Technical Method

Antibiotic sensitivity testing: A modification of the Stokes method using a rotary plater

C. H. PEARSON AND J. E. M. WHITEHEAD *From the Public Health Laboratory, Coventry and Warwickshire Hospital, Coventry*

Many of the sources of error in testing the antibiotic sensitivity of bacteria by the technique of disc diffusion can be overcome if a method is used in which each of the antibiotic-containing discs is controlled with a standard sensitive strain of bacteria. This requirement is fulfilled in the method described by Stokes (1968) and incorporated as method B in Broadsheet 55 of the Association of Clinical Pathologists on antibiotic sensitivity tests by diffusion methods (Stokes and Waterworth, 1972). This method permits up to four tests to be made on each plate if the ordinary (circular) 85 mm Petri dishes are used. The prevalence of multiple antibiotic resistance among bacteria, however, frequently necessitates the testing of strains against more than four antibiotics, and although up to six tests per plate can be made by the Stokes method if square 100×100 mm dishes are used (Felmingham and Stokes, 1972), these have the disadvantages of costing

between four and five times as much as ordinary dishes and of needing more culture medium. A modification of the Stokes method is described, in which a rotary plater is used for the inoculation of the plates, so enabling up to six tests to be performed on the usual 85 mm dish and still allowing for the inclusion of a control culture on each.

Method

A metal rod, having two locating pins 5 mm apart, is mounted firmly and horizontally 30 mm above the dish (fig 1). This serves to guide the swabs used for inoculation so that the areas spread on each plate are consistent and intermixing of control and test organisms is prevented. The test organisms—either as a primary or as a pure culture—are inoculated onto the plate with a swab by spreading radially from the centre (fig 2), using the rod as a guide, so as to cover approximately two-thirds of the agar surface, while the plate revolves on a rotary plater¹ (Williams and Bambury, 1968), modified to revolve at 150 revolutions per minute.

The control culture—on a preimpregnated swab—is spread as a peripheral ring using the rotary plater and guide rod, so as to leave an uninoculated ring intervening between it and the central area spread with the test culture. Up to six discs are placed equidistant from one another on the uninoculated

¹Supplied by Denley Instruments Limited, Bolney Cross, Bolney Sussex.

Received for publication 3 January 1974.

Fig 1

Fig 1 Rotary plater and guide rod.

Fig 2

Fig 2 Inoculating the plate by radial spread from the centre as plate rotates.

Fig 3

Fig 3 Positioning the discs on the uninoculated ring using a cardboard template under the plate as a guide.

Fig 4 Sensitivity test plate after incubation. Central area is test culture, peripheral ring is the control.

Fig 4

ring with a hypodermic needle, using a template to guide their positioning and a rack to preserve their sequence (fig 3).

A sensitivity test plate inoculated by this method is illustrated in fig 4 and shows that ready comparison of the zone radii of the test culture (central area of growth) may be made with those of the control (peripheral ring of growth) for each disc. The results are interpreted in the same manner as described for method B of Broadsheet 55 (December, 1972 revision).

Comment

The need for antibiotic sensitivity tests to be adequately controlled and the suitability of the Stokes method as a simple means of achieving this in routine practice has been commended by others (Garrod, Lambert, and O'Grady, 1973). This modification extends its usefulness by enabling more tests to be done in Petri dishes of ordinary shape and size without recourse to additional or unconventional culture plates and the consequent extra expenditure of financial and technical resources, although against this must be offset the capital cost (£26.40) of the modified rotary plater. The more economical use of laboratory plastic ware in a time of shortage of plastics has also to be borne in mind.

The speed of rotation of the standard model of the

commercially available machine is 58 revolutions per minute; this was found to be unsuitable for producing a uniform inoculum quickly enough to be acceptable during busy periods. Increasing the speed to 150 revolutions per minute corrected this without scattering organisms from the swab to adjacent uninoculated areas of the agar surface, for no growth was obtained on the outer ring when it was deliberately left uninoculated on a number of occasions after inoculating the central area alone with different organisms.

In routine use the modification has proved a well matched alternative to method B in terms of ease and time of performance, with fewer instances of intermixing of control and test cultures in the spreading of the plates.

References

- Felmingham, D., and Stokes, E. J. (1972). Sterile swabs as essential equipment for rapid reliable antibiotic sensitivity tests. *Med. Lab. Technol.*, **29**, 198-200.
- Garrod, L. P., Lambert, H. P., and O'Grady, F. (1973). *Antibiotic and Chemotherapy*, 4th ed., p. 496. Churchill Livingstone, London and Edinburgh.
- Stokes, E. J. (1968). *Clinical Bacteriology*, 3rd ed., p. 179. Arnold, London.
- Stokes, E. J., and Waterworth, P. M. (1972). Antibiotic sensitivity tests by diffusion methods. Association of Clinical Pathologists Broadsheets 55 (revised December 1972).
- Williams, R. F., and Bambury, J. M. (1968). Mechanical rotary device for plating out bacteria on solid medium. *J. clin. Pathol.*, **21**, 784-786.