

Serological grouping of streptococci by a slide coagglutination method

ROGER G. FINCH AND IAN PHILLIPS

From the Department of Medical Microbiology, St Thomas's Hospital Medical School, London

SUMMARY A new method for the serological grouping of streptococci by coagglutination with specific antibodies adsorbed to protein A-containing staphylococci has been assessed. A total of 242 strains of streptococci, including β -haemolytic streptococci of groups A, B, C, F, and G, *Streptococcus pneumoniae* and *Strep. faecalis* were studied. All streptococci of groups A, B, C, and G, groupable by standard methods, were correctly grouped by coagglutination, although 7.3% showed varying degrees of cross-agglutination. Two β -haemolytic strains of *Strep. faecalis* produced coagglutination with group C streptococcal reagent. The method appears to be quick, accurate, reproducible, and simple to perform.

Several methods have been described for the serogrouping of streptococci by precipitation of the group-specific carbohydrate with group-specific antiserum. These differ in the techniques used to extract the group antigen and include the use of hot acid (Lancefield, 1933), enzymes (Maxted, 1948; Ederer *et al.*, 1972), hot formamide (Fuller, 1938), autoclaving (Rantz and Randall, 1955), and hot trichloroacetic acid (Slade, 1965).

More recently, a coagglutination method has been described in which formaldehyde- and heat-treated Cowan I staphylococci are coated with rabbit gamma globulin specific for each streptococcal group. The adsorbed antibody has its Fc portion attached to the staphylococcal protein A and its antigen-combining Fab parts directed outwards. Serogrouping by this method is claimed to be accurate, rapid, and simple (Christensen *et al.*, 1973).

Group specific antibodies adsorbed to protein A-containing staphylococci have recently become commercially available for grouping streptococci of groups A, B, C, and G (Phadebact Streptococcus Test, Pharmacia Diagnostics AB, Uppsala, Sweden).

We have evaluated the test on fresh clinical isolates, in a clinical microbiological department, and have compared the results with those obtained by conventional precipitation techniques.

Material and methods

Streptococci were obtained from clinical samples sent to the Departments of Clinical Microbiology, Received for publication 8 July 1976

St Thomas' Hospital and the London Hospital. All β -haemolytic streptococci were grouped by either the enzyme extraction method (Maxted, 1948) or acid extraction method (Lancefield, 1933). In addition to the streptococci of groups A, B, C, F, and G strains of *Strep. pneumoniae* and *Strep. faecalis* were similarly tested.

The reagents were prepared, in accordance with the manufacturer's instructions, by resuspension in 2 ml of reconstituted phosphate buffered saline, pH 7.4, followed by centrifugation at $1500 \times g$ for 10 minutes; the supernatant was decanted and the reagent resuspended in 2 ml of buffer in bijou bottles supplied with dropper caps, and stored at 4°C until used.

For the test, each streptococcus was grown in 2.5 ml Todd-Hewitt broth with aerobic incubation at 37°C overnight. One drop of each reagent was placed on a glass microscope slide (76 \times 25 mm) and a standard loopful (0.006 ml) of the broth suspension was mixed with each group-specific reagent. The slide was gently rocked and the time taken for maximum coagglutination was noted and graded strong (+++), moderate (++) or weak (+). Whenever coagglutination occurred with more than one reagent, that producing the greater or more rapid degree of coagglutination was considered to denote the serogroup. All samples were tested 'blindly' by the same person (RGF).

Results

A total of 242 strains of streptococci was tested. This

Table 1 Time (seconds) and strength of coagglutination for serogroupable streptococci

Time (s) for maximum coagglutination	Group A			Group B			Group C			Group G		
	+++	++	+	+++	++	+	+++	++	+	+++	++	+
0- 15	1			13			2			2		
16- 30	7	22	1	15	1		1	7		7	7	
31- 45	1	11	2	6	3		1	6		1	5	3
46- 60	3	11	6		2			4	1		3	1
61- 90		5	1		1			2	1		3	
91-120		1						5				4

Strength of coagglutination: strong (+++), moderate (++), weak (+).

included group A (72), group B (41), group C (30), group F (12), group G (36), *Strep. pneumoniae* (16), and *Strep. faecalis* (35).

Table 1 summarises the strength of the reaction and the time taken to reach maximum coagglutination for groups A, B, C, and G. There was no coagglutination with group F streptococci. All *Strep. pneumoniae* coagglutinated with group C reagent with the exception of one isolate which coagglutinated with both C and B reagents. The strains of *Strep. faecalis* tested produced no coagglutination with the exception of two β -haemolytic strains which produced (+ +) coagglutination with group C reagent and one non-haemolytic strain that produced (+ +) coagglutination with group G reagent.

Five strains were initially incorrectly identified by routine laboratory testing and correctly identified by coagglutination. This was confirmed by regrouping the strains by the enzyme extraction method (Maxted, 1948). These results are summarised in Table 2.

Strains producing coagglutination with more than one reagent are shown in Table 3.

Discussion

The serogrouping of streptococci is regularly carried out in many diagnostic clinical microbiology laboratories. It is of value in diagnosis and management of disease and for epidemiological studies.

Coagglutination methods, in which antisera are adsorbed to protein A-containing staphylococci, have been used for the serotyping of *Strep. pneu-*

Table 2 *Streptococci incorrectly identified by routine testing and correctly identified after coagglutination*

Strain	Incorrect identification	Coagglutination result	Correct identification
STG 2	G	C	C
STC 3	C	B	B
STC 1	C	G	G
STB 6	B	G	G
LHB 3	B	No reaction	<i>Strep. faecalis</i>

Table 3 *Streptococci producing cross-coagglutination showing strength and time (s) of reactions*

Strain	Definitive serogroup	Cross-agglutinating groups
5187	A +++ (10)	C + (30)
5204	++ (30)	+ (40)
5361	++ (60)	+ (60)
GP 11	B +++ (15)	G + (45)
260	+++ (15)	G + (120)
FPA 35	+++ (30)	A + (120)
LHC 5	C +++ (35)	B ++ (35)
STC 6	++ (30)	A + (120)
5447	++ (90)	G + (115)
5043	+++ (120)	G + (120)
STG 12	G +++ (25)	B + (40)
STB 6	++ (30)	C + (60)
STG 17	++ (35)	B + (45)

Strength of coagglutination: strong (+++), moderate (++) , weak (+).

moniae (Kronvall, 1973), *Neisseria meningitidis* (Olcén *et al.*, 1975), and mycobacteria (Juhlin and Winbald, 1973), the serogrouping of streptococci (Christensen *et al.*, 1973), and the serodiagnosis of *Neisseria gonorrhoeae* infection (Danielsson and Kronvall, 1974).

The test as performed here was simple and rapid and the results were reproducible. Group B streptococci gave the strongest and most rapid degree of coagglutination (range 10-70 s) (Figure) and group G streptococci the weakest and slowest (range 10-120 s), but none was difficult to interpret although occasional strains required two loopfuls of suspended organisms to produce coagglutination.

Cross-agglutination occurred in 7.3% of the groupable streptococci to a similar extent in all groups. However, this did not give rise to problems of interpretation. No strain produced coagglutination with all reagents, although if this should occur retesting is recommended by the manufacturer after the addition of freshly prepared trypsin to the broth suspension to a final concentration of 1 mg/ml and incubation for 30-60 minutes aerobically at 37°C.

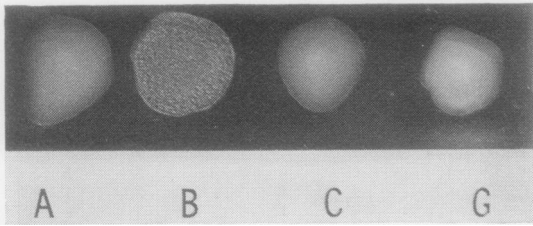


Figure Coagglutination reaction of group B streptococcus with the group-specific staphylococci. Strength of reaction (+++).

This was not necessary for the strains tested here.

The three strains of *Strep. faecalis* that produced coagglutination did so repeatedly with both intact cells in the broth suspension and its enzyme extract (Maxted, 1948) although the strength of coagglutination was diminished in both to (+) and the response time considerably prolonged. Two of these strains producing coagglutination with group C reagent also produced β -haemolysis on blood agar, thus introducing a risk of incorrectly identifying these organisms.

The coagglutination of all strains of *Strep. pneumoniae* tested with group C reagent is in accordance with the known similarity between the capsular antigens of *Strep. pneumoniae* and other streptococci (Austrian *et al.*, 1972) and confirms the findings of Christensen *et al.* (1973). This phenomenon is unlikely to produce difficulties in interpretation.

The test was quick and simple to perform and the results were reproducible. The reagents tested maintained their activity for two months when stored at 4°C; this should be adequate for use in a clinical microbiology laboratory. The results of the test were more easily read than those of the standard Lancefield grouping technique, particularly for group B strains.

We wish to thank Pharmacia (Great Britain) Ltd for generous supplies of reagents, and Dr J. Kensit for assisting in the collection of streptococci.

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