Use of the Streptosec test for grouping beta-haemolytic streptococci

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SUMMARY The Streptosec test, which embodies the coagglutination principle for grouping beta-haemolytic streptococci, was used against 72 streptococci previously grouped by precipitin methods. Only two of the 72 strains failed to react. The test is easy to manipulate, represents a considerable saving in time and effort, and produces results with an acceptable degree of accuracy.

Various methods have been described for the serological grouping of beta-haemolytic streptococci. The classical method described by Lancefield¹ is a precipitation reaction utilising antisera and the carbohydrate group antigen extracted from the streptococcal cell wall. The test is time-consuming, and this may be one of the reasons why streptococci are not grouped in bacteriology laboratories as often as they should be. The precipitation test was performed originally in capillary tubes, and many laboratories still use these, but in the last few years several workers have reported that precipitin reactions can also be successfully detected by gel diffusion techniques.^{2 3}

An additional grouping procedure, slide agglutination of trypsinised streptococcal suspensions, was described in the 1950s by Rosendal,⁴ and this method is attractive in that it is technically easy to perform.

Counterimmunoelectrophoresis (CIE) has been used extensively in the past few years.⁵ It has the advantage of being economical in the use of antigen and is very sensitive. If a broth culture is used this can eliminate the need for extraction of antigen, though the test is normally used with the extract and allows results to be read within a few hours. This would seem to have the greatest potential for CIE as it does not offer any advantage over capillary precipitation if overnight cultures and extracts are used.

The fluorescent antibody (FA) technique can also be employed for rapid identification of streptococcal groups. It is rapid, does not require pure cultures, and has been recommended by several workers.^{6 7}

In recent years, latex agglutination has also been added to the battery of streptococcal grouping

methods and has been described as an effective and rapid test.⁸

Some years ago Christensen and colleagues⁹ described a method for grouping streptococci with specific antibody adsorbed to protein-A-containing staphylococci. In the following year, Edwards and Larson¹⁰ described a coagglutination technique using specifically sensitised protein-A-containing staphylococci. In this test the group antisera are bound to the protein-A on the cell wall via the Fc structures of the gammaglobulin, thus orientating the Fab-located antibody-combining sites outwards. When corresponding antigen is added coagglutination occurs with the antibody-coated staphylococci.¹¹

The coagglutination method is very useful as it is quick and easy to perform. It also eliminates the need for antigen extraction and makes possible the identification of streptococcal groups from the primary plate.

Streptosec is a commercially produced coagglutination kit, and this paper discusses its use for grouping beta-haemolytic streptococci.

Material and methods

Streptosec is presented as a box which can be used for 50 tests. Each box contains 50 slides, each with four reaction areas marked A, B, C, and G, and 200 plastic spatulas. Each reaction area consists of dried reagent and comprises antibody to either group A, B, C, or G streptococci, bound to the cell surface of heat- and formaldehyde-treated Cowan-1 staphylococci.

Seventy-two beta-haemolytic streptococci of known groups were tested as follows: group A, 8; group B, 37; group C, 14; group G, 13. These strains were obtained from the Streptococcus

Reference Laboratory, Colindale, London, and from clinical specimens obtained from either the Simpson Memorial Maternity Pavilion or the Royal Infirmary, Edinburgh. All had been grouped by precipitation methods.

Strains were spread on blood agar plates and incubated aerobically at 37°C overnight. One colony was picked and transferred to 2 ml Todd-Hewitt broth (THB) which was incubated for 4 hours.

Of the streptococcal suspension 0.05 ml was dropped on each of the four reaction areas. The suspensions and dried reagents were mixed using a plastic spatula; a fresh spatula was used for each area to avoid contamination. The plate was then gently rocked for 60 seconds.

Colonies were also tested directly from the blood plate. These were suspended in 0.25 ml THB which was shaken vigorously. The suspension was then tested as above. Fifty-one groups were thus examined.

Results

Table 1 compares the times taken for coagglutination reactions to occur with streptococcal strains incubated for 4 hours in THB and with the same strains picked directly from a blood agar plate. (Seventy-two strains were tested after incubation in THB. No reaction occurred with two of these strains. One of them had been identified as group G by precipitation methods; the other had given equivocal results with these methods, and there is doubt as to whether it belonged to group A, B, C, or G. Fifty-one strains were tested using the direct method.) Over 90% of those grown in THB showed a positive reaction within 60 seconds or less, whereas only around 50% of those tested directly from the blood agar plate reacted as quickly. Two strains grown in THB gave coagglutination with more than one reagent. Nine more gave multiple coagglutination when tested from the primary plate.

The intensity of the coagglutination reactions is shown in Table 2. The strongest reactions were seen with those strains incubated in THB. Reactions with those plated directly were mostly of moderate intensity although a substantial proportion (24%) gave only a weak reaction.

Table 1 *Coagglutination reaction times*

Seconds	THB incubation		Direct plating	
	Number	%	Number	%
Under 20	48	66.6	17	33.3
Under 60	66	94.3	25	49.0
Over 60	4	5.7	26	51.0

There were no significant intergroup differences either in reaction times or in strength of coagglutination reactions.

All strains whose reactions took longer than 60 seconds gave a positive reaction within 5 minutes.

Table 2 *Strength of coagglutination reactions*

Reaction	THB incubation		Direct plating	
	Number	%	Number	%
Strong	33	47.1	7	13.7
Moderate	36	51.5	32	62.8
Weak	1	1.4	12	23.5

Discussion

Of the 72 streptococcal strains grown in THB, two did not produce coagglutination with the appropriate reagent and two coagglutinated more than one reagent. The manufacturers of Streptosec recommend, however, that if coagglutination occurs with more than one reagent the greater or more rapid degree of coagglutination should be taken as the correct streptococcal group. With the two THB strains there was certainly a stronger reaction with the relevant reagent. (If equal degrees of coagglutination occur the manufacturers recommend that the streptococcal suspension should be trypsinised.) Strains tested directly from the plate produced many more multiple-coagglutination reactions. In the above series, therefore, two groupings from 72 strains were missed, an error of around 3%, which in comparison with the other methods of grouping is within acceptable limits.

Most reactions occurred in less than 60 seconds with the suspensions grown in THB but were much slower in those made directly from the blood agar plate. Also, the intensity of reactions was greater when the streptococci were grown in THB.

Because of the relative infrequency of cross reactions and because of the intensity and speed of the reactions it is recommended that cultures should be tested only when grown in THB. Cultures should not be tested directly from a plate unless there is a considerable degree of urgency for results.

Although it is suggested in the test protocol that only one streptococcal colony should be picked for testing, the findings in the above series were that two or three colonies produced a satisfactory degree of growth more consistently. It was not found necessary to measure accurately 0.05 ml of the streptococcal suspension for mixing with the reagent; satisfactory results were obtained using one drop from a Pasteur pipette.

The Streptosec test is very easy to perform and

gives quick results with a high degree of accuracy. It obviates any need for extraction of the streptococcal carbohydrate antigen with consequent savings in time and labour. It is hoped that with the production of such test kits a far greater proportion of beta-haemolytic streptococci will be grouped in service laboratories. (Another commercial kit using sensitised staphylococci—the Phadebact Streptococcus Test—is produced by Pharmacia Diagnostics, Uppsala, Sweden.)

The Streptosec kits were supplied by Organon Teknika Ltd, Cromwell Road, St Neots, Huntingdon, Cambs.

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