this antiphagocytic action in guinea-pigs and rats is due to the large amounts of exopolysaccharide produced by these bacteria. Hence, the ability of mucoid bacteria to escape the normal alveolar macrophage-mediated clearance mechanisms of the lung appears to be due primarily to a ‘barrier’ effect of the slime. In addition, it has been shown that slime from mucoid type Ps. aeruginosa will inhibit phagocytic killing of some bacteria by rabbit polymorphonuclear leucocytes. 6 If it is possible to inhibit production of the exopolysaccharide or to destroy it after formation, normal clearance mechanisms may be able to operate. An alternative is to stimulate macrophage activity so that phagocytosis is able to take place in the presence of the slime. We think that these findings are important in the study of the mucoid form of Ps. aeruginosa in chronic respiratory disease. The results may partly explain the difficulty in clearing mucoid organisms from the lung and the increase in severity of cystic fibrosis associated with the presence of this organism.

While it has long been recognised that capsules can confer virulence on some bacteria, there are no reports of previous studies demonstrating this using alveolar macrophages and the mucoid form of Ps. aeruginosa. This may be explained by the difficulty that exists in working with the mucoid form of this organism, as spontaneous reversion to the non-mucoid form occurs in vitro. 7 Selection of the colony from solid media is important, particularly when non-mucoid forms are present in the culture. Finally, when long-term in vitro exposure to macrophages is attempted, spontaneous reversion to non-mucoid forms allows phagocytosis to take place, giving the false impression that mucoid forms have been engulfed.

References


Correlation between two commercial streptococcal grouping kits

In recent years, a number of rapid streptococcal grouping kits have become commercially available. We have evaluated one, the Streptosec test (Organon Teknika), which has recently been marketed, by comparing it with another, the Streptex test (Wellcome Reagents), a method in current use in our laboratory that has been shown to be satisfactory and reliable.

Streptosec is a coagglutination method employing antibodies to the streptococci (groups A, B, C, and G) bound to Cowan type I staphylococci. The four reagents are stained and dried on to wells on a white plastic tile. Both tests were carried out according to the manufacturers' instructions as follows:

Streptex A heavy suspension of beta-haemolytic streptococci was made in 0.4 ml (400 µl) of extraction enzyme in a test tube. The suspension was incubated at 56°C for 1 hour and then centrifuged at 1200 g for 10 minutes. Using a Pasteur pipette, 1 drop of the clear supernatant was added to each of the six circles on the glass tile provided with the kit. One drop of each well-mixed streptococcal latex suspension (groups A, B, C, D, F, and G) was added to the appropriate circle on the tile and mixed with the bacterial extract with a wooden stick. The slide was rocked gently for up to 2 minutes and examined for agglutination.

Streptosec Three to four streaks of growth were taken from a dense culture of beta-haemolytic streptococci on blood agar with a wire loop and suspended in 250 µl of Todd Hewitt broth. The suspension was mixed for a few seconds in a vortex mixer; 50 µl of suspension was placed next to the blue spot in each reaction area of the plate. The liquid and dried reagent were mixed using a plastic spatula (provided with the kit) and spread over the reaction area. The plate was rocked gently for 60 seconds and examined for coagglutination. When cross reactions occurred, the suspension was trypsinised and retested.

Eighty-four fresh clinical isolates of beta haemolytic streptococci found to belong to group A, B, C, or G by the Streptex method were grouped by the Streptosec method. With Streptex 23 were group A, 29 group B, 8 group C, and 24 group G. The same results were obtained with Streptosec. Thus, in our hands, Streptosec provided a reliable alternative for the grouping of beta haemolytic streptococci belonging to groups A, B, C, and G.

Immunoperoxidase staining

Recently, my MLSOs have been anxious to develop immunoperoxidase methods, and I have discovered that they were overlooking the reagent diaminobenzidine tetrahydrochloride, which was described by Mason et al. (J Clin Pathol 1980; 33:609-16). This reagent, of course, has been mentioned in previous papers.

The formula of this reagent suggests that it is related to the carcinogenic hydroxylamines, some of which, particularly benzidine, are prohibited for laboratory use. There is no indication either in the papers or in the catalogues of reagents that these substances are carcinogenic.

In the most recent publication, Safety in Pathology Laboratories, benzidine is included among the carcinogens, and it is recommended that methods using these chemicals should be discontinued (HM).