JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1979, p. 641-646
0095-1137/79/11-0641/06$02.00/0

Vol. 10, No. 5

Evaluation of Commercial Latex Agglutination Reagents for Grouping Streptococci

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Received for publication 14 August 1979

A total of 155 strains of beta-hemolytic streptococci were serologically grouped by conventional techniques (Lancefield extraction and capillary precipitin testing) and by latex agglutination (LA). Agreement between conventional and LA techniques was 97% when the instructions of the manufacturer for the LA technique were followed. Agreement of 99% was obtained when modified autoclave extracts were used as antigens in the LA procedure. A total of 82 strains of non-beta-hemolytic streptococci were also tested by conventional, prescribed LA, and modified autoclave procedures. The agreement between conventional techniques and both LA procedures was 76%. However, when serological cross-reactions in the conventional grouping procedures were considered as errors, the accuracy of identification of both LA procedures was 88% among the non-beta-hemolytic strains. Of 13 strains of *Streptococcus bovis*, 10 did not react with the LA group D reagent but were serogroup D by conventional techniques. More *S. bovis* strains were grouped by the LA technique when extracts of 20 ml of broth cultures were used as antigens; however, cross-reactions were observed with non-group D strains when this technique was applied to them.

The standard procedure for determining the group carbohydrate antigen of the streptococci is to prepare extracts (i.e., acid-heat, formamide, and autoclave) from the growth of strains in broth. This extract is then reacted with specific grouping antisera in a capillary precipitin test (5). A slide agglutination test with cell suspensions has been described but has not gained wide acceptance (15). Recently, however, Christensen and his colleagues (3) described a slide agglutination test with group-specific antisera conjugated to protein A-rich staphylococcal cells. This procedure facilitated the agglutination reaction and was termed co-agglutination (CA) (3). A commercial CA product has been made available, and several clinical evaluations have indicated that the reagents perform very well in the identification of group A, B, C, and G streptococci (1, 2, 8, 10, 16, 17, 18).

Several modifications of the original CA procedure have been reported. Most of the modifications were techniques that shortened the time required for antigen preparation. Several reports suggested a 3- to 4-h incubation period for antigen preparation (2, 8, 16, 17). Other investigators described antigen preparations that were made directly from the growth on blood agar plates without incubation of the antigen (4, 17, 20). These modifications have a distinct advantage over conventional techniques, which require an additional 24 h for identification.

Several investigators have prepared their own CA reagents which resulted in reagents much less expensive than those available commercially (4, 11, 12, 14, 20). Maxted et al. (14) described a slide agglutination technique which does not require staphylococcal cells. However, he prepared staphylococcal cells to add to his reaction mixtures of streptococcal cells and grouping antisera when these reagents failed to react. He concluded that this procedure worked as well as the CA reagents.

Szilagyi and his colleagues (19), using the commercially available CA reagents, reported that group B streptococci could be identified from selective broth cultures without agar plate isolation. This procedure eliminated the need for isolation of the streptococci.

Kirkegaard and Field (12) prepared group B typing reagents for the CA procedure. Acid-heat extracts, however, were prepared from selected colonies.

Recently, Lue et al. (13) reported on an agglutination test with latex particles used as carriers for the streptococcal group-specific antisera. Simple suspensions of cells in trypsin were prepared and used as antigens. Reagents for group A, B, C, and G were prepared and reacted with streptococcal suspensions prepared from one to five isolated colonies. This procedure was comparable to the CA procedure.

None of the above procedures have included

attempts to prepare specific slide agglutination reagents for group D and F streptococci. These two reagents are necessary to complete the serological testing of streptococci because group D and F streptococci are found in human infections. This report includes an evaluation of a commercially available latex slide agglutination kit designed to identify group A, B, C, D, F, and G streptococci.

MATERIALS AND METHODS

Strains. The 238 streptococcal strains were from the Center for Disease Control (CDC) streptococcal culture collection. All strains were clinical isolates received from various city and state public health laboratories and private institutions in the United States. They represented a distribution of isolates that may be encountered in clinical laboratories. All strains were extracted by the Lancefield procedure and serologically grouped by capillary precipitin tests (6). Group D and viridans streptococci were identified by previously described physiological tests (5, 7).

LA. The latex agglutination (LA) reagents (Streptex) were provided by Max Moody of the Burroughs Wellcome Co., Research Triangle Park, N.C. The grouping kit was evaluated by following the instructions of the manufacturer. Pronase for the extraction procedure was provided with the kit. Inocula for preparing the pronase extract were taken from pure cultures (coded to insure anonymity) on Trypticase soy-sheep blood agar (TSA-SBA) that had been incubated overnight at 35°C. A heavy suspension of cells was made by transferring at least three sweeps of culture with a bacteriological loop to 0.4 ml of pronase solution. The suspension was mixed and incubated at 56°C for 1 h, after which the extracted cells were sedimented by centrifugation. The clear supernatant containing the extract was then tested for reactivity with the latex reagents. A Pasteur pipette was used to obtain one drop of the extract which was placed in each of the six circles on a washable glass tile provided with the kit. One drop of each of the six group-specific reagents (groups A, B, C, D, F, and G) was then placed in each of the appropriately labeled circles. The contents of each circle were mixed with a wooden toothpick-like stick provided with the kit. A separate stick was used for each circle. The glass tile was then rocked gently for up to 2 min or until a definite agglutination was observed. No magnification was used to interpret the reactions.

In a second procedure, a modification of the Rantz and Randell autoclave extraction method, growth from the same Trypticase soy agar-sheep blood agar plates used for the pronase extraction procedure was used (16). Each extraction procedure was performed by different technicians, and neither technician knew the results obtained by the other. A very heavy suspension of cells was prepared from each culture by transferring at least three sweeps of growth from the plate to 0.2 ml of physiological saline solution in a Durham fermentation tube. The tube was plugged with cotton and autoclaved for 15 min at 121°C. The tube was later centrifuged to sediment the cells. The clear supernatant (extract) was then tested for reactivity with the latex reagents. Small drops of both extract and latex reagents were used to modify the grouping procedure. Whereas free-falling drops of reagents were used with the pronase extract (according to the instructions of the manufacturer), approximately half the amounts of reagents were used with the modified procedure.

If the pronase and autoclave extracts were both negative or if discrepant reactions were observed with the latex grouping reagents, a second extract was prepared with a heavier suspension of cells according to the instructions of the manufacturer. In addition, several pronase extracts were prepared from the growth obtained from 20 ml of Todd-Hewitt broth (THB) cultures incubated overnight (18 h) at 35°C. The growth from the 20 ml of broth was harvested by centrifugation, extracted with pronase, and reacted with the latex reagents according to instructions of the manufacturer.

A second modification of the LA test was performed with 100 of the strains. Clear centrifuged supernatants from overnight 5-ml THB cultures were tested for reactivity with the latex reagents. Free-falling drops of both reagents were used in this modification. The reagents were mixed, and the results were read as previously described for the pronase extraction procedure.

RESULTS

The results of the double-blind study in which the pronase and modified autoclave extraction procedures were performed from the growth of streptococcal strains of the blood agar plates are shown in Table 1. Among the β-hemolytic strains, agreement between the conventional grouping procedure (Lancefield extract, capillary precipitin tests) and the extraction procedures was 97% with pronase and 99% with autoclave extracts. Pronase extracts of two group A streptococcal strains did not give positive agglutination reactions when first tested. After retesting according to the instructions of the manufacturer, heavier suspensions of these two strains gave positive group A agglutination reactions. One pronase extract of a group C streptococcal strain reacted in the same manner as the two group A strains. Group F LA reactions were obtained with pronase extracts of four beta-hemolytic strains which were nongroupable by conventional techniques. With the autoclave procedure, only one of these strains demonstrated a group F reaction by LA. These reactions probably should not be counted as errors because all the beta-hemolytic nongroupable strains by conventional techniques used in this study were identical to group F streptococci morphologically and physiologically. Thus, with the exception of the group F antigen, the group F streptococci and the beta-hemolytic non-

TABLE 1. *The number of streptococci identified from growth on blood agar plates by two extraction procedures and grouped with commercial LA reagents*

Streptococci	Lancefield extract, capillary precipitin	Pronase extract, LA		Autoclave extract, LA	
		No. correct	No. negative and errors	No. correct	No. negative and errors
Beta-hemolytic group[a]					
A	39	39[a]	0	39	0
B	34	34	0	34	0
C	22	22[a]	0	22	0
F	10	10	0	10	0
G	35	35	0	35	0
None	15	11	4F[b]	14	1F
Non-beta-hemolytic group					
B	3	3	0	3	0
D (*S. faecalis*)	23[c]	23	0	23	0
D (*S. faecium*)	5[c]	4	0	4	0
D (*S. bovis*)	13	3	10 Neg[d]	3	10 Neg
C (viridans)	7	1	6 Neg	1	6 Neg
F (viridans)	6	3	3 Neg	4	2 Neg
G (viridans)	2	2	0	2	0
None (viridans)	23	23	0	22	1F

[a] Two group A and one group C strain did not react on initial testing; however, all three strains were correctly identified on repeat testing.

[b] F, False-positive reaction with group F reagent.

[c] One *S. faecalis* strain reacted with both group D and G latex reagents. The Lancefield extract of this strain was negative in a capillary precipitin test with group G antisera. Extracts of one *S. faecium* strain did not react in capillary precipitin or LA tests.

[d] Neg, Negative reactions in all latex reagents.

groupable streptococci used were identical.

Among the non-beta-hemolytic strains, the agreement between the conventional grouping procedure and both extraction procedures was 76% with the latex grouping reagents (Table 1). Pronase and modified autoclave extracts of all three nonhemolytic group B strains reacted with the latex reagents. Furthermore, 27 of 28 enterococcal strains (*Streptococcus faecalis* and *S. faecium*) were correctly identified by both extraction procedures and latex group D reagent. Only 3 of 13 pronase and modified autoclave extracts of the *S. bovis* strains, however, reacted with the latex group D reagent.

The results of testing pronase extracts obtained from the growth of the streptococcal strains grown overnight in 20 ml of THB are listed in Table 2. This table also lists the results of testing overnight supernatants as antigens for the LA test. Pronase extracts of all 39 strains of beta-hemolytic streptococci tested reacted correctly with the latex reagents. Broth supernatants of all the group A and C strains tested, however, failed to react with the latex reagents. The latex reagents correctly identified 9 of 9 group B, 8 of 9 group F, and 8 of 9 group G when broth supernatants of these strains were tested.

Only a few strains of non-beta-hemolytic streptococci were tested with the pronase extract from 20-ml growth (Table 2). Pronase extracts of more *S. bovis* strains (9 of 11) reacted with the latex reagents when the extracts were made from 20 ml of broth than when the same strains were extracted from the growth on agar plates (3 of 13). However, more viridans strains (8 of 16) also falsely reacted with the group D latex reagent when extracted by pronase from the 20-ml growth than with the group D reagent when extracted by pronase from the agar plate growth (0 of 37). This indicates that the latex group D reagent contains a cross-reacting antibody.

When the broth supernatants of the group D *S. faecalis* and *S. bovis* strains were tested with latex reagent, the group D antigen was detected in all the supernatants. None of the supernatants of three group D *S. faecium* strains, however, reacted with the group D latex reagent. In addition, the broth supernatants of 10 of 33 strains of viridans strains reacted with the latex reagents when they should not have.

DISCUSSION

The development of slide agglutination tests for grouping streptococci has given clinical microbiologists a tool which enables them to identify the different serogroups of streptococci within 18 to 24 h of receiving the specimen. No sophisticated laboratory equipment is necessary,

TABLE 2. *The number of streptococcal strains identified by LA reagents and pronase extracts of the growth of strains in 20-ml THB and THB supernatants*

Streptococci	Pronase extracts from 20-ml THB growth			THB supernatants		
	No. tested	No. correct	Errors	No. tested	No. correct	Errors
Beta-hemolytic group						
A	10	10	0	11	0	11 Neg[a]
B	9	9	0	9	9	0
C	5	5	0	3	0	3 Neg
F	6	6	0	9	8	1 Neg
G	8	8	0	9	8	1 Neg
None	1	1	0	7	7	0
Non-beta-hemolytic group						
B	1	1	0	1	1	0
D (*S. faecalis*)	2	2	0	6	6	0
D (*S. faecium*)	NT[b]			3	0	3 Neg
D (*S. bovis*)	11	9	2 Neg	11	11	0
C (viridans)	7	1	6 Group D	7	1	1 Neg 5 Group D
F (viridans)	4	3	1 Neg	4	2	2 Neg
G (viridans)	NT			1	1	0
None (viridans)	5	3	2 Group D	21	16	5 Group D

[a] Neg, Negative reaction with all latex reagents.
[b] NT, No strains tested.

and the procedures for performing the tests are simple. The accuracy of commercially available CA reagents for identifying group A, B, C, and G streptococci has been reported to be above 95% by several authors (2, 10, 16, 17, 18). We originally reported only a 77% accuracy for the commercially available CA reagents (R. R. Facklam and J. F. Padula, Abstr. Annu. Meet. Am. Soc. Microbiol. 1977, C17, p. 38). The results of our recent evaluation of this same product, however, are similar to those of other investigators. The best antigen preparations were 18-h THB or Trypticase soy broth supernatants. Of the streptococci tested, 99% were correctly identified, and no cross-reactions were detected (unpublished data). Trypsinization was not necessary for the supernatant antigen preparations. When 4-h or 18-h cell suspensions were used as antigens, however, trypsinization was often necessary because of multiple reactions. Multiple reactions in the commercially available CA reagents have been reported by other authors (1, 10, 16, 18). Only one multiple reaction was detected during our evaluation of the LA reagents. Pronase and autoclave extracts of one *S. faecalis* strain reacted with both group D and G latex reagents. Because Lancefield extracts of this strain did not react with CDC group G antiserum, we can only assume that the latex group G reagent contains a cross-reacting antibody or that the isolate in question possessed a cross-reacting antigen not detectable with CDC group G antiserum.

One disadvantage of the CA reagents is that reagents for detecting group D or F streptococci are not available. Another disadvantage is that the CA reagents can be used only for testing β-hemolytic strains. If group D or viridans strains are tested, they are often incorrectly identified (16, 17). The LA reagents contain all six group antibodies and can be used to test non-beta-hemolytic streptococci, but the results should be interpreted with caution. No erroneous identifications were noted when the extracts were made from agar plate growth. Several viridans species were erroneously identified as group D streptococci when the extracts were made from the 20-ml broth growth. The failure to detect the group D antigen in all the strains of *S. bovis* with the LA reagents is disappointing, but it should not be considered a serious drawback to using the reagents. Clinical microbiologists must determine whether group D streptococci are enterococci or nonenterococci, regardless of the grouping procedure. The microbiologist must determine the tolerance of all group D strains to 6.5% NaCl broth. We suggest that microbiologists who perform any kind of grouping procedure test all non-beta-hemolytic strains for bile-esculin reactions and tolerance to 6.5% NaCl broth. The reactions and interpretations are described elsewhere (6).

The discovery that extracts and broth supernatants of some viridans streptococci react with grouping reagents is not surprising. Most of these reactions represent cross-reacting antibod-

ies in the grouping reagents. For example, only one of the seven viridans strains that reacted in the capillary precipitin test with the CDC group C antiserum showed a line of homology in double-gel diffusion tests with our reference beta-hemolytic group C strain. This indicates that our group C antiserum contains a cross-reacting antibody. The results also indicate that the failure of the latex group C reagent to detect these same antigens is not an error. The latex group C reagent apparently does not contain the same cross-reacting antibody that the CDC group C precipitating antiserum does. The pronase extracts and broth supernatants of these same strains contain an antigen that reacts with the latex group D reagent; this indicates that the latex group D reagent may contain the same cross-reacting antibody as the CDC group C antiserum. These results indicate that, if serological grouping reactions of the non-beta-hemolytic streptococci are to be meaningful, the grouping reagents need to be tested for cross-reacting antibodies with extracts of viridans strains. Currently, this procedure is not done; however, our results show the benefits of testing the grouping reagents with extracts of viridans strains.

We conclude that the LA reagents accurately detected the antigens in pronase extracts of the growth of beta-hemolytic streptococci on blood agar as well as in THB. The latex reagents were also accurate in detecting the antigens in our modified autoclave extracts of beta-hemolytic streptococci grown on agar plates. In our opinion, all three of the procedures described above identified 100% of the beta-hemolytic strains. The latex reagents were not as accurate in identifying the non-beta-hemolytic streptococci. Because of the selected distribution of strains used in this study, it is probably inaccurate to predict the percentage of correct identifications. We feel, however, that these strains may be close to the distribution of streptococcal species encountered in clinical laboratories. The only errors of misidentification among the non-beta-hemolytic streptococci extracted from agar plate growth by both pronase and autoclave techniques occurred when the latex group D reagent failed to detect group D antigen from 10 of 13 *S. bovis* strains. Thus, 88% of the non-beta-hemolytic streptococci were properly identified by the latex reagents and both extraction procedures with the growth from agar plates.

At this time, we cannot recommend the latex reagents for use with the pronase extract of THB growth or THB supernatants of non-beta-hemolytic streptococci. Future lots of the group D latex reagent that do not contain the cross-reacting antibody we detected among viridans strains may be prepared. The broth supernatants of beta-hemolytic strains were not useful because the latex reagents did not detect the group A and C antigens in the broth.

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