Multiple Syringe Inoculator for Agar Plates

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The design and operation of a manually operated multiple syringe inoculator was described. Either 9 or 21 inoculations of constant volume could be made simultaneously. Up to 100 plates could be inoculated in 15 min with excellent reproducibility. No contact occurred between the inoculating needles and the agar surface. Construction was simple and inexpensive, with minimal maintenance.

Multiple inoculation has become an essential technique for screening the responses of large numbers of bacterial strains to nutrients or antimicrobial agents. Many devices have been developed for this purpose, as reviewed by Hartman (3). Although the replica-plating technique, which was velveteen (5), has been widely employed in taxonomic investigations (8), it requires many master plates and frequent reinoculations. Bacterial colonies cannot be replicated consistently, due to variability in the size of the inoculum and spreading growth on the master plate after several impressions have been made.

Inoculation with loops or rods is more laborious because of the necessity for flaming. The contact between the inoculating rods and agar surface is not clearly visible, and the agar is often punctured. As a result, the inoculators, after penetration of the medium, carry trace amounts of agar from one plate to another. Furthermore, the risk of cross-contamination and airborne contamination is considerable. To overcome these drawbacks, semiautomatic or automatic devices have been developed. An automatic multipoint inoculator with hood (4, 9) has overcome the time constraint of most inoculators by using two motors which rotated, raised, or lowered two sets of loops that were alternately inoculating or being sterilized.

Techniques for multipoint inoculation using Pasteur pipettes or syringes have also been reported. Pasteur pipettes or capillary tubes operate by capillary action, and when in contact with the surface of an agar plate, deposit a droplet of inoculum (2, 6, 7). The multiple dropper, using syringes, discharges drops by raising the plate until the drops make contact with the agar surface (1, 10). The problem of drops running on the plate is increased because the points project downward.

Design criteria. We required a reliable apparatus, of simple design and construction, which would rapidly produce the same sized drop for several hundred plates; simplicity of operation and reproducibility of results were essential. The inoculum should not splash, run, or streak, and the risk of contamination should be minimal.

To evaluate the response of bacterial isolates to single organic substrates, refined and precise methods were essential. No contact could be allowed to occur between the inoculator and the agar surface, as trace elements could be transmitted and initiate growth in the second medium. Such a false-positive growth response could provide seriously inaccurate and misleading results.

In our studies, it was necessary to rapidly inoculate several hundred isolates on 250 different types of media. Each isolate had to produce a clearly defined area of growth that could be compared easily and accurately with control plates.

Construction and features of the multipoint inoculator. The multipoint inoculator that was developed had several important advantages: simplicity of design, ease of construction, minimal cost, and negligible maintenance requirements. This inoculator could be built in the most modestly equipped laboratory. The prototype inoculator was made of wood and painted with enamel, which withstands limited autoclaving. The apparatus consisted of six components, as shown in Fig. 1 and 2: an inoculator holder, A; the front plate or guard, B; the syringe holders, C; the syringe pressure plate, D; the temporary restrainers, E (Fig. 1); and the syringe inoculators (Fig. 2) (3-ml disposable syringes, Becton-Dickinson & Co. Canada, Ltd., Mississauga, Ontario).

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When assembled, the syringe pressure plate D was inserted into box A (25.5 cm long, 36.0 cm wide and 10.5 cm high). The syringe holder C (23.0 by 26.5 by 1.2 cm, length by width by height) was placed on the plate D, which has the same dimensions as plate C, so that the four corner posts fitted into the four corner holes of plate C. The semicircular sponges (3.6-cm height) on plate D, which acted as springs, were Dispo plastic foam plugs used for openings 35 to 45 mm OD (Scientific Products, American Hospital Supply Corp., McGraw Park, Ill.). The 3-ml syringes were held in the vertical position with tips facing up and the plunger resting on plate D. The small black marks in the outer-most positions in the corners of plate C (Fig. 1) were pressure marks made by the large screws pressing against plate C. The temporary restrainers E (21.5 by 3.5 by 1.2 cm, length by width by height) were kept in place to maintain the space after the initial adjustments had been made. These were eventually removed. The leveling adjustments were made with the turn screw by eye, so that each drop appeared to be the same size. This was confirmed by inoculating a plate containing basal medium. The turn screws were simple to operate, and the inoculating procedure was easily learned. Plate B, which acted as a guard against accidental contact with plate C, was then secured into place. The apparatus was wiped with disinfectant regularly during use and was occasionally autoclaved.

**Inoculation procedure.** The bacterial suspension was drawn into a 3-ml syringe. After removal of air bubbles, the syringe was inserted into a hole in plate C from the reverse side.
After a complete set of syringes (9 or 21) were filled, the temporary restrainers, E, used as space maintainers were inserted beside the syringes. Plate D was placed on top of the syringe plungers, and pressure was applied to release droplets from the syringe. The unit (C, D, and E) was inverted gently and inserted into the inoculator-holder, A. The two pieces of wood, E, were then carefully removed (Fig. 2), and the cover plate B was fastened to the front of A. The first droplets were adjusted with the four screws at each corner. Adjustment by screws was necessary for every two to three inoculations, as the residual pressure was sufficient to expel a second, and possibly a third, drop. Inoculation was performed as shown (Fig. 3). Control plates containing mineral salts were used to adjust droplet size. Growth as observed on glucose agar (Fig. 4), with the nine-point inoculator and 15-cm petri plate, is shown. After the inoculator had been adjusted, the inoculum was prepared for continuous use without re-flaming, cooling, or repeated dipping. It was possible to inoculate 100 plates in 15 min with this apparatus.

The disadvantages observed with other inoculators have been reduced considerably. The inoculating plate can be adjusted quickly and accurately, and contact between droplet and plate is visible. The agar surface is not touched by the inoculator. Due to the inverted position of the plate during inoculation, airborne contamination is reduced, and it is eliminated if the inoculator is used in a laminar-flow hood. Resulting growth is discrete, and cross-contam-
ination between strains is rare.
Several thousand inoculations have been made with this apparatus and it is, for our studies in taxonomy, a most efficient and accurate, manually operated multiple inoculator.

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