Capillary-action replicator

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Studies involving repeated inoculations of agar surfaces with different isolates of microorganisms are greatly facilitated by some form of replicating apparatus. Using such a device, individual inoculations are made easily, precisely, and rapidly: in addition, since the plate surfaces are exposed less frequently for simultaneous batch inoculations than for multiple single inoculations, the possibilities for contamination are reduced. The type of replicator used is determined by the nature of the growth of the organisms and the type of medium employed. When the test isolates are basically unicellular and capable of growing on solid media, the transfer of inocula onto test surfaces can be readily effected by solid heavy gauge needles, pins, or rods which can be heat sterilized between successive runs. Such passive transfer of viable material can be of value in studies with bacteria or yeasts and can also be used in inoculating test media with conidia produced aerially by filamentous moulds.

Effective as such methods can be, however, they lack the precision afforded by the transfer of small volumes of cell suspension with a capillary action replicator. The apparatus described below was designed to permit the inoculation of large numbers of agar media in petri dishes with approximately equal quantities of cell suspension. The test organisms were yeasts, but the technique could be used for any aqueous suspensions of viable cells capable of growth on solid media. It is a natural development of the methods previously described by Massey and Mattoni (1965) and by Hartman and Pattee (1968), differing mainly in the introduction of a simple but effective control of the plate inoculation procedure.

Description

Basically, the apparatus (fig 1) consists of two autoclavable polypropylene sheets 7 mm thick (F-1258, Labfor, Ace Scientific) held in position by means of two stainless steel bolts (5.5 cm long and 6 mm diameter) but separated by 3 mm thick stainless steel washers. These constitute the capillary holders and are clamped firmly in place by two nuts. Holes,

Fig. 1 The assembled replicator showing capillary tubes charged with suspensions of microorganisms.

2 mm in diameter, are then drilled through both thicknesses of the sheets in the required pattern. In the replicator illustrated, 12 holes were made, which permitted the spacing of inocula centres 2.5 cm apart. Other patterns were devised according to need and incorporating eight to 30 holes. The pattern shown permitted ready access to test inocula which were pipetted into sterile autoclavable test-tube closures, eg, Bellco 'Kap-uts', size K-16 (fig 1). These inocula reservoirs contained about 2 ml of cell suspension and were maintained in position with soft wire or 3 M autoclavable tape (1222). It will be noted that 12 such reservoirs fit readily within a 9 cm glass petri dish base. Before use, sufficient clusters of inoculation reservoirs were prepared to accommodate all the cell suspensions to be tested. Each cluster was covered with the lid of a glass petri dish and autoclaved. Between re-charging of the capillary inoculation tubes, aseptic conditions were

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maintained by covering the inocula reservoirs with the lid of the petri dish.

Before replica plating the paired capillary holders were held vertically in a burette clamp and sterile 9 cm capillary tubes (OD 2 mm) inserted through the bottom holes. The outside diameters of the capillary tubes must be less than that of the holes in the capillary holders so that vertical movement is possible. Each capillary tube was prevented from falling out of the holes by sliding a short section (about 4 mm) of teflon tubing (2.2 mm OD) as a collar over the top end. This was best achieved by holding the collar with a pair of fine forceps as the capillary tube was eased through each hole. The collar permitted some vertical movement of the tubes during the plate inoculations and this effectively eliminated the possibility of both spatter and clogging which would otherwise occur if one or more capillary tubes were inadvertently driven into the test agar. It was found easier and quicker to attach these collars than to form a retaining hook at the top ends of the capillaries in a fine point burner. Moreover, the collars permitted a more accurate alignment of the lower ends of the capillaries in a horizontal plane. As shown in fig 1 about 1 cm of each tube protruded above the top surface of the upper capillary holder. The holes prepared to accommodate the capillary tubes were made with an electric drill attached to a simple drill press stand. The latter was subsequently used as an integral portion of the replicator itself. Before use the capillary holders with the tubes in place were clamped securely into the drill-retaining guard holes (fig 1). The entire replica assembly can now be moved vertically by operating the spring-loaded plunger arm. This affords a much greater degree of control to be exercised in inoculating the test media than can be achieved by hand-held plates.

The petri or other dishes containing the test media or the inocula reservoirs are brought up to a suitable working distance by a laboratory jack which is placed on the base of the press stand. Before charging the capillary tubes with test suspensions, their lower ends are aligned by placing an inverted dry sterile top lid of a petri dish onto the surface of the jack and raising this under the capillary tubes until one or more makes contact. The remaining tubes are quickly and effectively aligned by pushing down the top ends in turn with a sterile scalpel blade, until contact with the lid is made. The apparatus is now ready for use.

Use

A cluster of inocula reservoirs containing the test suspensions is placed on the platform of the jack

Fig. 2 Surface of Sabouraud's agar inoculated 24 times without recharging using a single capillary tube containing a suspension of *Candida albicans*. Arrows indicate the sequence of individual inoculations. Plate incubated for 48 hours at 37°C. Note absence of 'spatter' and relatively uniform colony size.

and the protective petri dish lid is removed. After aligning so that each capillary tube is placed over the centre of its inoculum reservoir, the plunger arm is depressed so that the tubes are charged with the test suspensions. After carefully releasing the plunger arm, the cluster of inocula reservoirs is replaced by the first of the petri dishes containing the test media. About 25 to 30 plates can be inoculated from the capillary tubes before they have to be re-charged with fresh test cell suspension. Figure 2 shows colonies of *Candida albicans* developing from cells deposited serially by a single capillary tube on a Sabouraud's agar plate with this method. It will be noted that there is relatively little variation in size between the colonies and no evidence of accidental spatter inoculation.

For each group of test isolates, a separate series of sterile capillary tubes and inocula reservoirs has to be provided. Initially a number of the paired capillary holders were prepared: capillary tubes were inserted and aligned. Then each replicator assembly was placed within an aluminium container (Inter-

national centrifuge cup 353A), covered with aluminium foil and autoclaved. In practice, however, contamination has never been a problem, and it has been found practicable to remove the used capillary tubes after one run and replace these carefully with fresh sterile ones. If the capillary tubes had been charged with pathogenic microorganisms, this handling procedure might constitute a hazard to the operator. In that event, the lower ends of the used capillary tubes should be immersed in an appropriate disinfectant contained in a suitable vessel such as a 600 ml beaker. The height of the vessel would be adjusted with the laboratory jack so that the capillary tubes are introduced below the surface of the disinfectant before their removal. Each tube would be individually removed by applying forceps below the lower polypropylene sheet of the capillary holder and carefully pulling downwards until the tube is freed from the teflon collar and slides into the disinfectant. This method effectively safeguards against splashing. If the number of cell suspensions

to be tested is large, the apparatus could be readily modified by preparing larger rectangular capillary holders and patterns as recommended by Hartman and Pattee (1968) and using appropriately sized plates.

Using the apparatus as described, it was found possible to inoculate the surface of 190 plates with 12 inocula on each plate in less than two hours.

The main advantages of this apparatus are its relatively low cost, its simplicity of operation, and its reproducibility of performance.

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Letters to the Editor

Immunofluorescent Techniques for the Diagnosis of Respiratory Syncytial Virus

Professors Court and Gardner in their recent letter (*J. clin. Path.*, 1973, 26, 312) find the immunofluorescent examination of nasopharyngeal secretions for combined detection and identification of respiratory syncytial (RS) virus 97% efficient whereas we have diagnostic results in only 45% (Urquhart and Walker, 1972). Differences in technique and reagents to explain these results are not apparent from published reports (Gardner and McQuillan, 1968; McQuillan and Gardner, 1968) although we note that others using this technique (Hers, 1963) experienced variable results with positive diagnoses in 38% to 82%. However, we agree that immunofluorescence is specific.

Conversely in applying immunofluorescence for combined detection and identification of RS virus in tissue cultures we find 95% overall agreement with standard tests (Urquhart and Martin, 1970) whereas they find only 87% comparative efficiency (Gardner and McQuillan, 1968; McQuillan and Gardner, 1968). Again details of technique and the time at which tests are applied are important and could account for these differences. An analysis of variables in this method of

application shows a major problem to be small numbers of infective virus particles in inocula (Urquhart and Martin, 1970).

While further evaluation of both of these applications proceeds in individual laboratories we may say that for identification of tissue culture isolates showing cytopathic effect, the immunofluorescent test is 100% efficient (Urquhart and Martin, 1970).

This alone represents significant improvement in laboratory efficiency and ensures continuation of immunofluorescence as an essential laboratory procedure in the diagnosis of RS virus infection.

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Trypsin-treated Human Erythrocytes for Rubella Haemagglutination Inhibition Tests

The haemagglutination inhibition (HI) test is the most sensitive and practical test for the detection of rubella antibodies and is now in general use in virological laboratories. The cells most often employed in the test are 1-day-old chick erythrocytes since they have a high sensitivity to specific agglutination.

We wish to draw the attention of laboratory workers to the report by Quirin (1972) of the usefulness of trypsin-treated human O erythrocytes instead of chick cells for the rubella HI test. He compared rubella HI antibody titres obtained by trypsin-treated human ery-