CAMP-Disk Test for Presumptive Identification of Group B Streptococci

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The modification of the CAMP test for group B streptococci involved substituting a paper disk impregnated with partially purified beta-hemolysin for the staphylococcal culture that was the source of beta-hemolysin in the original test. The disk is placed onto a sheep blood agar plate beside the streak of *Streptococcus* being tested. The plate is then incubated aerobically at 35°C. A positive reaction consists of a lunar-shaped clear zone that appears within 24 h in the dark beta-hemolysin zone surrounding the disk. A double-blind study of 135 randomly coded streptococcal isolates showed complete agreement between the CAMP-disk test and the standard Lancefield precipitin test. All group B streptococci tested had positive reactions, and all strains tested from streptococcal groups A, C, D, and G were negative. The CAMP-disk test is a simple and convenient way to identify presumptively group B streptococci.

Of the several presumptive tests described for group B streptococci, the hippurate (1) and CAMP (2, 10) tests appear to be the most accurate and the most thoroughly evaluated (3, 4). The hippurate test identified over 99% of all of the group B strains tested at the Center for Disease Control (CDC). This test itself, however, takes 2 days to complete, includes several time-consuming manipulations, and must be verified by yet another test to differentiate groups B and D streptococci. In comparison, the CAMP test, which can be read within 18 h, requires minimal manipulations, and very rarely gives false-positive reactions with other streptococcal groups, identified over 98% of the group B strains tested at CDC (unpublished data).

The CAMP test is based on the fact that group B streptococci produce a factor (CAMP factor) that acts synergistically with staphylococcal beta-hemolysin (β -lysin) on sheep or ox erythrocytes (2, 10). The test consists of streaking the *Streptococcus* in question perpendicular to and discrete from a streak of β -lysin-producing *Staphylococcus aureus* on a sheep blood agar plate (SBAP). After overnight incubation at 35°C, the group B *Streptococcus* can be presumptively identified by the zone of complete hemolysis where the CAMP factor and β -lysin have diffused and converged.

Incorporating staphylococcal β -lysin into paper disks and thus obviating the need for always keeping a culture available would simplify the presumptive identification of group B streptococci in a clinical laboratory, whether

for diagnostic purposes or for epidemiological screening. This report describes the production and evaluation of such disks.

MATERIALS AND METHODS

Preparation of partially purified staphylococcal β -lysin. A description of the properties of β -lysin by Haque and Baldwin (5, 6) was useful in designing the experiments described below. The overnight growth of *S. aureus* strain SS697 on a Trypticase soy agar slant was resuspended in 3.5 ml of physiological saline (optical density of 0.59 at 500 nm). One milliliter of bacterial suspension was added to each of three 250-ml bottles of Todd-Hewitt broth adjusted to pH 5.2, 5.5, or 7.6. After overnight incubation at 35°C, the pH 5.2 and 5.5 Todd-Hewitt broths contained very light bacterial growth, and the pH 7.6 Todd-Hewitt broth contained heavy growth. Each bottle was centrifuged, and its supernatant fluid was passed through a membrane filter (Millipore Corp.; pore size, 0.22 μ m). Each sterile filtrate was tested separately for β -lysin activity as follows.

A filter paper disk (6.35 mm in diameter; Schleicher & Schuell Inc., Keene, N.H.) was allowed to touch a β -lysin filtrate until it was saturated with fluid. Prepared disks were placed on an SBAP, made with a 5% suspension of washed sheep erythrocytes in Trypticase soy agar and 5 mm deep. After the plates were incubated overnight at 35°C, the blood agar adjacent to each disk was examined for the dark zone characteristic of β -lysin. Because the best β -lysin activity occurred with disks impregnated with the pH 7.6 filtrate, the other two were eliminated from any further purification steps.

To concentrate and partially purify the β -lysin, 2 volumes of acetone that had been prechilled to -20°C were added slowly and with constant stirring to the pH 7.6 filtrate. After an additional 1-min

period of stirring, the cloudy fluid was centrifuged for 1 h at $10,000 \times g$ at 4°C . The precipitate was dissolved in 6 ml of 0.01 M phosphate-buffered saline, pH 7.6, containing 0.001 M MgCl_2 (PBS-Mg). This partially purified β -lysin was approximately 25 times more concentrated than the crude-culture filtrate (estimate based on volume reduction).

Preparation of CAMP-disks. Twofold dilutions of the partially purified β -lysin were made in PBS-Mg. Filter paper disks impregnated with various test dilutions of β -lysin were placed on SBAPs, and the diameter of the dark zone surrounding each disk was measured after overnight incubation at 35°C . Although zones occurred around disks saturated with dilutions as high as 1:16, the undiluted β -lysin was used in subsequent experiments. Approximately 0.02 ml of undiluted β -lysin was absorbed by each disk, and the resulting zone diameter was approximately twice as large as the disk diameter; that is, a 6-mm disk had a 10- to 13-mm zone (including the disk). These disks will hereafter be called CAMP-disks.

CAMP-disks were divided into three groups and desiccated as follows. Group 1 was placed in an evacuation chamber for 3 days at room temperature. Group 2 was evacuated for 3 days at 4°C . Group 3 was lyophilized and sealed in vacuo. Half of each group was stored with silica gel desiccant at room temperature, and the other half was stored with silica gel at -20°C . Subsequent tests showed equivalent β -lysin activity in all three groups, and the disks remained active after storage for 3 years at -20°C . Therefore, the CAMP-disks used in a double-blind evaluation of the test were prepared by the most convenient way: desiccation for 21 h at room temperature and storage with silica gel desiccant at -20°C .

Evaluation of CAMP-disk test. A preliminary study of stock streptococcal strains representing groups A through U and alpha-hemolytic strains representing streptococcal species *S. pneumoniae*, *S. mutans*, *S. uberis*, *S. salivarius*, *S. mitis*, *S. sanguis*, and *S. acidominimus* had revealed only one CAMP test-negative group B *Streptococcus* and three CAMP test-positive strains from groups other than B. The latter strains were in groups E, M, and U, but since these groups are not often encountered in a clinical laboratory, the decision was made to use only groups A, B, C, D, and G in the evaluation of the CAMP-disk test. A total of 135 strains, obtained from and identified by the Streptococcus Laboratory, CDC, by Lancefield grouping (7, 12), were used in the following double-blind study.

Each strain was streaked on a rabbit blood agar plate, which was then incubated overnight at 35°C . The streptococcal growth was streaked onto an SBAP approximately 5 mm from the edge of a CAMP-disk. As many as six strains, two per CAMP-disk, could be conveniently inoculated onto each SBAP, which was then incubated overnight at 35°C . Because a preliminary study with stock strains showed that group A as well as group B streptococci had positive CAMP-disk test reactions when the plates were incubated either anaerobically or in a candle jar, all plates in this study were incubated aerobically.

RESULTS

A typical CAMP-disk test is shown in Fig. 1. A lunar-shaped area of complete hemolysis in the dark β -lysin zone surrounding the CAMP-disk indicates a positive CAMP-disk reaction and, hence, a presumptive group B *Streptococcus*. The hemolytic reaction extended throughout the depth of the 5-mm SBAP, and this observation was used as the basis for distinguishing a positive reaction from the intermediate reaction exhibited by some group A strains. The results of evaluating the CAMP-disk test with 135 streptococcal strains in a double-blind study are shown in Table 1. There was complete agreement between the CAMP-disk test and the Lancefield precipitin test.

DISCUSSION

Group B streptococci are isolated from a variety of human pathological material (13) but, in particular, are the etiological agents of an apparently greater proportion of cases of neonatal septicemia and meningitis than in the past (14). Prompt and accurate identification of group B isolates will hopefully lead to more specific therapy and improved prognoses (8). The standard method for identifying group B streptococci is the Lancefield precipitin test (7, 12). The immunofluorescence technique (11) is a good alternative method, especially because it identifies nonhemolytic group B streptococci and those in cultures heavily contaminated with other organisms. It has the further advantage of speed: a diagnosis can be made within 2 to 6 h after obtaining the culture. However, because many commercially prepared group B antisera and immunofluorescence reagents are of poor quality, many laboratories are using presumptive tests either instead of or in addition to serological tests.

Since it was first described in 1944, the CAMP test has been used to identify presumptively group B streptococci (2). The advantages of the CAMP test over other presumptive tests include simplicity, accuracy, and speed. Until now, the tests required that a viable β -lysin-producing strain of *S. aureus* be streaked on SBAP with the *Streptococcus* being tested, with a synergistic enhancement of hemolysis in the area between the growth of the two strains constituting a positive reaction. The experiments described in this paper were done to eliminate the need for the *Staphylococcus* culture. Paper disks were impregnated with β -lysin, desiccated, and stored for over 3 years without any discernible loss of activity. When a CAMP-disk was incubated on SBAP with a group B *Streptococcus*, the synergistic hemolysis appeared as a lunar-shaped clear area in the

FIG. 1. Example of CAMP-disk test with streptococcal groups A (top left streak), B (center left), C (bottom left), D (top right), and G (center and bottom right). The lunar-shaped area of complete hemolysis throughout the depth of the blood agar within the β -lysin zone (barely visible in photograph) surrounding the center CAMP-disk is interpreted as a positive reaction.

TABLE 1. Numbers of streptococcal strains identified as presumptive group B by the CAMP-disk test

Serological group	No. of strains	
	+	-
A	0	25
B	26	0
C	0	28
D	0	26
G	0	30

dark β -lysin zone surrounding the disk. A double-blind study of 135 streptococcal strains representing groups A, B, C, D, and G showed complete agreement between the standard Lancefield serological test and the CAMP-disk test.

Accuracy of the CAMP-disk test depends on observing the following precautions. Blood agar plates must contain a 5% suspension of washed sheep erythrocytes and be 5 mm deep. The dark β -lysin zone surrounding a 6-mm CAMP-disk should be 10 to 13 mm in diameter (including the diameter of the disk). Test streptococcal strains should be streaked, in pure culture, approximately 5 mm from the edge of the CAMP-disk. The inoculum should be sufficient

to produce confluent growth. Inoculated plates should be incubated aerobically at 35°C and must be read within 24 h. A reaction is considered positive only if the synergistic hemolysis in the β -lysin zone extends throughout the depth of the blood agar. Control strains of streptococcal groups A, B, C, D, and G should be used to assure the reactivity of each new lot of CAMP-disks and as a guide for laboratorians unfamiliar with the test.

If CAMP-disks were prepared and stored in advance or if they were available commercially, the CAMP-disk test would be as convenient to use for presumptively identifying group B streptococci as the bacitracin test (9) is for presumptively identifying group A streptococci.

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