Micromethod for Identification of Anaerobic Bacteria: Design and Operation of Apparatus

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A replicator is described for transferring 48 bacterial cultures into separate wells of microtiter plates. The device was designed for determination of carbohydrate fermentation patterns of anaerobic bacteria but should be useful for other applications. A simple device for filling microtiter wells with media is also described.

As part of a microsystem for testing carbohydrate fermentation patterns of large numbers of anaerobic bacteria (3), we designed a replicator, similar to the handheld device of Fung and Hartman (1), for transferring 48 different bacterial cultures into separate wells of microtiter plates. The replicator had to fulfill the following requirements: (i) be small enough to fit easily into the entry port of most anaerobic glove boxes; (ii) be sufficiently simple and stable to be operated easily with gloved hands; (iii) be capable of stab inoculation of the agar-filled wells, instead of surface inoculation; (iv) have a low contamination rate; and (v) be easily sterilized. This paper describes both a device that fulfilled these requirements and a modification of a commercial device for filling the microtiter wells with media. A shop sketch of the device is available upon request.

MATERIALS AND METHODS

Design of the replicator device. A view of the complete replicator device is shown in Fig. 1. The device is similar in principle to a Steer's replicator (2) modified for inoculation of microtiter plates by stab inoculation of the agar-filled wells. A head containing 98 brads (Fig. 2), based on the same principles described by Fung and Hartman (1), is charged with inocula between inoculation of each microtiter plate, and each prong holds a different bacterial culture. The separate cultures are contained in glass tubes in a master plate (Fig. 3). A shield device (Fig. 4 and 5) covers the brads except when they are pushed into the inoculum tubes or into the agar wells of a plate. The shield prevents aerosol contamination from brad to brad and assures that exact alignment is attained with the glass inoculation tubes by fitting exactly over the tubes prior to insertion of the brads.

Operation of the replicator device. For our purpose of testing anaerobic bacteria, the inoculation process is done inside an anaerobic chamber (Coy Manufacturing Co., Ann Arbor, Mich.) and consists of first filling the 48 sterile glass tubes in the master plate with the inocula. We test only 48 cultures at one time in a "checkerboard" pattern and leave the remaining 48 wells uninoculated as a control for contamination. The 48 glass tubes are filled by the use of Pasteur pipettes fitted with rubber bulbs. A sterile plastic lid is used to cover the master plate except when the tubes are actually being filled. Care must be taken to prevent the formation of aerosols during the filling process.

Exact alignment of the sterile replicator head and the sterile master plate (autoclave sterilized, 121 C for 15 min) is performed by inserting a microtiter plate on each side of the master plate on the replicator tray and adjusting the screws at each end of the tray. Front to back alignment is accomplished by adjustment of the two bolts that run through the base and are attached to the bottom of the tray. After the plates are aligned, the inoculation process is started. A plate containing medium is placed on the replicator tray to the operator's right of the master plate (Fig. 6). The master plate is then pushed firmly against the plate of medium and the replicator head is gently depressed, charging the brad with inocula. The plate to be inoculated is then used to push the master plate against the adjustment screw at the other end of the tray, thus aligning the plate of medium for inoculation (Fig. 1). The head is gently depressed, the shield contacts the plate, and further depression of the head pushes the brads into the wells. This procedure is then repeated for each substrate to be tested.

Construction details of the replicator device. Figure 7 shows a bottom view of the frame of the replicator. The base was made from 1/4- by 1/2-inch (ca. 0.32- by 1.3-cm) strap iron formed into a rectangle (9.5 by 30 cm). This base was drilled and tapped to take 1/4-inch-diameter (ca. 0.6-cm) bolts. These bolts were threaded through nuts brazed to the bottom of a sheet metal tray (8.7 by 40 cm) with a 6-mm-high lip on both the front and the back. A nut was brazed to each end of the tray and fitted with a screw, which was used to limit the sideways travel of the microtiter plates. The bolts beneath the tray provided for front to back alignment.

A slide device for holding the replicator head was
FIG. 1. Front view of complete replicator.

FIG. 2. View of replicator head (right) and shield (left) that is attached to the head by screws in the corner of the head. The grooves for attachment of the head can be seen on the right and left sides of the head.
attached to the base (Fig. 8 and 9). This consisted of two steel rods (1/4-inch diameter) brazed 7 cm apart to the base. A slide was made from strap iron to hold the replicator head by means of two V-shaped protrusions on the front and a pointed screw at the end of the 12-cm-long bar (Fig. 10). The three points of contact fitted into three notches cut into the replicator head (Fig. 2), and the head was secured by tightening the pointed screw. The replicator head was suspended on the slide with a weak spring attached to the top of the slide.

The replicator head consisted of a 1/8-inch-thick, rectangular aluminum plate in which holes just smaller than the diameter of 1-inch-long steel brads were drilled in the exact pattern of the wells of a microtiter plate. A brad was driven into each of these holes (Fig. 2 and 11). A shield was made to cover the brads except when they were either in the microtiter plates or in the inoculum plate. This consisted of a retangular aluminum-alloy plate 1/8 inch thick in which holes were drilled to correspond exactly in diameter and spacing to the wells of a microtiter plate (Fig. 2). The shield was then suspended from the replicator head by four screws that screwed into taps at each corner of the shield. The screws went through holes in the replicator head which were larger in diameter than the screw threads; this allowed the shield to move up and down easily (Fig. 4-6).

A master plate to hold the inoculum tubes was made from a 1.9-cm-thick piece of aluminum alloy that had the exact dimensions of a microtiter plate. Holes 1.5 cm deep were drilled into this plate in the

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**Fig. 3.** Master plate with 48 glass tubes.

**Fig. 4.** View of shield attached to head; brads extend out of shield.

**Fig. 5.** View of shield attached to head; brads covered by shield.
FIG. 6. *Front view of replicator in first position for inoculation process.*

FIG. 7. *Bottom view of replicator showing frame and adjustment bolts.*
exact configuration of the wells of a microtiter plate. Glass tubes 2 cm long were made from glass tubing (7-mm outer diameter by 5-mm inner diameter); these fit exactly into the holes without wobbling (Fig. 3).

Design and operation of the media dispenser. This apparatus is essentially a handheld modification of a commercial device (Cooke Engineering Co.) for aseptically filling microtiter plates. Eight 18-gauge needles with tips and hubs removed were brazed to a 13.5-cm-long piece of 7-mm stainless-steel tubing so that the needle centers were the same distance apart as the center of the microtiter wells. One end of this tube was closed off and a Luer-Lok connector was brazed to the other end. This device was then connected directly to an automatic pipetting syringe (Cornwall, Becton-Dickinson Co.). Alignment of the needles with the wells was no problem since the needles could be placed into the wells (Fig. 12). A single depression of the plunger of the adjustable syringe delivered 0.2 ml to each of the eight wells. We normally filled four plates with each substrate and then flushed the dispenser thoroughly.
FIG. 11. Top view of replicator head.

FIG. 12. Device for filling microtiter plates.
with sterile boiling water, followed by several flushes with the next medium to be tested.

RESULTS AND DISCUSSION
The replicator device described in this paper has been in weekly use in this laboratory for the past 3 years without any major problems. Earlier models which did not have a shield to prevent aerosol contamination had an unacceptably high contamination rate; the addition of the shield eliminated this problem. The glass tubes used to hold the inoculum can be omitted when the operator becomes adept at filling the wells with inocula. The tubes allow the operator to replace accidentally contaminated tubes prior to starting the replicating procedure.

We have only used this replicator for determination of carbohydrate fermentation patterns of anaerobic bacteria, but it should be useful for other procedures that require repeated multiple inoculations. The device would, of course, be even simpler to operate aerobically outside of a glove box. The simple eight-prong device that we have used for filling microtiter plates also should be useful for dispensing any type of liquid into a microtiter plate. We found this device to be much faster to use than the commercial device, and it was easier to flush between changes of media.

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