Laboratory Evaluation of a Rapid Four-Hour Serological Grouping of Groups A, B, C, and G Beta-Streptococci by the Phadebact Streptococcus Test

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Grouping of beta-hemolytic streptococcal isolates by staphylococcal coagglutination was performed with the Phadebact Streptococcus Test to determine whether such isolates could be accurately grouped serologically within 4 h after examination of the primary isolation plates. Of 132 clinical isolates, 131 were correctly grouped by the Phadebact method using the Lancefield precipitation method as the accepted standard. Of the correctly grouped streptococci, 119 were definitively grouped within 4 h after examination of the primary plates, and the remaining 12 isolates were grouped within 24 h. Since the Phadebact Streptococcus Test contains coagglutination reagents for groups A, B, C, and G, those isolates that failed to react were considered as positive for groups other than the four included in the test system. There were 23 such isolates in this study. Lancefield grouping of these isolates indicated that nine were group F, five were group D, and the remaining nine were not groupable with the Lancefield reagents employed in this study. The one Phadebact "failure" involved an isolate that produced a 4+ reaction with the Phadebact group A reagent and a 4+ reaction with Lancefield group F reagent.

Until recently, most clinical laboratories wishing to serologically group beta-hemolytic streptococci had to use the method described by Lancefield (11). The Lancefield method requires considerable amounts of a technologist's time and frequently produces results that are difficult to read. Thus, it has become common practice to simply identify a beta-hemolytic streptococcal isolate as either a "group A" or "non-group A" streptococcus based on the results of the bacitracin disk susceptibility pattern. Several recent studies have demonstrated that beta-hemolytic streptococci other than the group A organisms not only produce serious infection in man, but are also important epidemiologically (5, 8, 9, 16). As a result, it is no longer acceptable to identify a beta-hemolytic streptococcus simply as group A or non-group A.

Biochemical methods for identifying the beta-hemolytic streptococci have met with varying degrees of success. The bacitracin disk susceptibility test has been demonstrated by Moody (13) to be fairly reliable but to produce different results based on the commercial source of the disks. A recent study (6) employing both bacitracin disks and trimethoprim/sulfamethoxazole susceptibility disks to differentiate between group A and B streptococci, as well as to differentiate these two groups from

the remaining groups, has also demonstrated failures. The biochemical approach presented by Cowan and Steel (2), although adequate, involves a large battery of biochemical media and, as such, becomes impractical for most laboratories.

In 1973, Christensen et al. (1) reported a relatively simple method for serologically grouping several of the beta-hemolytic streptococcal groups by the use of dead staphylococci rich in "A protein," on whose surface group-specific streptococcal antisera had been adsorbed. This method is called coagglutination and has been reported by several investigators as being accurate, reliable, and simple to perform (3, 4, 7, 12).

In 1976, a coagglutination method for grouping members of groups A, B, C, and G became commercially available in the United States as the Phadebact Streptococcus Test (Pharmacia Diagnostics, Piscataway, N.J.). The purpose of this study was to determine the accuracy of this product as compared with the Lancefield method and, in addition, to employ a shortened incubation period to determine whether definitive grouping of the isolate could be accomplished on the day after receipt of the specimen.

MATERIALS AND METHODS

Each clinical specimen used in this study was

received on a dacron-tipped cotton swab in silica gel. Upon receipt, the swab was used to streak approximately one-sixth of a Columbia sheep blood agar plate containing 10 mg of colistin per liter and 15 mg of nalidixic acid per liter (Columbia CNA plate) and an improved sheep blood agar plate (Pfizer Diagnostics, Clifton, N.J.). The inoculum was then spread over the remaining surface of both plates with a sterile bacteriological loop. Two Taxos A disks (BBL) were placed on each plate, one in the area inoculated with the swab and the other in the area inoculated with the loop. Both plates were then incubated at 35°C under anaerobic conditions (GasPak [BBL]) for 18 to 24 h. After incubation, the plates were examined for the presence or absence of beta-hemolytic streptococcal colonies. If beta-hemolytic colonies were present, a typical colony was selected for Gram stain to confirm that the morphology of the organism was that of a streptococcus. Once confirmed as a streptococcus, the Taxos A disks were examined for any zones of inhibition of growth of the beta-hemolytic colonies. If present, these growth inhibition zones were measured as the diameter of the zone and expressed in millimeters.

Once it had been established that the primary plate(s) contained a beta-hemolytic streptococcus, four to five well-isolated, typical, morphologically similar colonies were picked and used to inoculate a tube containing 2 ml of Todd-Hewitt broth and a tube containing 8 ml of T-soy broth (both from Scott Laboratories). The Todd-Hewitt tube was inoculated first since it required the larger inoculum.

After 4 h of incubation at 35°C, 6 to 7 drops of the Todd-Hewitt cell suspension were removed with a sterile Pasteur pipette and used to serologically test the organism with the Phadebact reagents. The remainder of the Todd-Hewitt culture was reincubated overnight and tested again the following day with the Phadebact system. To test with Phadebact, the laboratory must have a small glass plate with several wells or rings, such as the type of plate that is used for the performance of the febrile agglutination slide test performed in most serology laboratories. The test is carried out by placing 1 drop of each Phadebact reagent into a separate well, adding 1 drop of the test culture, mixing well with applicator sticks, and then rotating the mixtures for 1 min. The reactions are examined with side illumination against a dark background, and each reagent-organism mixture is examined for complete agglutination.

The T-soy broth suspension was incubated overnight, processed according to the method described by Watson et al. (17), and then serologically grouped as described by Lancefield (11) with reagents for groups A, B, C, D, E, F, and G obtained from Wellcome Reagents Ltd.

The serological testing was performed by three individuals who recorded their results independently. Only after three procedures had been completed were the results compared and evaluated.

Stock strains of beta-hemolytic streptococci belonging to groups A, B, C, D, F, and G were obtained from B. Watson at Massachusetts General Hospital. These strains were given coded numbers and were

tested in exactly the same manner as the clinical isolates.

RESULTS

A total of 1,036 clinical specimens from different sources were cultured, and 132 were found to contain beta-hemolytic streptococci. The positive specimens consisted of 106 throat, 2 umbilicus, 5 vaginal, 1 cervical, 2 blood, 3 ear drainage, 2 eye (conjunctiva), 10 wound, and 1 urine.

All 132 isolates were recovered from the Columbia sheep blood CNA plates, whereas only 118 were recovered or observed from the improved blood plates. In all 14 instances, the inability to either observe or recover the beta-hemolytic streptococci from the improved blood plates was due to heavy overgrowth of the plates with gram-negative enteric bacilli.

The Phadebact system yielded 76 group A clinical isolates, 14 group B, 14 group C, 5 group G, and 23 isolates nongroupable by the Phadebact test. Of these 23 isolates, the Lancefield method indicated that 9 were group F, 5 were group D, and 9 were not groupable. Table 1 indicates the number of isolates correctly grouped by the Phadebact method at 4 and 24 h and the results obtained by the Lancefield method.

The two isolates demonstrating discrepancies in Table 1 were carefully reexamined for possible mixed culture. Single colonies were selected and used for testing, and unambiguous results were obtained from these single-colony cultures.

The 21 stock strains used in this study included 7 group A, 2 group B, 2 group C, 3 group G, 2 group F, and 5 group D cultures. All were correctly identified by both the 4- and 24-h Phadebact test as well as by the Lancefield technique. The group D and F cultures produced negative reactions with the Phadebact system but were considered as correct since this system does not include reagents for these two groups.

TABLE 1. Comparison between Phadebact and Lancefield serological grouping

No. of strains	Group	No. correct with Phadebact at:		No. grouped by Lancefield
		4-h reading	24-h reading	
76	A	71	75	76
14	B	12	14	14
14	C	9	14	14
6	G	5	5	5 ^a
22	Nongrouped	22	23	8 ^a group F 5 group D 9 negative

^a Lancefield reagents produced a 4+ reaction for both groups F and G; thus, one culture was not counted as either and is not included in this table.

According to the Phadebact instructions, multiple-agglutination reactions can occur, but the reagent demonstrating the fastest and most complete coagglutination should be considered as the positive reaction. Multiple agglutinations were observed with great frequency in this study, especially for the strains examined after only 4 h of incubation. Of the 75 group A isolates, 68 demonstrated very weak agglutination in the Phadebact C reagent. All of those isolates found by the Lancefield method to be group D organisms demonstrated very weak agglutination in all four Phadebact reagents, whereas those found by the Lancefield method to be either group F or nongroupable demonstrated negative reactions with the Phadebact reagents.

In this study, the 4-h Phadebact reading produced four group A isolates, two group B, five group C, and one group G that either failed to agglutinate in any of the Phadebact reagents or produced equal agglutination in all four reagents. This problem was eliminated in the 24-h readings of the same broth cultures.

DISCUSSION

The commercial availability of coagglutination reagents for grouping beta-hemolytic streptococci belonging to groups A, B, C, and G now offers the clinical laboratory the capability of accurately grouping most beta-hemolytic clinical isolates. The Phadebact Streptococcus Test has been available in the United Kingdom for some period of time so that both Maxted et al. (12) and Farrell and Amirak (4) have had an opportunity to evaluate it.

In their evaluation, Farrell and Amirak reported on the use of a 4-h broth culture rather than the 24-h broth culture recommended by the manufacturer. The idea that a laboratory might be able to produce a definitive grouping of a beta-hemolytic streptococcus on the day after the receipt of the culture, as reported by Farrell and Amirak, was very stimulating to me for two reasons. First, the hospital where this study was performed is located in a major resort area and, as such, has a very large non-referred outpatient service. Normally, the patient comes directly to the outpatient service, where he is examined and a culture is taken, if necessary. The patient is then instructed to call back for the culture results 2 or 3 days later. Since the patient is usually a long way from home and his own physician, this 2-day delay frequently produces considerable concern on the part of the vacationing patient, which often results in a considerable amount of time being spent on the telephone by both the outpatient

service and the laboratory. This is nonproductive technologist time and has been estimated to be equivalent to approximately 4 technologist h per day during the summer vacation season. It was felt that, by reducing the time interval between when the patient is seen and when results are available by 1 full day, much of the wasted telephone time could be regained as productive technologist time. Our personal experience indicates that, thus far, this is the case.

The second reason for my interest in having the capability of producing a grouping the day after the receipt of the specimen is that this capacity would allow the laboratory to generate data during a time-frame that makes the data clinically relevant and potentially important epidemiologically.

This study employed both a Columbia sheep blood agar CNA plate and a standard sheep blood agar plate, because many types of specimens often contain organisms, in addition to beta-hemolytic streptococci, that can mask the presences of streptococcal colonies. The Columbia CNA plate inhibits the growth of the gram-negative aerobic rods and, in this study, allowed 100% (132/132) recovery of streptococci. The standard sheep blood agar plate was included since it was not known whether the CNA-type plate had any effect on either the growth or the hemolytic properties of beta-streptococci. The results of this study indicate that CNA does not have any effect on the beta-hemolytic streptococci. Anaerobic incubation was employed on the basis of the data of Murray and co-workers (14), who demonstrated a better recovery rate of non-group A beta-hemolytic streptococci from anaerobic plates.

In this study, definitive grouping of 92.6% (119/132) of the beta-hemolytic streptococci was accomplished with the Phadebact reagents on the day after receipt of the specimen, and 99% of streptococci were grouped within 48 h after receipt of the specimen. It must be indicated, however, that these figures are based on the premise that streptococci that did not produce a reaction with the Phadebact system can be identified as "beta-hemolytic streptococci, not groups A, B, C, or G."

The one isolate that had to be considered as a Phadebact failure involved an organism that produced a 4+ reaction with Phadebact group A reagent and a 4+ reaction with Lancefield group F reagent. One other isolate failed to produce any reaction with the Phadebact reagents but did produce a 4+ reaction with both Lancefield groups G and F. According to the work of Jablon and co-workers (10) and the work of Ottens and Winkler (15), who found

that, in addition to group-specific antigens, many strains of group A also contain a carbohydrate antigen (type II) that is also found in both group F and G strains. In the case of the one isolate considered to be a Phadebact failure, this type II antigen may well account for the discrepancy, since the Phadebact system does not contain reagent for the group F streptococci.

Several investigators (3, 4, 8), as well as the manufacturer, have reported on the frequent multiple-agglutination phenomenon encountered with the Phadebact system. However, these investigators and the manufacturer's instructions indicate that the correct coagglutination reaction is rapid, complete, and produces large clumps of reagent, whereas the false reactions are slow to develop, incomplete, and produce a fine aggregate. In this study, we noted multiple-agglutination reactions with 68 of the 75 group A isolates. There was no difficulty in obtaining the correct grouping in any of the 68 instances. This multiple-agglutination reaction was not noted with any of the group B, C, or G isolates. Interestingly, all five isolates that proved to be group D organisms by the Lancefield method produced very weak reactions in all four Phadebact reagents.

The results of this study indicate that the Phadebact Streptococcus Test offers the clinical laboratory an accurate, reliable, and easy-to-use method for serological grouping of the group A, B, C, and G beta-hemolytic streptococci. In addition, this kit offers those laboratories that may wish it a method of serologically grouping the majority of beta-hemolytic streptococcal isolates within 30 h after receipt of the specimen. This study did not consider a cost-benefit analysis of the Phadebact test versus grouping with Lancefield's method. Such an analysis may be of interest to laboratories with a large volume of streptococcal cultures.

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