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Identification of Streptococcal Groups A, B, C, and G by Slide Co-Agglutination of Antibody-Sensitized Protein A-Containing Staphylococci

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A total of 98.7% of streptococci, groups A, B, C, and G, isolated from various sources was correctly identified by the co-agglutination technique. The active components in this technique are protein A-containing staphylococci coated with antibodies specific for group A, B, C, and G streptococci. A suspension of streptococci belonging to one of these four groups co-agglutinates with the antibody-sensitized staphylococci specific for this group. The technique is extremely rapid and simple and requires no special equipment. It should therefore be a valuable alternative to other techniques used in the grouping of streptococci and is shown here to be as reliable as the Lancefield technique.

Streptococci associated with human disease are most often identified as group A, B, C, or G streptococci (8).

Grouping of streptococci can be performed according to the precipitin method of Lancefield (5) or to some of its modifications. In these techniques the group-specific polysaccharides from the cell wall are extracted from a liquid, overnight culture. This extraction procedure is time consuming, but the technique has proven to be highly reproducible. The precipitin method is an international standard method, but other techniques such as immunoelectroosmophoresis (7) and immunofluorescence are available.

Grouping of streptococci by the immunofluorescent technique is a rapid method and it is now used by many major laboratories. The group-specific antibodies are mixed with whole cells, and the fluorescent pattern is inspected in an ultraviolet light microscope. The reliability of this technique is good but is dependent on well-trained laboratory personnel and on a high degree of standardization to eliminate false-positive results (2).

Grouping of streptococci with the slide co-agglutination technique is extremely rapid (3) and is applied here in grouping streptococci isolated from various sources. The co-agglutination technique is based on the properties of protein A-containing staphylococci to bind the Fc part of antibodies, thus causing the antigen-binding site of the antibodies to orient outwards from the staphylococci. When mixing staphylococci coated with group-specific antibodies against streptococci with the corresponding streptococci, a co-agglutination occurs, which is visible to the naked eye within 1 to 3 min.

Bacterial strains and growth conditions. The origins of the bacterial strains are given in Table 1. Strains were kept either in the freeze-dried state or in blood-containing broth. For examination they were cultivated on blood agar and tested for purity and confirmation of the diagnosis by a modified precipitin method of Lancefield (4). The same method was used for the test described (see below). For the co-agglutination test a bacterial colony was transferred to 2 ml of Todd-Hewitt broth (BBL) and incubated overnight at 37°C.

Precipitin method. In the precipitin method a single colony is taken from the blood agar and incubated in 40 ml of Merck-Standard I broth (containing a special peptone, yeast extract, and dextrose) for 24 h at 37°C. The extraction of the precipitating antigens (C-polysaccharide) is performed by autoclaving the concentrated bacteria suspension (0.5 ml of saline) for 20 min at 121°C (6). After centrifuging, the clear supernatant is corrected to pH 7.0 to 7.2 by using neutral red as an indicator and is ready for precipitation. This is done in self-made capillaries (inner diameter about 1.5 mm, length about 3 to 4 cm) using available group serum as an antibody (Wellcome Reagents, Ltd., London). Within 1 to 15 min a white precipitate can be seen between the antigen and antibody layer by inspection against dark background.

Grouping of streptococci by co-agglutination. Phadebact Streptococcus Test containing

TABLE 1. *Total number and origin of investigated streptococcal strains*

Group according to precipitin method	Origin								Total
	Human	Cattle	Pig	Horse	Dog	Guinea pig	Unknown	Laboratory reference strains	
A	28							2	30[a]
B	30	30							60[b]
C	10	5	1	10		1	1	2	30[c]
G	19				9			2	30[d]
Total	87	35	1	10	9	1	1	6	150

[a] One strain α-hemolytic, 29 strains beta-hemolytic.
[b] All strains show hemolytic type between α and β.
[c] Five strains α-hemolytic, 25 strains beta-hemolytic.
[d] Two strains α-hemolytic, 28 strains beta-hemolytic.

lyophilized group A, B, C, and G reagents was supplied by Pharmacia Diagnostics AB, Uppsala, Sweden. The reagents are essentially prepared according to Christensen et al. (3). *Staphylococcus aureus*, Cowan I (NCTC no. 8530, ATCC no. 12598), is grown in Casaminocasein hydrolysate-yeast extract broth (1) and killed by treatment with formaldehyde and heat. The washed, sterile suspension of staphylococci is coated with specific antibodies and then freeze dried. The reagents are reconstituted in a buffer included in the test and washed once by centrifugation (1,500 × *g* for 10 min) before use.

One drop of each reagent was placed on a glass slide and one drop of an overnight culture of *Streptococcus* was added to each reagent drop. Reagent and test bacteria were mixed by the tilting of the glass slide 15 to 30 times during 1 min. Throughout the mixing time the drops were inspected in transillumination and against a dark background, and appearance of co-agglutination in one of the drops was recorded. In cases of no or weak co-agglutination, the slide was kept on the table for an additional 2 min, whereafter the result was set, although instructions with the product specify 1 min for the reaction.

In 148 (98.7%) of 150 investigated streptococcal strains, identical identification was obtained with the precipitin technique and with the co-agglutination technique (Table 2). The majority of the strains were beta-hemolytic, but alpha-hemolytic strains were also present. One A strain from the latter group showed doubtful agglutination in both A and B reagent and could therefore not be grouped by the co-agglutination technique. One beta-hemolytic G strain showed weak co-agglutination in all four reagents even after 3 min of reaction time. All the other A strains (29 out of 30), all 60 B strains, all 30 C strains, and 29 out of 30 G strains were correctly identified with the co-agglutination technique.

TABLE 2. *Comparison of grouping obtained with co-agglutination technique and precipitin method according to Lancefield*

Co-agglutination technique	Precipitin method			
	A	B	C	G
A	29			
B		60		
C			30	
G				29
Not groupable	1			1

The group identity of streptococci could in the majority of the cases be settled within 1 min after contact with the reagents. Some strains, 4 out of 30 group C streptococci and 11 out of 30 group G streptococci (10%), needed an observation time of 3 min until safe grouping could be performed. Many strains (97 out of 150) showed weak co-agglutination in reagents other than their group-specific reagent (Table 3). However, this cross-reactivity did not appear until the reaction time exceeded 1 min and this incorrect co-agglutination was always significantly weaker than the reaction in the group-specific reagent. No misinterpretation of the results was therefore made.

Comparison of grouping A, B, C, and G streptococci with the precipitin method of Lancefield and the co-agglutination method shows that 148 out of 150 strains were correctly identified with the latter technique. Complete agreement between the two methods has been reported (3). The main advantage with the co-agglutination

TABLE 3. *Results obtained with the co-agglutination technique*[a]

Group according to precipitin method	No. of strains	Degree of co-agglutination with:[a]																									
		Anti-A						Anti-B						Anti-C						Anti-G							
		++++	+++	++	+	±	−	++++	+++	++	+	±	−	++++	+++	++	+	±	−	++++	+++	++	+	±	−		
A	30	29				1				4	8	1	17			8	7	5	10			5	6	3	16		
B	60			2	7		51	60								1	8		51			21	19	3	17		
C	30			3	11		16			1	9		20	17	9	2	1	1[b]				2	8		20		
G	30			2	7	4	17			5	6	3	16			5	4	6	15	13	6	5	5	1			

[a] ++++, −, Scale of strongest to weakest; numbers indicate the number of strains.
[b] Poor culture growth.

method is that a normal overnight culture of streptococci is used, and no special extraction methods are necessary. The result is read within 1 to 3 min, and no special equipment is needed. The occurrence of cross-reactions is a disadvantage and can sometimes be eliminated by treatment of the overnight culture by trypsin (1 mg/ml) for 1 h at 37°C. However, cross-reactions do not appear during the 1st min. The co-agglutination is always much stronger than the cross-reactions, and therefore good identification can be performed.

No cross-reaction in any of the four reagents has been observed with group D streptococci. Therefore group D streptococci was omitted in the present investigation.

Disturbing auto-agglutination of streptococci in the co-agglutination method has been reported by Christensen et al. (3), who therefore treated the streptococci with trypsin for 1 h at 37°C. In the present investigation no trypsination was found to be necessary.

The main advantage of the co-agglutination test in comparison to the Lancefield precipitin method is to be seen especially for smaller medical laboratories, because a special extraction procedure needing an autoclave is not necessary. Being rapid, simple, and reliable, the co-agglutination technique becomes a valuable alternative to the precipitin method of Lancefield.

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