repeated centrifugation and/or a series of sieving steps (Krakower and Greenspon, 1951; Spiro, 1967; Fong and Drummond, 1968; Gang, 1970; Misra, 1972). Nørgaard described a technique utilizing density gradient centrifugation. Although high purity and ultrastructural preservation were seen, the procedure required renal perfusion with a special medium, and yields of pure glomeruli were relatively low (10 to 20%). The current procedure is simple and rapid and requires neither renal perfusion nor special media or equipment. Because of the ultrastructural preservation and relatively high yield, we think this technique should have wide application in the study of glomerular metabolism and is suitable for both immunological and virological studies.

References

- Fong, J. S. C., and Drummond, K. N. (1968). Method for preparation of glomeruli for metabolic studies. *Journal of Laboratory and Clinical Medicine*, **71**, 1034-1039.
- Gang, N. F. (1970). A rapid method for the isolation of glomeruli from the human kidney. *American Journal of Clinical Pathology*, **53**, 267-269.
- Jørgensen, F. (1967). Electron microscopic studies of normal visceral epithelial cells. *Laboratory Investigation*, **17**, 225-242.
- Krakower, C. A., and Greenspon, S. A. (1951). Localization of the nephrotoxic antigen within the isolated renal glomerulus. *Archives of Pathology*, **51**, 629-639.
- Latta, H. (1970). The glomerular capillary wall. *Journal of Ultrastructural Research*, **32**, 526-544.
- Misra, R. P. (1972). Isolation of glomeruli from mammalian kidneys by graded sieving. *American Journal of Clinical Pathology*, **58**, 135-139.
- Nørgaard, J. O. R. (1976). A new method for the isolation of ultrastructurally preserved glomeruli. *Kidney International*, **9**, 278-285.
- Reynolds, E. S. (1963). The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *Journal of Cell Biology*, **17**, 208-212.
- Spiro, R. G. (1967). Studies on the renal glomerular basement membrane. *Journal of Biological Chemistry*, **242**, 1915-1922.

Requests for reprints to: Dr John L. Portis, Department of Health, Education, and Welfare, Public Health Service, National Institute of Allergy and Infecting Diseases, Rocky Mountain Laboratory, Hamilton, Montana 59840, USA.

A modified device for replica plating

KENT CROSSLEY AND KAREN MEAD *Department of Internal Medicine, St Paul-Ramsey Hospital and Medical Center, St Paul, Minnesota, USA*

Replica plating, described 26 years ago by Lederberg and Lederberg (1952), is a standard technique for the selection of bacterial mutants. The method utilises a template covered with sterile cotton velveteen. By pressing the surface of a solid medium on which bacterial colonies are growing against the velveteen and then pressing the velveteen against an uninoculated medium, bacteria may be transferred from surface to surface in a way that retains the spatial orientation of the original colonies.

The principal difficulty with this technique is smudging and loss of colonial identity, which occurs

Received for publication 18 October 1978

as the velveteen collects moisture from the agar surface (May and Houghton, 1965). Although pre-drying the plates and increasing the concentration of agar in the medium decreases this problem to some extent, it is difficult to entirely eliminate smudging.

In the course of some studies designed to determine the nature of aminoglycoside resistance in strains of *Staphylococcus aureus* we encountered significant difficulties with smudging during attempts to replica plate with velveteen. It is likely that modifications in the manufacture of this material during the past quarter century were responsible for these problems. Sources in the fabric industry indicate that the pile thickness, fabric finish, and chemical treatment of velveteens have changed appreciably in recent years.

As a result of the difficulties we encountered with velveteen, we attempted to find a fabric that would be suitable for replica plating but would not become saturated with moisture during the procedure. By replacing velveteen with the 'hook-half' of Velcro

Fig. 1 Surface of the 'hook-half' of Velcro. (To make the hooklets more distinct in this photograph, a white powder has been dusted on the Velcro.)

Fig. 2 The replicating device.

Fig. 3 A portion of a plate from which replicates have been made. Note the marks in the agar made by the Velcro hooklets.

Technical methods

(Velcro USA, Inc, Manchester, New Hampshire), we have been able to eliminate smudging.

Velcro is an all-nylon tape prepared in two complementary forms which is used for closure devices on garments and for fastening fabrics. One strip of this material is a dense, irregular surface (the female or loop section) and the other (the male or hook section) is made of regularly spaced, U-shaped protrusions of a stiff monofilament nylon. These hooklets are 1.5 mm in height and are spaced 1.25 mm (centre to centre) in rows. There are approximately 65 hooks per square centimetre (Fig. 1).

For our purposes, we obtained an industrial 2 inch wide hook tape. A narrow border of the backing which is not equipped with hooks was cut off with scissors. Two complementary semicircles were cut from the material and glued to a wooden block using industrial heat-resistant adhesive. The block was circular and slightly smaller in diameter than a standard 100 mm petri dish (Fig. 2). The devices were simple to construct and could be autoclaved multiple times without difficulty.

In addition to eliminating smudging, there is another advantage to replica plating with Velcro. As the surface of a medium is pressed against the Velcro, small 'needle marks' are made in the surface of the agar. Thus one can easily determine if the entire surface of the medium has been in contact with the replicating device. A minor disadvantage to the methodology is that, because the nylon hooks are discontinuous, it is possible that some colonies will

not be replicated. This has not been a problem because the spacing of the hooks is such that most colonies of usual size are successfully replicated (Fig. 3).

Because of difficulties encountered with velveteen, we cannot directly compare the traditional technique with the use of Velcro. In replicating over 100 000 colonies, we have found the device described here to be simple, accurate, inexpensive, and easy to use.

Although Velcro has been successfully utilised in replica plating of a filamentous fungus (Kaplan and Walls, 1971), we are unaware of others who have used it in bacteriological investigation. Certainly other applications for this material (for example, determining minimal bactericidal concentration from agar dilution antibiotic susceptibility plates) are possible.

References

- Kaplan, P. J., and Walls, D. (1971). A technique for replica plating *Coprinus lagopus*, a filamentous fungus. *Genetical Research*, **17**, 279-281.
- Lederberg, J., and Lederberg, E. M. (1952). Replica plating and indirect selection of bacterial mutants. *Journal of Bacteriology*, **63**, 399-406.
- May, J. W., and Houghton, R. H. (1965). A simple multipoint inoculator. *Laboratory Practice*, **14**, 168.

Requests for reprints to: Dr Kent Crossley, Department of Medicine, St Paul-Ramsey Medical Center, St Paul, Minnesota 55101, USA.

Letters to the Editor

Relative sensitivities of the indirect conjugate method and the antibody bridge method using PAP

The paper on demonstration of immunoglobulins in cryostat and paraffin sections (Curran and Gregory, 1978) is a very useful study of the effects of fixation and paraffin embedding and the relative merits of fluorescein and peroxidase as labels, in immunocytochemistry. It has answered several questions raised by conflicting reports in this field. The finding of similar sensitivities for FITC conjugates and PAP is of relevance to the general question of the relative sensitivities of the indirect conjugate method and the antibody bridge method.

Many studies have been carried out comparing FITC with peroxidase conjugates, and these show that peroxidase is at least as sensitive as fluorescein (eg, Benson and Cohen, 1970; Dorling *et al.*, 1971; Boorsma *et al.*, 1976). It has been reported that the antibody bridge method using PAP is 100 to 1000 times more sensitive than the indirect FITC conjugate method (Sternberger *et al.*, 1970) and 'at least 20 times more sensitive' than the indirect peroxidase conjugate method (Burns, 1975). However, these comparisons with PAP were made with conjugates of unstated antibody content and labelling ratios and are therefore inconclusive (Johnson *et al.*, 1978).

The labelling ratio and antibody content of PAP is constant (Sternberger *et al.*, 1969). The activity of enzyme should

be the only variable between batches, and this can be expected to be minimal if the described methods of preparation and storage are followed. Curran and Gregory's conclusion that FITC is as sensitive as PAP would then appear to be soundly based. This, together with the results of careful comparisons of FITC with peroxidase conjugates referred to earlier, strongly suggests that the indirect conjugate method using either fluorescein or peroxidase is no less sensitive than the antibody bridge method using PAP.

J. DORLING
Division of Cell Pathology
Clinical Research Centre
Watford Road
Harrow, Middlesex
HA1 3UJ