

Rapid Grouping of Beta-Hemolytic Streptococci by Latex Agglutination

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Latex agglutination was compared with fluorescent-antibody staining with group A conjugate and Lancefield precipitation for grouping of beta-hemolytic streptococci. Latex agglutination correctly grouped 98.8% of 82 group A streptococci and more than 95% of 187 group B, C, or G streptococci. Occasional cross-reactions occurred between groups A and C and groups B and G.

The determination of the Lancefield group of hemolytic streptococci by means of the precipitin test is time consuming and requires the preparation of an extract from an overnight culture of the isolate (12). Nonserological screening procedures such as bacitracin susceptibility are not totally reliable for the identification of group A streptococci; some non-group A streptococci are inhibited by bacitracin, whereas certain group A strains are bacitracin resistant (6).

It has been shown that streptococci can be grouped by a rapid slide agglutination procedure using particles coated with group-specific antibody. Christensen was able to group streptococci by utilizing killed suspensions of staphylococci coated with group-specific antiserum (5). Variable results obtained in our laboratory with these sensitized staphylococcal suspensions led us to investigate the use of latex as an alternate carrier particle.

MATERIALS AND METHODS

Preparation of antiserum. Rabbits were immunized with vaccine strains of Lancefield groups A, B, C, and G provided by the Center for Disease Control, Atlanta, Ga. The schedule of injections followed the recommendations of the Center for Disease Control. The immunoglobulin fraction of each serum was prepared by sodium sulfate precipitation.

Sensitization of latex particles. Latex particles were sensitized by a modification of the method of Severin (13). One volume of the working dilution of the globulin fraction was added to one volume of 0.81- μ m-latex-particle suspension (Difco Laboratories). The mixture was incubated in a 37°C water bath for 2 h, after which the suspension was centrifuged at 2,500 rpm for 15 min. The supernatant was discarded, and the pellet was suspended in one or more volumes of glycine-buffered saline containing 0.1% bovine serum albumin (Sigma Chemical Co.) and 0.1% sodium azide. Sensitized suspensions were stored at 4°C. The working concentration of each globulin fraction was determined by using serial dilutions prepared with

glycine-buffered saline to sensitize batches of latex. Each batch was then tested against a standardized suspension of a stock culture of the homologous organism. The dilution giving prompt and clear-cut agglutination was used to prepare the latex reagent.

Procedure for grouping with latex. One to five colonies of the organism to be tested were suspended with the aid of a Vortex mixer in 0.2 ml of 0.1% trypsin (1:250; Difco) in glycine-buffered saline. The suspension was permitted to stand at room temperature for 1 h and was then used for testing. One drop of the suspension from a Pasteur pipette was mixed on a slide with one drop of the sensitized latex and observed for 1 min while the slide was rocked back and forth. Reactions were observed against a dark background by using oblique transillumination (Fig. 1).

Cultures tested. Sixteen strains of beta-hemolytic streptococci were obtained from the Center for Disease Control. A total of 267 clinical isolates of beta-hemolytic streptococci grown on CNA agar containing 5% sheep blood (Scott Laboratories, Inc.) were obtained from specimens submitted to the Clinical Microbiology Service. Another laboratory provided 10 additional isolates of beta-hemolytic streptococci ungroupable with a commercially available coagglutination product. Five clinical isolates of *Streptococcus pneumoniae*, four group D streptococcal isolates, and two strains of streptococcus viridans group were also tested with each of the four latex reagents.

Protocol. All strains were tested by latex agglutination. Group A strains were confirmed by fluorescent-antibody staining with group A conjugate; other groups were confirmed by the Lancefield precipitin method. Latex testing of the clinical isolates was performed from colonies on primary isolation plates; about 10% of these were mixed cultures.

RESULTS

The comparative results of the three grouping procedures are shown in Table 1. The agreement between the latex and the fluorescent-antibody or Lancefield procedures for each group is shown in Table 2. One of the group A strains was misidentified as group G when tested in mixed

culture; retesting this isolate in pure culture gave correct results. Three of the group B isolates were misidentified as group G. Two of the group C isolates from the Center for Disease Control

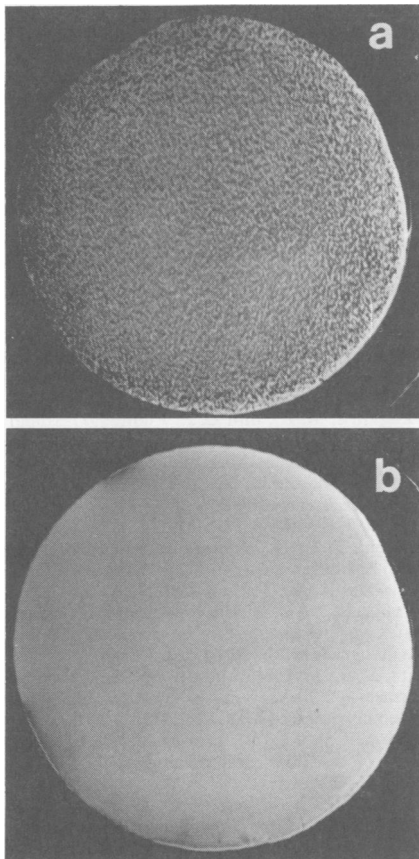


FIG. 1. (a) Positive latex agglutination with group A streptococcus and homologous suspension and (b) negative reaction with group C suspension. $\times 2.5$.

TABLE 1. Strains reacting with homologous latex reagent and confirming test^a

Group	No. of strains reacting with:		
	Latex agglutination	Confirming test	
		FA	Lancefield
CDC strains			
A	8	8	ND
B	4	Negative	4
C	2	Negative	4
Clinical isolates			
A	73	74	ND
B	93	Negative	97
C	46	Negative	48
G	32	Negative	34

^a FA, Fluorescent-antibody procedure; CDC, Center for Disease Control; ND, not done.

TABLE 2. Agreement of latex agglutination with confirming test

Group ^a	No. of strains		% Agreement
	Tested	Identified by latex	
A	82	81	98.8
B	101	97	96.0
C	52	48	92.3
G	34	32	94.1

^a Group A was confirmed by the fluorescent-antibody method; groups B, C, and G were confirmed by the Lancefield precipitin method.

failed to react with the latex; one group C clinical isolate was misidentified as group A, and another group C isolate gave equivocal reactions with both A and C latex reagents. Two of the group G isolates failed to react with the homologous latex reagent. Fourteen strains were identified as group F by the Lancefield method; 11 of them did not react with any of the latex reagents, whereas 3 were misidentified as group G.

Of the 10 isolates that failed to react with the commercial coagglutination product, 3 were found to be group A, 1 was group B, and 5 were group G by latex; all were confirmed by Lancefield. The remaining isolate failed to react with latex and was found to be a group F by Lancefield. Of the 29 isolates tested in mixed culture from the primary plates, 28 were correctly grouped by the latex procedure.

Each of the five strains of *S. pneumoniae* reacted with the group C latex reagent, as did one of the two streptococcus viridans group. No cross-reactions occurred between the four group D streptococci and any of the latex suspensions.

DISCUSSION

Specifically sensitized latex suspensions have been used to detect antigens in body fluids for the diagnosis of purulent meningitis (15) due to *Haemophilus influenzae* (11), *Neisseria meningitidis* (13), and *Cryptococcus neoformans* (2). Trichinosis (10), echinococcosis (4), histoplasmosis (1), sporotrichosis (3), and systemic *Candida albicans* infections (14) have also been detected by the use of specifically sensitized latex particles. Latex agglutination has also been used to diagnose septicemia and in one recent study showed a better detection rate than culture or counter-immunoelectrophoresis (7).

Although the latex agglutination test is based on the same principle as the coagglutination test, there are significant differences. The preparation of the coagglutination suspension is subject to variables such as cultural conditions, incubation atmosphere, and formaldehyde and heat

treatments (8) which can lead to irregular results. The staphylococcal suspensions are sensitized with whole serum, whereas the latex suspensions are sensitized with the immunoglobulin fraction, which should result in increased sensitivity. The commercial coagglutination test requires broth subculture for 4 to 24 h, whereas the latex test can be performed directly from colonies.

The agreement of the latex test with the fluorescent-antibody procedure for the group A strains tested was 98.8%, and the overall agreement of the latex method with the Lancefield precipitin method for the four groups tested was over 95%. Discrepancies thus far detected are the result of occasional cross-reactions between groups A and C and groups B and G. The similarity of the group-specific carbohydrate of groups A and C has been shown by Krause (9).

Cross-reactions could almost always be resolved by retesting glycine-buffered saline dilutions of the organism suspension. Testing should be limited to isolated colonies of beta-hemolytic streptococci, since cross-reactions occurred between *S. pneumoniae* and streptococci of the viridans group and the group C latex reagent. No cross-reactions occurred with the limited number of group D streptococci tested.

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