Technical methods

A rapid bile solubility test for pneumococci

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The observation that pneumococci when grown in an anaerobic atmosphere with 10% CO₂ produce characteristically large colonies (Howden, 1976) called for a simple rapid confirmatory test; in this way a precise report could be issued on the morning of isolation.

Hawn and Beebe (1965) described a solubility test for aerobic pneumococcal colonies using a sodium deoxycholate solution (pH 7.0). Aerobic colonies rarely exceed 1 mm in diameter; consequently, we found the results with this method difficult to interpret.

With the large anaerobic colonies, however, that problem did not occur. The size of the colony permitted the careful placing of a loopful of bile in such a way that one part of the colony could be seen to dissolve, the other part serving as a negative control.

It was found that a 2% solution of sodium deoxycholate (pH 8.2) (SD) gave the most rapid reaction with the minimal effect on the blood in the culture plate.

After several comparisons the following proved to be the optimum method for routine cultures:

- (1) Samples were inoculated on to horse blood Columbia agar and incubated overnight at 37°C in an anaerobic environment containing 10% added carbon dioxide (Fig. 1).
- (2) A loopful of bile salt (SD) was placed on the suspected pneumococcal colonies, and the plate was returned to the incubator.
- (3) After 15 minutes, the dissolution of treated colonies compared with the adjacent ones could be clearly distinguished (Fig. 2).
- (4) If, after a second period of 15 minutes' incubation, the colony had not dissolved, the test was considered to be negative.

Initially, results were confirmed by the optochin sensitivity and standard bile solubility methods

(Lund, 1960). Of 202 consecutive pneumococcal isolates examined, only two gave discrepant results. These had both been isolated from eye swabs, produced colonies less than 1 mm after anaerobic culture, were sensitive to optochin, but were only partially bile soluble. Dr M. T. Parker, to whom the strains were referred, confirmed that they were pneumococci but probably rough variants.

A miscellaneous collection of strains comprising 65 alpha-haemolytic streptococci, 4 Enterobacteriaceae, 4 *Haemophilus influenzae*, 5 neisseria, 6 staphylococci, 3 diphtheroid bacilli, and 3 cultures of beta-haemolytic streptococci were all negative by the rapid bile solubility test.

Fig. 1 Colonies of *Streptococcus pneumoniae* on Columbia blood agar after overnight incubation anaerobically at 37°C.

Fig. 2 The same view after the dissolution of the colony third from right with bile solution at 37°C.

The combined use of anaerobic culture with added carbon dioxide and the rapid confirmation by plate bile solubility test enabled clearcut results to be obtained even in the mixed flora from respiratory specimens. The method thus saves the time required to obtain pure cultures.

The test is simple to perform, gives rapid results, compares well with the standard identification methods, and is therefore recommended as a routine screening procedure for the diagnostic laboratory.

References

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Plastic embedding of transbronchial biopsy specimens for light microscopy

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Transbronchial biopsies are increasingly used in the investigation of pulmonary disease, but the interpretation of conventional paraffin sections of such material can be difficult. The specimens are small and consist of several fragments, each 2 mm or less in diameter. Air spaces are often torn, distorted, or collapsed. Furthermore, because of the limitations of paraffin wax as an embedding medium, finer details are obscured by the thickness of the section and shrinkage artefact.

In recent years it has been shown that, if tissue is embedded in synthetic resins, shrinkage artefact is minimised and sections 1 μ m or less in thickness are easily obtained (Green, 1970; Burns, 1973; Lee, 1977; Philpotts, 1977). It is thus possible to prepare sections that provide a simple and useful intermediate step between light and electron microscopy. Histological preparations of this type are now used routinely in many centres, particularly in the diagnosis of lymphoreticular and glomerular disease. However, they have not previously been applied to the study of pathological processes in the lung.

This paper deals with two methods for embedding transbronchial biopsy material, which we have been evaluating in our laboratory: the first uses hydroxyethyl methacrylate, and the second an epoxy resin first described by Spurr in 1969. Both these techniques are applicable to larger biopsies or postmortem material with appropriate minor modifications.

Material and methods

It must be emphasised that many of the reagents used in the two techniques described below are toxic, carcinogenic, explosive, or inflammable. They must be handled with extreme care, and a fume cupboard is mandatory. All the materials mentioned below are available from BDH Chemicals Ltd, Poole, or from TAAB Laboratories, Emmer Green, Reading.

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