APPLIED MICROBIOLOGY, Aug. 1969, p. 193-197

Vol. 18, No. 2
Printed in U.S.A.

Time, Cost, and Efficacy Study of Identifying Group A Streptococci with Commercially Available Reagents

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Received for publication 24 March 1969

During the 12-month period primary throat, wound, and skin cultures, tentatively identified as B streptococci, were submitted by 10 different clinical laboratories for evaluation. A total of 692 beta-hemolytic streptococci were isolated from cultures submitted and examined in parallel by the fluorescent-antibody, precipitin, and bacitracin techniques. An evaluation of the specificity and sensitivity in conjunction with basic and personnel costs was determined for each method. The standard Lancefield precipitin method was established as the standard by which the bacitracin and fluorescent antibody techniques were compared. With some variation depending on the commercial source of the disc, approximately 7% of the strains examined produced false reactions with the bacitracin disc. False-negative reactions were rarely noted by the group A fluorescent antibody technique (0.5%), but an appreciable number of other Lancefield groups (B, C, and G) were nonreactive with homologous conjugates.

The need to recognize clinical streptococcal infections correctly is well known not only because of the immediate disease but also because of the important possible sequelae of rheumatic fever and acute glomerulonephritis associated with parasitism by these organisms. The clinical manifestations of streptococcal infections of the respiratory tract are protean, and diagnosis based upon clinical evidence (4, 8) is frequently incorrect. Inasmuch as the prevention of rheumatic fever is predicated upon prompt and adequate antibiotic therapy, greater reliability must be placed on laboratory methods to identify these infections accurately. Inasmuch as streptococci only in certain groups are capable of producing disease in humans, it becomes important to define and recognize such groups by appropriate laboratory techniques. To be useful clinically, the laboratory test should be reliable, rapid, easily performed, and economically feasible. Currently, the fluorescent-antibody, precipitin, and bacitracin disc methods are utilized for the laboratory identification of pathogenic groups of streptococci causing clinical diseases.

This investigation was undertaken to evaluate specificity and sensitivity of commercially available reagents with each method in conjunction with basic and personnel costs.

MATERIALS AND METHODS

During these studies, primary cultures from throat, wound, and skin tentatively identified as beta-hemolytic streptococci were submitted by ten different clinical laboratories within the Oklahoma City area. All isolates of beta-hemolytic streptococci, a total of 692, were examined in parallel by the fluorescent-antibody, precipitin, and bacitracin techniques.

Bacitracin disc technique. Each isolated strain of beta-hemolytic streptococci was subcultured to a sheep blood-agar plate. A bacitracin disc from four commercial sources (specifically prepared for identification of group A streptococci), designated as 1, 2, 3, or 4, was placed over the heavily inoculated area of the plate and incubated for 18 to 24 hr at 37 C. Strains were evaluated as sensitive (Group A) when any zone of growth inhibition occurred from the edge of the disc. Absence of zone of growth inhibition was used to designate other than group A streptococci.

Fluorescent microscopy. Cultures to be examined were grown in Todd-Hewitt broth for 4 hr at 37 C, centrifuged, and suspended in buffered saline. Duplicate air-dried smears, fixed in 95% ethyl alcohol, were examined with conjugated antisera to groups A, B, C, D, F, and G obtained from two different commercial sources. Optimal staining titers were determined on

each lot of antiserum with homologous reference cultures obtained from the American Type Culture Collection. Smears were stained 30 min in a moistened chamber, rinsed for 10 min in three changes of buffered saline, blotted dry, and mounted with buffered glycerol. Smears were examined with a Leitz SM fluorescence microscope equipped with HBO-200 Osram mercury vapor lamp, BG-12 pass filter, and OG-1 barrier filter. Fluorescent-antibody reactions were read and graded visually from +1 to +4 by the method of Moody, Ellis, and Updyke (12).

Precipitin test. Overnight broth cultures (40 ml of Todd-Hewitt broth) were centrifuged, the supernatant discarded, and the sediment hydrolyzed with 0.4 ml of 0.2 N HCl in a boiling water bath for 10 min. The extract was cooled and centrifuged, and the supernatant was adjusted to a *p*H of 7.0 to 7.2 with 0.2 N NaOH. Each extract was tested with commercially available antisera for groups A, B, C, D, F, and G by drawing 2 to 3 cm of antiserum into a 10-cm capillary tube, mixing with an equal amount of extract, placing into a platicine block, and examining for evidence of a precipitate within 20 to 30 min (6). The antisera from two different commercial sources were examined separately and in parallel.

Cost procedures. To determine accurately the time required for each technique, the "technical time," "elapsed time," and the number of specimens examined were recorded daily. Time actually used by the technician in the performance of a procedure was considered technical time. Elapsed time was that time utilized in the performance of a technique which required a period of waiting before the next step in the procedure could be instituted. Overnight incubation of cultures was not considered an elapsed time.

Expendable supplies were prorated to an individual test based upon current costs. Cost of permanent type equipment was amortized for the actual time in use.

RESULTS

Table 1 is a summary of results obtained from examining 692 strains of beta-hemolytic streptococci by bacitracin discs obtained from four different commercial sources. Of the total examined, 85.8% were group A, 4.4% group B, 5.1% group C, and 2.9% group G. Other serogroups amounted to 0.7% of the strains examined. On the basis of these tests and depending on the source of disc, 0.5 to 5.4% of group A were resistant to bacitracin, whereas 16.1 to 25.8% of group B, 36.9 to 71.5% of group C, and 5.0 to 60.0% of group G were sensitive to bacitracin. Table 2 shows the percentage of false reactions encountered with bacitracin discs from the four different commercial sources and corrected for the population studied. For example, although numerous false-positive reactions were detected with group C streptococci, this group represented only 5.1 % of the streptococci examined.

The Lancefield precipitin method was the standard against which the bacitracin and fluorescent-antibody methods were compared. As shown in Table 3, 594, or 85.8%, of the isolates were positive for group A by the precipitin method. Groups B, C, and G accounted for 4.4, 5.1, and 2.9% of the isolates, respectively. During these studies, precipitin antisera were obtained from two different manufacturers and evaluated comparatively. Results obtained suggested no basic differences between the two products; however, certain lots of antisera produced barely perceptible precipitates, which resulted in some difficulty in reading. This phenomenon was observed with lots obtained from both commercial sources. It was noted, however, upon reexamination of the same acid hydrolysate with different lots of antisera, that

TABLE 1. *Comparison of results of bacitracin discs from four commercial sources*

Lancefield group by precipitin method	Source of disc	Strains inhibited/strains examined	Per cent false positive	Per cent false negative
A	1	588/594		1.0
	2	565/594		5.4
	3	553/555		0.5
	4	574/594		3.4
B	1	8/31	25.8	
	2	5/31	16.1	
	3	7/31	25.0	
	4	5/31	16.1	
C	1	27/38	71.5	
	2	13/38	42.1	
	3	26/38	70.3	
	4	14/38	36.9	
G	1	12/20	60.0	
	2	1/20	5.0	
	3	12/20	60.0	
	4	3/20	15.0	

TABLE 2. *False reactions encountered with bacitracin discs from four different commercial sources corrected for population studied*[a]

Disc	Per cent false negative	Per cent false positive	Total per cent false reactions
1	0.9	6.5	7.4
2	4.8	3.1	7.9
3	0.4	6.4	6.8
4	2.9	4.0	6.9

[a] Percentage based upon 692 strains examined of which 594 were group A.

quantitatively greater precipitation with larger flocculation was obtained.

The results obtained by comparing the fluorescent antibody test with the Lancefield grouping procedure are shown in Table 3. As noted, excellent correlation was achieved with the group A conjugate. Only three group A strains failed to react, resulting in 0.5% false-negative reactions. On the other hand, a greater number of nonreactive strains to homologous conjugates was encountered with groups B, C, and G. Although a relatively small number of group B streptococci was examined (31 strains), only 18 reacted specifically, resulting in 41.9% false-negative reactions. All isolates of groups B, C, and G were examined with group A antisera. Only 3 (3.3%) of the non-group A strains reacted with the group A conjugate. As was the case with the precipitin antisera, various lots of fluorescent-antibody sera were obtained from two different manufacturers and evaluated separately and in parallel. No significant differences could be detected between the two products; lots from one source consistently were of lower titer.

Resumes relating to time and costs for each of the three methods are shown in Table 4. Current costs of expendable supplies, i.e. media, antisera, and bacitracin discs, were prorated to an individual test basis in arriving at the basic item cost. The cost of permanent type equipment such as a centrifuge, fluorescent microscope, and incubator was amortized for actual time in use while performing the test. It should be noted that, because of the variability in breakage of reusable glassware, these items were not included in the total cost per test, but listed separately. Likewise the cost of the mercury vapor lamp was not included in the cost of the fluorescent-antibody procedure because of the variability in the life span for each lamp.

TABLE 3. *Results of fluorescent-antibody reactions with cultures confirmed by precipitin tests*

Lancefield group	Number examined	Per cent false negative reactions
A	594	0.5
B	31	41.9
C	38	18.4
G	20	15.0

TABLE 4. *Cost and time analysis for each technique*

Cost	Bacitracin technique	FA[a] technique	Precipitin[b] technique
Basic item	$0.245	$0.091	$0.37
Depreciation	0.035	0.04	0.04
Per test	0.280	0.131	0.41
Resuable glassware		1.67	1.98
Technical time	2.48 min	8.10 min	9.71 min
Elapsed time	1.67 min	9.20 min	23.09 min
Time per test	4.15 min	17.30 min	32.80 min

[a] Average based upon 15 tests/day
[b] Average based upon 10 tests/day

TABLE 5. *False positive reactions of 66 staphylococci examined by bacitracin, fluorescent-antibody, and precipitin techniques*

Technique	Per cent false positive readings
Bacitracin disc	
Disc 1	25.8
Disc 2	7.6
Disc 3	35.8
Disc 4	10.6
Fluorescent antibody	
Group A	0.0
Group B	6.1
Group C	1.5
Group G	3.0
Precipitin grouping	
Group A	19.7
Group B	47.0
Group C	13.5
Group D	4.5
Group F	1.5
Group G	7.5

The total time to complete a test was lowest for the bacitracin disc technique. As noted, the total time per test was separated into the individual components of technical time (time the technician was actually performing a procedure) and elapsed time (in which a period of waiting was necessary before proceeding to the next step).

During these studies, a number of staphylococci were submitted to the laboratory in error as streptococci; therefore, it was of interest to determine the reactions of each technique to staphylococci (Table 5). Of the 66 strains examined, false-positive reactions occurred with all procedures except anti-group A fluorescent antibody.

DISCUSSION

It is well documented that rheumatic fever is a preventable complication of streptococcal infections if an accurate diagnosis is made and

therapy is promptly initiated. Massell and coinvestigators (9) initially suggested that penicillin therapy might prevent rheumatic fever. Subsequent studies including those of Denny et al. (2) and Wannamaker et al. (19) confirmed that adequate penicillin therapy prevented initial attacks of rheumatic fever. The effect of therapy in the prevention of acute glomerulonephritis is less well documented, but apparently is partially effective (17). Inasmuch as the clinical diagnosis may be misleading (4, 11) and a high carrier rate of hemolytic streptococci may be encountered (15, 16), it becomes imperative that accurate bacteriological diagnosis be made so that proper therapy can be instituted.

Maxted (10) reported the use of discs prepared with 5 units of bacitracin per ml to differentiate group A streptococci from other beta-hemolytic streptococci. Subsequently, Levinson and Frank (7) reported on the use of discs prepared with 1 unit of bacitracin per ml. With the availability of commercial bacitracin discs, few laboratories will prepare their own discs; therefore, it was of applied interest to examine the specificity of the test with discs obtained from four commercial sources. During these studies, of the 594 strains of group A streptococci examined, 1.0 to 5.4% false-negative reactions were noted depending on the disc used.

As noted in Tables 1 and 2, the greatest error was noted with nongroup A strains being sensitive to bacitracin. Although these strains represented only 14.2% of the streptococcal isolates, a significant number were sensitive to bacitracin. These findings are consistent with previous reports by Estela and Shuey (3) and Streamer et al. (18).

A greater incidence of resistant group C, but not B or G, strains was encountered than previously reported by Maxted (10) and Levinson and Frank (7). Discs from sources 1 and 3 consistently produced greater false-positive reactions and fewer false-negative reactions. Throughout these studies, the percentage of false reactions remained fairly constant for discs utilized from each source. It is assumed that the amount of bacitracin incorporated into discs was constant for each manufacturer but varied among the different manufacturers. Based on these studies, it would appear that, if standardization in the amount of bacitracin incorporated into the disc could be achieved, the probability of false reactions could roughly be estimated. Storage of discs for extended periods of time likely will increase the technique error; therefore, it is assumed that this type of error could be reduced if discs were dated.

The sensitivity and specificity of the fluorescent-antibody test for group A was considered equal to the precipitin test. With the exception of minor variations in the dilutions of antisera for maximum fluorescence, equally satisfactory results were obtained with the products of two commercial suppliers. However, as supplied, many lots of antisera examined were of sufficiently low titer that appreciable dilution would significantly reduce the sensitivity of the test. Although large populations of groups B, C, and G were not examined by the fluorescent-antibody test, significant false-negative reactions were encountered especially with group B.

Time and cost analyses for each technique, as shown in Table 4, indicate that, of the three procedures, expendable materials was lowest for the fluorescent-antibody technique. As noted, the total time (both technical and elapsed time) was proportionally higher for the fluorescent antibody and precipitin procedures, respectively, than the bacitracin disc technique. In view of the appreciable increase of technician's salaries in recent years, the salary component obviously represents significant differences between the three procedures. Time required to obtain a pure culture of streptococci for examination was not included in the total time per test. Inasmuch as the fluorescent-antibody test is not prefaced by pure-culture technique, time expended for the latter must be taken into consideration.

As shown in Table 5, 66 random strains of staphylococci were examined for false-positive reactions by the three techniques. Although it is assumed that this error will not occur when a Gram stain and catalase determination are performed, these data indicate that significant false-positive reactions occurred, with the exception of group A fluorescent-antibody examination. Since antibodies against staphylococci may occur in nonimmunized rabbits (1), it was expected that some staining of staphylococci with group A conjugate would be encountered. Failure of the group A antisera to stain the staphylococci examined could be attributed to low levels of fluorescence which were diluted out or to specific absorption techniques by the manufacturer, or both.

The fluorescent-antibody test has been applied to direct throat swabs and 2-hr broth cultures (13). This study and others (5, 13, 14) demonstrate that the fluorescent-antibody test making use of group A conjugate is specific and sensitive. However, unless an appreciable number of specimens are examined daily the time and cost for examining several specimens daily would likely be prohibitory. Additionally, as pointed out by Moody et al. (13), the reliability of the fluorescent-antibody test is predicated upon the

use of trained personnel, reliable equipment, standardized reagents, and adequate controls. In laboratories where sufficient cultures do not warrant the fluorescent-antibody procedure, an appropriate compromise can be achieved by examining strains resistant to bacitracin with precipitin grouping for false-negative reactions.

ACKNOWLEDGMENT

This investigation was supported by Public Health Service grant no. PH-108-65-207(P) from the Division of Chronic Diseases, Heart Disease Control Program.

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