Standardization and Evaluation of the CAMP Reaction for the Prompt, Presumptive Identification of *Streptococcus agalactiae* (Lancefield Group B) in Clinical Material

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Primary cultures of clinical material were screened for the presence of colonies suspected of being *Streptococcus agalactiae* (Lancefield group B). Sixty-three such cultures and 108 other isolates of beta-hemolytic streptococci (groups A, C, and G), encountered during the first 3 months of the investigation, were studied by Lancefield grouping, sodium hippurate hydrolysis, and a standardized CAMP test. All streptococci were inoculated perpendicularly to streaks of a beta-toxin-producing staphylococcus on sheep blood agar plates and incubated aerobically in a candle jar and anaerobically at 37°C. Plates were examined after 5 to 6 and 18 h of incubation. The production of a distinct “arrowhead” of hemolysis was indicative of a positive CAMP reaction. All group B streptococci produced a positive CAMP reaction in the candle jar or anaerobically, usually within 5 to 6 h, and aerobically after 18 h of incubation. All group A streptococci produced a positive reaction only under anaerobic conditions. Groups C and G streptococci were negative under all atmospheres. The CAMP reaction is a prompt and reliable procedure for the presumptive identification of group B streptococci when a candle jar atmosphere is used during incubation.

Christie et al. (6) observed a lytic phenomenon which occurred when hemolytic or non-hemolytic group B streptococci were grown in a zone of staphylococcal beta-toxin activity on sheep or ox blood agar plates (BAP). Under anaerobic conditions the effect was more marked. However, they did not state what atmosphere was used to test group A, C, and G streptococci, which did not produce this phenomenon. Munch-Petersen et al. (20) then designed a test to detect the lytic phenomenon. A beta-toxin-producing staphylococcus was streaked across the center of a sheep BAP. Strains of streptococci were streaked at an angle to, but not touching, the staphylococcal inoculum. The BAP was incubated at 37°C and, after overnight incubation, was observed for positive or negative reactions. Murphy et al. (21) proposed the lytic phenomenon be called the CAMP (initials of the original authors) reaction. Later the term “CAMP” was given to other procedures. For example, group B streptococci were used to detect beta-toxin-producing strains of animal staphylococci (27); and Maassen and Wulkow (15) found that epsilon-toxin-producing staphylococci produced increased hemolysis in a zone of staphylococcal beta-toxin activity.

A variation of the original CAMP reaction has led to confusion as to how the test should be performed. Esselveld et al. (8) inoculated streptococci at an angle of 35 to 45° to a streak of a beta-toxin-producing staphylococcus on sheep BAP. They noted areas of hemolysis shaped like “candle flames” in a positive CAMP reaction after incubation at 37°C for 18 to 24 h. They reported that 99.5% of group B and 80% of group A streptococci were positive. Wilson and Miles (26) reported that it was best to incubate the BAP anaerobically. Since 80% of group A streptococci were reported to produce a positive CAMP reaction, it follows that the test has not been used extensively for the presumptive identification of group B streptococci in clinical laboratories.

Group B streptococci have been implicated as the cause of a variety of clinical manifestations (4, 12, 17). The high morbidity and mortality of neonatal sepsis due to these streptococci has been particularly noteworthy (2, 3, 5, 7, 9-11, 13, 16).

An attempt was made in this study to standardize the CAMP test and to determine its accuracy in the prompt, presumptive identification of group B streptococci in the clinical laboratory.

**MATERIALS AND METHODS**

Primary cultures of clinical material were screened for the presence of colonies suspected of being group B...
streptococci over a 15-month period. Preliminary identification was based on colonial morphology and a relatively small zone of hazy beta hemolysis (4). In addition, all other beta-hemolytic streptococci isolated during the first 3 months of this investigation were also studied. The following tests were performed on each isolate.

(i) Lancefield grouping. Extracts were prepared by the autoclave method of Rantz and Randall (22). Antisera obtained from Baltimore Biological Laboratories (Cockeysville, Md.) were used in the performance of the precipitin test (24).

(ii) Sodium hippurate hydrolysis. Sodium hippurate broth was inoculated with a single colony and incubated at 37°C. Aliquots of the medium were removed at 8, 18, 24, and 48 h and tested for the production of benzoates according to the method of Ayers and Rupp (1).

(iii) CAMP test. Twelve milliliters of tryptone and soy peptone agar (Difco Laboratories, Inc. Detroit, Mich.), containing 5% citrated sheep blood, were put into disposable plates (15 by 100 mm). The depth of the blood agar in the center of the plates was approximately 1.5 mm. Packed cell volumes of the sheep blood used varied from 40 to 47%. The plates were kept in plastic bags, refrigerated at 4°C, and discarded if not used within 21 days. They were warmed to 37°C before use. An inoculating needle or the edge of a loop was used to streak Staphylococcus aureus 681, a strain known to produce a high level of beta-toxin (14), in a straight line across the center of the plate. Strains of streptococci to be tested were streaked in a straight line 2 to 3 cm in length and at a right angle to this inoculum, with care taken not to touch the staphylococcal streak. A prototype strain of group B streptococcus and a maximum of four unknown strains were tested on each plate. The plates were inoculated in triplicate and incubated at 37°C under the following conditions: aerobically in a candle jar and anaerobically in a Gas-Pak system (BBL). The candle and anaerobic jars were prewarmed to 37°C. The production of a distinct "arrowhead" of hemolysis constituted a positive CAMP reaction.

RESULTS

During this study, 171 beta-hemolytic streptococci were isolated. Lancefield grouping revealed 81 strains to be group A, 63 group B, 11 group C, and 16 group G. Fifty (79%) of the group B isolates were cultured from the genitourinary tracts of females. Included were isolates from the fetal membranes of one mother and the cervical os of a second. The newborn of these two mothers had the same streptococci. Group B streptococci were isolated from the nose, throat, skin, umbilical stump, and urine of a third newborn. The 108 isolates of groups A, C, and G streptococci were predominantly from throat cultures.

None of the group A, C, or G streptococci produced a positive result in the sodium hippurate hydrolysis test. This test was negative for all group B streptococci at 8 h, moderately positive at 18 and 24 h, and strongly positive at 48 h.

All group B streptococci produced a positive CAMP reaction under the three atmospheric conditions used. When incubated in a candle jar or anaerobically, arrowheads of hemolysis were usually detected in 5 to 6 h (Fig. 1). Under aerobic conditions, incubation for 18 h was often required before a positive reaction was produced. The 81 strains of group A streptococci produced arrowheads of hemolysis under anaerobic conditions, but not under aerobic or candle jar atmospheres. However, under the latter two atmospheres, increased hemolysis was observed in the zone of staphylococcal beta-toxin activity with most of these streptococci. Under anaerobic conditions, the arrowheads of group B streptococci were at least twice as large as those of group A. None of the group C or G streptococci produced arrowheads or increased hemolysis under the three atmospheric conditions used.

DISCUSSION

The high morbidity and mortality of neonatal sepsis due to group B streptococci requires prompt identification of these bacteria. Although serological grouping is the test for identifying group B streptococci, a disadvantage of the procedure is the requirement of at least overnight growth to produce sufficient cell mass for antigen extraction purposes. The hippurate hydrolysis test has been reported to be the most

![Fig. 1 CAMP test after incubation in candle jar at 37°C for 5 h. Horizontal streak is S. aureus 681. A, B, C, and G are, respectively, Lancefield groups A, B, C, and G streptococci. Arrowhead phenomenon is distinctly noted with the group B streptococcus.](http://jcm.asm.org/Downloaded from http://jcm.asm.org/ on August 14, 2017 by guest)
specific biochemical procedure for the identification of group B streptococci (4), but 24 to 48 h of incubation are required before sufficient benzoates are produced to yield a positive result. Prompt identification of group B streptococci can be made with the fluorescent antibody technique (19). However, the equipment is expensive and many clinical laboratories do not have it.

The CAMP reaction is a reliable test for the prompt, presumptive identification of group B streptococci. However, there are several important factors which must be considered when performing and evaluating the results of this test. Prompt results are obtained when the staphylococcal and streptococcal inocula are in an early stage of growth and the sheep BAP and candle jar are precwarmed to 37 C. Under these conditions, discernible arrowheads of hemolysis, a positive result, are usually seen after 5 to 6 h of incubation. An extended period of time may be required for a positive result when the test is performed under aerobic conditions. Group B streptococci will produce a positive result under anaerobic conditions, but so will group A. Citrated or defibrinated sheep blood must be used, as no reaction will occur with horse, human, rabbit, or guinea pig blood (6). The presence of beta-antitoxin in some batches of sheep blood will inhibit staphylococcal beta-toxin production and lead to false negative results. This can be avoided by using only erythrocytes (20). Any size plate may be used; however, the depth of the blood agar is important. As the depth of the blood agar is increased, the arrowheads of hemolysis become less distinct. An accurate test will not ensue if the surface of the BAP is wet or the inocula intersect. If inocula are not perpendicularly to each other, the production of arrowheads of hemolysis will not occur, and false positive results will occur if the unknown is not a streptococcus. For example, delta-toxin-producing Staphylococcus epidermidis will produce synergistic hemolysis when inoculated perpendicularly to a beta-toxin-producing staphylococcus (18).

Although nonhemolytic group B streptococci were not encountered in this investigation, human infections due to these organisms have been reported (25). Romero and Wilkinson (23) found that three of four such isolates enhanced the hemolysis produced by staphylococcal beta-hemolysin on sheep BAP. However, Butter and DeMoor (5) observed a negative CAMP reaction with two nonhemolytic group B streptococci of ichthyic origin.

Until the nature of the CAMP factor is known, it is recommended that the term “CAMP test” or “CAMP reaction” be reserved for the original observation by Christie et al. (6). The identification of beta-toxin-producing staphylococci, by using a prototype group B streptococcus, should be referred to as a “reverse CAMP test.” The potentiation of hemolysis in a zone of staphylococcal beta-toxin with other bacteria or their products should not be referred to as a positive CAMP reaction, but rather as a synergistic hemolytic effect.

Although S. aureus 681 was used in this study, any strain which produces a 4-mm or more band of beta-toxin activity (darkening) and less than a 2-mm band of complete hemolysis on sheep BAP is satisfactory for performing the CAMP test. However, 18 h of incubation may be required before a positive result is discernible.

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LITERATURE CITED