

Rapid Tube CAMP Test for Identification of *Streptococcus agalactiae* (Lancefield Group B)

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A rapid CAMP test for the presumptive identification of *Streptococcus agalactiae* (Lancefield group B) is described. Sheep erythrocytes, sensitized by staphylococcal beta-lysin and suspended in phosphate-buffered saline, were used to determine the lytic capacity of the neutralized supernatant fluids of 4-h broth cultures of streptococci being tested. A total of 96.2% of 130 group B streptococci gave positive CAMP tests, that is, lysis of the sheep erythrocytes after 10 min of exposure of streptococcal supernatants, whereas none of 381 non-group B streptococci tested produced any lysis. The test described provides presumptive identification of group B streptococci within 4 h and eliminates problems of intermediate reactions so that a positive test is indicative only of CAMP factor production.

The CAMP test is widely used for the presumptive identification of *Streptococcus agalactiae* (Lancefield group B), which has emerged in the last decade as an important cause of neonatal infections (15). The reaction on which the test depends consists of the lysis, by the CAMP factor of group B streptococci, of sheep erythrocytes (RBC) previously exposed to staphylococcal beta-lysin. A nutrient sheep blood agar plate is employed in the CAMP test as usually performed (2, 9, 10). A beta-lysin-producing *Staphylococcus aureus* strain is streaked diametrically across the plate, and then the streptococcus to be tested is inoculated at right angles to, but not touching, the staphylococcal inoculum, and the plate is incubated overnight at 37°C. The production of CAMP factor is manifested by a zone of glassy-clear hemolysis in the darkened area of blood agar adjacent to the beta-lysin-producing staphylococcus.

Most investigators have found little difficulty in producing a positive CAMP test with group B streptococci. However, a similar reaction can also be obtained with streptococci of other Lancefield groups, including up to 80% of group A strains under certain conditions (4). By employing defined conditions of atmosphere of incubation (3) and depth of agar (3, 14), a more specific test has been devised. Further, the nature of the medium, including its carbohydrate content and the atmosphere of incubation, have been studied with regard to optimum requirements for the production of both CAMP factor (12) and beta-lysin (6). Despite the interest over a number of years in this area, there does not yet appear to exist an accepted method which takes into account all the conditions known to

affect the production of both CAMP factor and staphylococcal beta-lysin and their combined action on sheep RBC.

In this paper is described a modification of the CAMP test. This method was found to provide a rapid, reliable, simple, and specific means of identifying group B streptococci.

MATERIALS AND METHODS

Streptococci. The strains used in this study were all beta-hemolytic streptococci isolated from throat, female genital, wound, urine, cerebrospinal fluid, and blood cultures at The Prince of Wales Hospital Microbiology Department between 1 January and 31 March 1979. These were Lancefield grouped (8, 13) using Burroughs Wellcome antisera. Of the 441 strains, 225 belonged to group A, 60 belonged to group B, 20 belonged to group C, 31 belonged to group D, 9 belonged to group F, 42 belonged to group G, 3 belonged to group K, and 51 strains could not be grouped. In addition, a further 70 group B streptococci obtained from earlier studies (18) were examined.

Rapid tube CAMP test. The rapid tube CAMP test is performed by exposing sheep RBC suspended in a fluid medium first to staphylococcal beta-lysin and subsequently to supernatants of broth cultures of streptococci.

Staphylococcal beta-lysin. Staphylococcal beta-lysin was produced by the technique of Haque and Baldwin (7). Beta-lysin-producing *S. aureus* strain S32A (1) was grown in a semi-solid agar medium composed of one part blood agar base (no. 2, Oxoid) and two parts nutrient broth (no. 2, Oxoid) with an agar content of 0.5% and incubated at 37°C in 20% CO₂. After overnight incubation, the agar was macerated and the culture was strained through sterile muslin gauze and then centrifuged twice at 3,000 × g for 10 min. The supernatant was filtered through a membrane filter (0.45-μm pore size; Millipore Corp.). The filtrate was stored in 20-ml aliquots at -4°C and,

under these conditions, remained stable for at least 12 months. When thawed for use, an aliquot of the preparation retained its activity for at least 4 weeks at 4 to 8°C. A titer of 64 was obtained when this preparation was used to lyse sheep RBC (7).

Phosphate-buffered saline. Physiological saline with added 0.02 M phosphate buffer (pH 7.3) was used as the suspending fluid for the sheep RBC and as the diluent for streptococcal culture supernatants. When the buffer was used as the diluent for staphylococcal beta-lysin, 0.001 M MgSO₄ was also added.

Sheep RBC. Sheep RBC suspended in Alsever buffer (Commonwealth Serum Laboratories) were triple washed in normal saline and, as packed cells, suspended in phosphate-buffered saline (pH 7.3) to make a 2% RBC suspension.

Sheep RBC—staphylococcal beta-lysin preparation. A 0.25-ml amount of the 2% sheep RBC suspension was mixed in a Wasserman tube with 0.5 ml of the staphylococcal beta-lysin preparation diluted 1:8. The mixture was incubated at 37°C for 30 min.

Streptococcal culture supernatants. The streptococcal culture medium contained (per liter) 25 g of brain heart infusion (Oxoid), 10 g of maltose, and 0.4 g of anhydrous disodium hydrogen phosphate. A 3-ml portion of this broth was inoculated with four colonies of each streptococcus strain grown overnight on nutrient horse blood agar plates prepared from tryptone soya agar (Oxoid). Survey strains were obtained from the original plate on which they were isolated and stored strains were obtained from fresh subcultures. The broths were incubated in a shaking water bath for 4 h at 37°C and then centrifuged, and the pH of the supernatant obtained was adjusted to 7.3 by the addition of NaOH at concentrations of 0.2 to 2 N.

Performance of test. Separate 0.25-ml volumes of prewarmed streptococcal supernatant, undiluted and diluted 1:2 and 1:4, were added to the incubated mixture of sheep RBC and beta-lysin contained in the Wasserman tubes. Care was taken to maintain the temperature at 37°C throughout the test period. A positive result was recorded when an estimated 50% hemolysis, or more, as determined visually (7), occurred in two or more tubes within 10 min. Comparisons were made with known positive and negative controls (vide infra) and scored as 0, +, ++, +++, or +++++ to correspond with estimated 0, 25, 50, 75, or 100% hemolysis. No lysis in any of the three tubes within 10 min was regarded as a negative result. Controls included supernatants from a known CAMP factor-producing group B strain, a group A streptococcus strain, and an uninoculated broth. Lysis should occur in unlysed control tubes only on cooling to 4°C for 30 min, i.e., hot-cold lysis due to the action of the beta-lysin on the sheep RBC (17).

RESULTS

When the test was carried out as described, of the 130 group B streptococci tested 125 (96.2%) produced a CAMP reaction, i.e., more than 50% lysis in two or more dilutions. In a 1:2 dilution 125 produced a reaction; in a 1:4 dilution, 114 produced a reaction. In none of the 381 non-group B streptococci tested was a positive

CAMP test recorded. Prolongation of the reading time beyond 10 min to 30 min revealed lysis in the first tube by a small number of group A and group C organisms.

In an attempt to improve the sensitivity of the test, the incubation period of the streptococcal broth cultures used to obtain the supernatant was extended beyond 4 h. Although this procedure enabled the five group B streptococci which had previously given a negative result to cause lysis, it led to a loss of specificity in that a high proportion of group A strains also produced a positive result under the same conditions.

DISCUSSION

There are a number of conditions to be observed for the satisfactory performance of any CAMP test. To ensure adequate staphylococcal beta-lysin activity for the CAMP reaction, the sheep RBC suspension should be washed free of staphylococcal beta-lysin antibodies which are often present in sheep blood (10), adequate concentrations of Mg²⁺ must be available (6, 17), and media and cultural conditions should be suitable for the production of the lysin (7). Further, the requirements for optimal CAMP factor production (12), in particular pH, peptone, and carbohydrate content, must be met by the same medium. In addition, even in the absence of CAMP factor, physical factors, including alterations in temperature, pH, and osmolarity, may induce lysis of the beta-lysin-affected sheep RBC (11, 16). This multiplicity of variables helps to explain the vagaries of CAMP tests, without even considering the problems of lysis due to non-group B streptococci. The tube CAMP test described here uses preformed staphylococcal beta-lysin and controls all the variables mentioned with the exception of that which the test is designed for, namely, CAMP factor production.

The tube CAMP test provides a rapid reliable and simple means of presumptively identifying group B streptococci within approximately 4 h provided only that four colonies can be obtained from the plate culture. Facklam et al. (5) used the plate CAMP tests of Darling (3) and Wilkinson (14) in a battery of tests to identify group A, B, and D streptococci. When the results of presumptive identification of group B streptococci by the CAMP test alone are considered, the results obtained by these workers are virtually identical with those reported here. However, in addition to its advantage of having the capacity to give a rapid result with most group B streptococci, the method of performing the CAMP test described here appears to have at least two other merits. The test is not expensive

in terms of materials or labor requirements, and it also eliminates the intermediate reactions which occur with many non-group B streptococci in the plate tests (4, 5). No false-positive reactions occurred with 381 non-group B streptococci in the tube test, which is simple to perform regardless of the number of strains to be tested.

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