab to the bottom of the serological tubes. A record of the orientation of the cultures in the master plate was kept on a mimeographed silhouette of the master plate. Dry air sterilization of the components in 15-cm glass petri dishes is recommended to prevent corrosion.

The system has been used successfully to test several thousand sewage treatment plant isolates (B. Lighthart and R. T. Oglesby, *in press*). Solid media were used to test for urease (Urea Agar, Difco) activity, Tween 80 and Tween 20 lipase activity (7), Simmon's citrate (Difco), anaerobic growth at 20 and 35 C, growth at 5 and 35 C, starch and gelatin hydrolysis (9), antibiotic sensitivity, and growth on seawater medium (Marine Agar-2216, Difco). Nitrate reduction (9) and sulfide production (6) were tested in minitubes, whereas Hugh and Leifson's oxidation-fermentation tests (4) and Litmus Milk (Difco) reactions were observed in the serological tube apparatus.

The efficiency of the system may be measured in terms of time necessary for a technician to inoculate one organism onto one medium. In our hands, the average time for 60 cultures to be prepared and inoculated onto 15 media in plates and tubes (i.e., 800 inoculations) was 6 sec per test per organism. With slight modifications, this system might be further automated for greater efficiency.

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LITERATURE CITED

1. Colwell, R. R., and C. Quadling. 1962. Miniature tube tests in diagnostic biology. J. Microbiol. **8**:813-816.
2. Gibbs, B. M., and F. A. Skinner. 1966. Identification methods for microbiologists. Academic Press, Inc., New York.
3. Hartman, P. 1968. Miniaturized microbial methods. Academic Press, Inc., New York.
4. Hugh, R., and E. Leifson. 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram-negative bacteria. J. Bacteriol. **66**:24–26.
5. Lovelace, T. E., and R. R. Colwell. 1968. A multipoint inoculator for petri dishes. Appl. Microbiol. **16**:944–945.
6. Pfister, R. M., and P. R. Burkholder. 1965. Numerical taxonomy of some bacteria isolated from antarctic and tropical waters. J. Bacteriol. **90**:863–872.
7. Sierra, G. 1957. A simple method for the detection of lypolytic activity of microorganisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek J. Microbiol. Serol. **23**:15–22.
8. Steers, E., E. L. Faltz, B. S. Graves, and J. Rider. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemotherapy **9**:307–311.
9. Society of American Bacteriologists. 1957. Manual of microbiological methods, p. 162. McGraw-Hill Book Co., Inc., New York.
10. Quadling, C., and R. R. Colwell. 1964. Apparatus for simultaneous inoculation of a set of culture tubes. Can. J. Microbiol. **10**:87–90.