

Technical methods

A micro-method for detecting toxins in pseudomembranous colitis

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The demonstration of a toxin, which produces a distinct cytopathic effect in tissue cultures, in the faeces of patients with pseudomembranous colitis was first reported by Larson *et al.* (1977). Subsequent work by Bartlett *et al.* (1978) and George *et al.* (1978) has demonstrated that the toxin is produced by *Clostridium difficile* and can be neutralised by antiserum to *Clostridium sordelii* toxin. Pseudomembranous colitis has a high mortality (Kappas *et al.*, 1978) and is particularly associated with gastrointestinal surgery. Because reports suggest that the condition can be treated with vancomycin (Tedesco *et al.*, 1978), screening patients with postoperative diarrhoea may be warranted. A simple inexpensive technique for routine screening for the faecal toxin is described.

Material and methods

Tissue cultures are grown in flat-bottomed, sterile, microtitre tissue-culture plates (Dynatech) sealed with sealing tape (Dynatech). In this laboratory HeLa cells are routinely used but the technique is

also successful with Hep₂ cells, human embryonic lung fibroblasts, and human amnion cells.

Faecal fluid is centrifuged and the supernatant is removed. Twenty-five microlitre aliquots are serially double diluted in maintenance medium using microdiluters (Dynatech) in transfer plates (Dynatech). These transfer plates (Figure) are perforated at the bottom of each well. Where necessary, an equal volume of antiserum can be added to the diluted faecal material in the transfer plate and allowed to react for 1 hour. When the transfer plate is placed in the tissue culture plate the faecal suspension drains out. Tissue culture plates initially contain 0.1 ml aliquots of maintenance medium per well, giving a fivefold dilution.

Results and comments

Provided large bubbles are not produced in the transfer plates by the microdiluters, the fluid drains without difficulty from the transfer plates to the tissue culture plates. Comparison of toxin titres in five faecal fluids and 10 broth cultures of *Cl. difficile* has shown the method to be of equal sensitivity to the routine tube method. Taking the end point as the tube or well in which 50% of cells showed the characteristic cytopathic effect, an identical result was obtained by the two methods with 12 of these samples. The titre of one sample was two fold higher by the tube method and the remaining two samples were two fold lower. We have used this method routinely for almost a year to

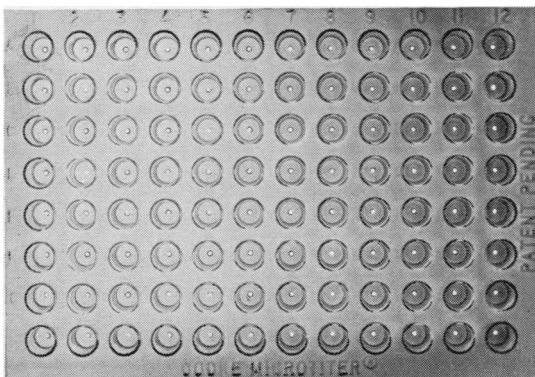


Figure Microtitre transfer plate in which dilutions are made showing the perforated wells. Fluid is retained by surface tension.

screen both faeces and bacterial cultures for toxin. The technique is quick to set up (one person can test 100 samples in a day without difficulty) and reduces the time needed to record results. It also produces considerable financial savings compared with glass tubes and pipettes, requires smaller volumes of cell suspension (we use 0.1 per well instead of the 1 ml required per tube), and economises on antiserum. Recently we have recycled transfer plates because they cost approximately £1 each. After use the transfer plate is soaked in chlorox, washed, and then rinsed repeatedly in distilled water, and finally dried in an incubator. We have not experienced contamination of cell lines due to this or detected carryover of toxin from previous tests. The basic principle can be varied to screen for toxin over a wide range of titres according to the volume and urgency of the work requested.

References

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A low-speed centrifugation technique for the preparation of grids for direct virus examination by electron microscopy

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The negative staining technique introduced by Horner and Brenner (1958) has been extremely useful as a rapid means of visualising virus particles from clinical specimens. However, limitations arise for three main reasons: too small a sample, too low virus concentration in the sample, and too much tissue debris relative to virus. We describe a simple technique for collecting specimens from skin lesions and preparing negatively stained grids for the electron microscope that greatly reduces the difficulties encountered in conventional techniques.

¹Type 10, 1 ml, Venture Plastics, Chessington, UK

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Method

Samples from 29 patients on wards and in casualty and outpatient departments who presented with skin lesions clinically suspicious of virus infection were examined. The lesions were uncapped with a scalpel blade and vesicle fluid, usually 2-5 μ l, was aspirated with a finely drawn Pasteur pipette. Flat-bottomed 6 mm diameter polythene tubes¹ were prepared by cutting down to a length of 15 mm. To each of two tubes 8 drops (200-250 μ l) of distilled water was added. Carbon-coated grids were lowered into the water at the side of the tubes and tilted so that the grids dropped to the bottom with the carbon/formavar film facing upward. The vesicle fluid was expelled into one tube by gentle rinsing in the distilled water and a single drop was transferred to the second tube, thereby giving a dilution of 1:9 between the tubes. When the lesions were dry the material on the tip of the scalpel blade was washed in 8 drops of distilled water which was transferred into an empty plastic tube and a grid emplaced as described. These tubes were put into 5 \times 1 cm glass tubes and centrifuged horizontally in an MSE minor bench centrifuge for 30 minutes at a speed of 3700 rpm, giving a calculated gravita-