

# Bacitracin and Coagglutination for Grouping of Beta-Hemolytic Streptococci

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Bacitracin may be used for presumptive differentiation of group A from other beta-hemolytic streptococci. Whatever criteria are used for the test, a small number of erroneous results will be obtained. With the 1,161 strains studied in this laboratory, using a zone of 10 mm or greater as indicative of group A was better than using Maxted's original criterion of any zone of inhibition for group A. Co-agglutination is a preferable alternative to bacitracin testing, providing a grouping result as quickly as the bacitracin test but with the advantage of giving a definite grouping result. With the 247 strains studied so far, coagglutination results have correlated exactly with results of conventional grouping by a precipitin method.

Since Maxted (11) first described the use of bacitracin for the presumptive identification of group A beta-hemolytic streptococci, most laboratories have adopted this test as part of their routine for identification of streptococci. However, the method followed in many laboratories varies from that described by Maxted in such factors as inoculum density, concentration of bacitracin, and interpretation of results.

In particular, most manufacturers of commercial bacitracin diagnostic disks quote zone sizes expected for group A streptococci, which is in complete contrast to Maxted and Levinson and Frank (10), who both showed that any zone of inhibition should be regarded as indicative of group A. Coleman et al. (4) showed that the need to measure zone sizes was dependent upon the strength of the bacitracin disks used. The results obtained with routine clinical isolates in this laboratory support the view that if bacitracin screening is used, zone sizes should be measured, thus reducing the number of streptococci wrongly identified by the test.

As an alternative to the use of the bacitracin screening test, an evaluation of coagglutination for grouping of streptococci using commercial reagents (Phadebact streptococcus kit) was carried out. The results show that coagglutination is a suitable method for grouping streptococci quickly, easily, and accurately and eliminates the need to use the bacitracin test.

## MATERIALS AND METHODS

**Strains.** All strains of beta-hemolytic streptococci studied were routine isolates from clinical specimens submitted from a large general hospital and general practitioners. There were 1,161 organisms studied over

an 18-month period in 1976-1977. Routine isolation was carried out on sheep blood agar plates incubated both aerobically and anaerobically at 35°C.

**Bacitracin testing.** A straight wire was used to touch four to five colonies and transfer the inoculum to a sheep blood agar plate. A dry cotton swab was then used to spread the inoculum uniformly across a segment of the plate. A second dry swab was used to spread the inoculum in two different directions, and then a wire loop was used to give a final fractionation to check for purity. Bacitracin disks (Taxo A, 0.04 U; BBL) were placed on both of the streaked segments, and the plates were incubated aerobically at 35°C for 18 to 24 h. The zone of inhibition was measured around the disk where the inoculum was semiconfluent (dense but not confluent). This procedure was evaluated and found to be reproducible for both inoculum density and size of zones obtained.

**Coagglutination.** Streptococci were inoculated into a modified Todd-Hewitt broth and incubated at 35°C for 18 to 24 h. Coagglutination was carried out using the Phadebact streptococcus grouping kit. The method was as described in the kit, except that the volumes used were drops from capillary tubes to conserve reagents. Trypsinization, if required, was carried out according to instructions in the kit.

**Serological grouping.** Extracts of streptococci were prepared by Lancefield's hot acid extraction method (9), using cultures incubated overnight at 35°C in modified Todd-Hewitt broth. Precipitation was carried out in capillary tubes, using commercial sera of groups A, B, C, and G (Burroughs Wellcome). Any extracts not giving a precipitate after 20 min were diluted 1:4, and the test was repeated.

## RESULTS

**Evaluation of the bacitracin test.** In the 18 months of the study, 1,161 beta-hemolytic streptococci were isolated from routine clinical specimens. All beta-hemolytic streptococci were

tested for sensitivity to bacitracin and were serologically grouped. The distribution of strains in groups A, B, C, and G can be seen in Table 1. The large number of group B streptococci (27%) is a reflection of the number of urines and genital swabs received from patients in gynecological wards and clinics. The 4.5% of strains shown as ungrouped did not react with antisera to groups A, B, C, or G in the precipitin test.

Zones of inhibition around bacitracin disks were measured, and the distribution of zone sizes for each group can be seen in Table 1. Only 2 of 547 (0.4%) group A streptococci were completely resistant to bacitracin. All but 1 of the remaining 545 group A strains had zones of inhibition with a diameter of 10 mm or more. The majority of strains had zone diameters of 15 to 19 mm.

Most of the non-group A streptococci showed no inhibition by bacitracin, with 294 of 318 (92%) group B, 32 of 74 (43%) group C, 82 of 170 (48%) group G, and 35 of 52 (67%) ungrouped strains being completely resistant. Altogether, 443 of 714 (72%) non-group A streptococci showed no zone of inhibition to bacitracin. Using Maxted's criterion that any zone of inhibition by bacitracin is presumptive identification of group A streptococci, the figures from this study showed false negative results for 0.2% of strains and 14.7% false positive results. Using the manufacturer's guidelines of reporting streptococci with zones of inhibition measuring 10 mm or more as presumptive group A, a false positive result was found with 11.5% of strains, whereas the number of false negatives (0.25%) was not significantly increased. An acceptable screening test should be sensitive enough to detect all positive results while still being specific enough to exclude most of the negative results. Therefore, the decision to use a zone of 10-mm diameter or greater for the bacitracin test makes the results more specific than if one used Maxted's original rule of accepting any zone of inhibition as indicative of group A.

**Evaluation of coagglutination.** Towards the end of this study, the Phadebact coagglutination method was tested in parallel with the precipitin technique. A total of 247 strains have been grouped (Table 2). A representative number of strains from each group were tested, and in all four groups there was 100% correlation between the two methods. Approximately 5% of strains required trypsinization to give a clear result in the coagglutination method, and most of these occurred towards the end of the life span of the reagents. Although coagglutination reagents are expensive compared with reagents for other grouping methods, the test is simple to perform and provides a grouping result in a

TABLE 1. Diameter of inhibition zones around bacitracin for streptococcal isolates

Group	No zone	No. of isolates with:				
		Zone diam (mm) of:				
		1-4	5-9	10-14	15-19	20
A	2		1	51	466	27
B	294		4	13	7	
C	32		9	19	13	1
G	82		23	40	24	1
Ungrouped	35		1	9	6	1

TABLE 2. Comparison of precipitin and coagglutination methods for serological grouping of streptococci

Group	No. of strains grouped	
	Precipitin result	Coagglutination result
A	99	99
B	86	86
C	15	15
G	38	38
Ungrouped	9	9

relatively short time. Therefore, it is justifiable to use coagglutination routinely in a clinical laboratory.

## DISCUSSION

Maxted (11) first reported that bacitracin could be used to differentiate group A and non-group A beta-hemolytic streptococci. He used filter paper squares soaked in a solution containing 5 U of bacitracin per ml. Levinson and Frank (10), using standard filter paper disks, showed that a solution containing 1 U of bacitracin per ml was equally as effective in differentiating group A streptococci. Both of these reports concluded that any zone of inhibition by bacitracin was presumptive identification of group A beta-hemolytic streptococci.

Subsequently, a number of workers have compared bacitracin to the fluorescent-antibody technique (1, 2, 6, 13, 14), precipitin methods (1, 2, 5, 6, 14), and coagglutination (1), with variable reports on the usefulness of bacitracin. Bacitracin has been assessed as part of a set of screening tests for pathogenic streptococci (7) and has been compared on primary versus secondary cultures for rapid differentiation of group A streptococci (12). All of these later authors have used commercially prepared bacitracin disks of differing strengths without direct comparison to the strength of bacitracin solution used by Maxted or Levinson and Frank. Some (2, 6, 7, 12, 14) have continued to use Maxted's criterion

that any zone of inhibition is presumptive evidence of group A, whereas others (1, 5, 13) have followed manufacturers' directions and measured zone size, assuming that a zone of 10-mm diameter or greater is required for presumptive identification.

Schaub et al. (15) were the first to quote sizes for the zone of inhibition, stating that a zone of 15 to 20 mm in diameter indicated a group A streptococcus but giving no data to support this conclusion. Several manufacturers quote zone sizes to be used with their bacitracin disks for differentiating group A streptococci, but again there are no data to support this. Petran (13) and Ederer et al. (5), using Taxo A disks (BBL) and a zone of 10 mm or more, found good correlation between bacitracin screening and serological methods. Facklam et al. (7), using the same disks but following Maxted's rule of any zone of inhibition, also found good correlation between bacitracin screening and serological grouping. Coleman et al. (4) recommended using the presence of any zone with a 0.04-U disk but use of a zone of 12 mm or more with a 0.1-U disk and showed similar results with both methods.

Many workers have stated the need to distinguish group A from other beta-hemolytic streptococci. Whatever criteria are used, reliance on the bacitracin test to detect group A streptococci will result in a small number of group A strains not being detected. The results in this study show that bacitracin can be used to screen for group A beta-hemolytic streptococci providing confirmatory tests are carried out to exclude erroneous results. Use of a semistandardized inoculum and measurement of the zone of inhibition around a 0.40-U bacitracin disk have shown that a zone diameter of 10 mm or greater is reasonable presumptive evidence of a group A streptococcus. The use of these criteria makes the test more specific without any decrease in sensitivity when compared with criteria using any degree of inhibition as an indicator of group A. The number of misidentifications of streptococci as presumptive group A will be reduced. However, the results of bacitracin testing should only be used as a final identification when no confirmatory or alternative tests are available.

In 1973, Christensen et al. (3) first described the use of coagglutination for serological grouping of streptococci. The basis of the test is that specific group antibody is attached to staphylococci via the immunoglobulin Fc-protein A reaction. The staphylococci are then used as antibody-coated particles to be mixed with streptococci in a simple slide agglutination test. Christensen found that the method correlated with conventional grouping procedures and was ac-

curate, rapid, and simple. Hahn and Nyberg (8) and Arvilommi (1) also showed a very high correlation between coagglutination and precipitation reactions, although the latter worker found that a rather large proportion (5 to 60%, depending on group) of strains needed trypsinization before giving a satisfactory result in the coagglutination method.

In testing 247 strains of beta-hemolytic streptococci, this study found 100% correlation between coagglutination and precipitation methods. The test is simple, requires only a few minutes to perform, and produces a grouping result 24 h after isolation of the organism. This is as rapid as the bacitracin test, but the result is more accurate and gives a grouping result rather than a separation of group A from non-group A strains. The reagents are relatively expensive, but the savings in labor costs over other grouping procedures make the cost of performance similar to other methods.

Coagglutination is therefore a suitable method to use routinely in place of bacitracin screening. Use of coagglutination eliminates the problems of standardization and interpretation inherent in disk diffusion tests such as the bacitracin test.

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