CAMP Test Protocols

Created: Monday, 09 October 2006

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Information

History

The "lytic phenomenon" between *Staphylococcus aureus* and *Streptococcus agalactiae* (group B), the basis for the CAMP test, was first observed on cultures of milk being tested for hemolytic*Streptococcus* during an outbreak of scarlet fever. Colonies of *Streptococcus* were only surrounded by zones of complete hemolysis when they were growing in close proximity to colonies of β-hemolytic*Staphylococcus* (3). Further studies showed that this enhanced hemolysis was a result of the production of a heat stable, filterable agent produced by only *Streptococcus agalactiae* (group B). Nongroup B *Streptococcus* failed to exhibit this enhanced hemolysis when grown near colonies of β-hemolytic *Staphylococcus* (3).

Munch-Petersen used this phenomenon to develop a test to identify *Streptococcus agalactiae* (group B), a major cause of mastitis in milk samples. He observed that "when two streptococcus colonies grow 5 mm from the β-producing staphylococcus and 5 to 6 mm from each other, the lysed area may become half moon shaped" (9).

Murray, in his paper published in 1952, was the first to refer to this test as the CAMP test, named after the first researchers (Christie, Atkins, Munch-Petersen) to observe this reaction. Murray inoculated the test organism (*Streptococcus*) in a straight line at right angles to the β-hemolytic*Staphylococcus aureus*. At the time of Murray's paper the CAMP test was the standard test for the identification of Lancefield group B (*S. agalactiae*) in milk as a means to screen for mastitis infections in cows (10).

Darling developed a standardized method of inoculating and incubating the CAMP test (see protocol section). The CAMP test was found to be effective for the "prompt and reliable" identification of*Streptococcus agalactiae* (group B). This test replaced other tests used to identify *Streptococcus agalactiae* (group B) in the clinical lab as results could be observed in as little as 18 hours and required few manipulations (4). The reliable CAMP test was found to rarely give false positives with other *Streptococcus* (11).

Purpose

The CAMP test is used to identify *Streptococcus agalactiae (*group B) (CAMP positive) and to differentiate it from *Streptococcus*

pyogenes (group A) (CAMP negative) and nongroup B*Streptococcus* (CAMP negative) (4).

Theory

The β-lysin produced by β-hemolytic *Staphylococcus aureus* acts synergistically with the CAMP factor produced by both β-hemolytic and nonhemolytic *Streptococcus agalactiae* (group B). This synergistic reaction results in an enhanced and very visible zone of hemolysis in the region between the two cultures. The synergistic zone is not observed in group A, C, and G *Streptococcus* (8).

RECIPE

The CAMP test is performed on standard sheep blood agar plates, trypticase soy agar +5% sheep blood. Atlas protocol Blood Agar Plates-recipe

PROTOCOL

Staphylococcus aureus is inoculated onto a sheep blood agar plate by making a narrow streak down the center of the plate with a loop or the edge of a needle (4). A strain of *S. aureus* known to produce a high level of β-toxin should be used (4, 8). The test organism (suspect group B *Streptococcus*) is streaked in a straight-line inoculum at right angles to the *S. aureus*. The *Streptococcus* streak should be within 2 mm of, but not touching, the *S. aureus* streak (Fig. 1) (4). The plates are incubated at 35°C for 24 hours. Incubation in ambient air is recommended to reduce the number of false positives (8).

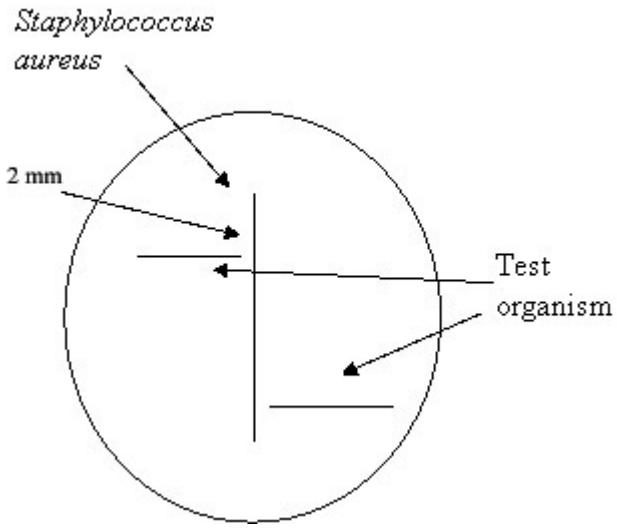

FIG. 1. Streaking pattern for the CAMP test.

A positive result is indicated by an "arrowhead"-shaped enhanced zone

of β-hemolysis. (See Atlas Protocol Blood Agar Plates-interpretation of β-hemolysis) in the area between the two cultures with the "arrow point" toward the *S. aureus* streak. No enhanced zone of β-hemolysis is observed in a CAMP negative reaction (8) (Fig. 2).

CAMP Test
Atlas images-CAMP test for the identification of *Streptococcus agalactiae (*group B)

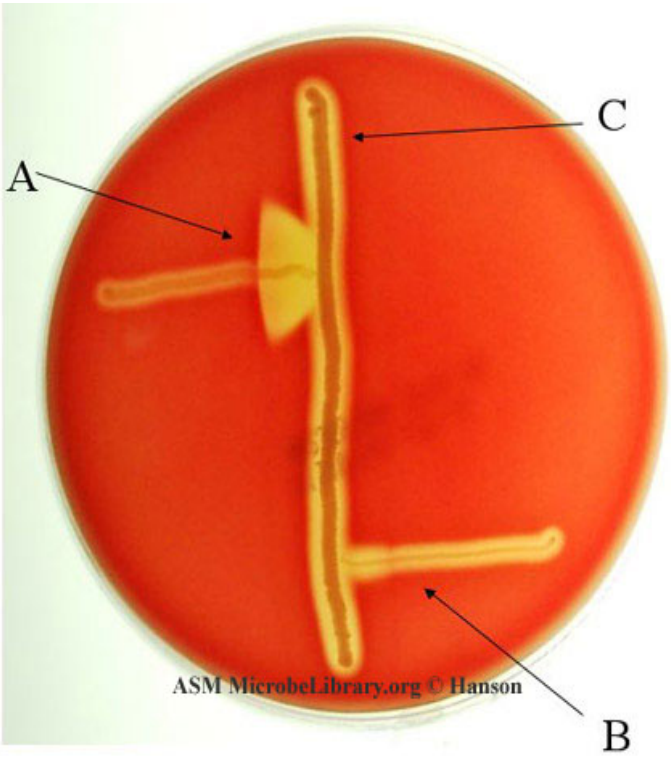

FIG. 2. CAMP test for the identification of *Streptococcus agalactiae (*group B). (A) *Streptococcus*(group B) shows a positive CAMP reaction. (B) *Streptococcus pyogenes* (group A) shows a negative reaction when inoculated at a right angle to (C) *Staphylococcus aureus*.

Other applications of the CAMP test.

CAMP test for the identification of *Listeria monocytogenes*

A version of the CAMP test was first used by Groves to identify pathogenic *Listeria monocytogenes*. He found that most of the pathogenic *L. monocytogenes* he tested were also CAMP positive (5). To perform the CAMP test for the identification of *L. monocytogenes, L. monocytogenes* is streaked at a right angle to the streak of β-

hemolytic *Staphylococcus aureus* on a sheep blood agar plate. It is recommended that the plate be incubated in ambient air at 35°C for 24 hours (8). A positive CAMP reaction is indicated by a weaker enhanced zone of β hemolysis and a smaller less obvious rectangular zone of hemolysis (5) (Fig. 3). *Listeria monocytogenes* is a biosafety level 2 organism and should be handled with the necessary precautions (2) (see safety section). Atlas Images CAMP Test for the identification of *L. monocytogenes*

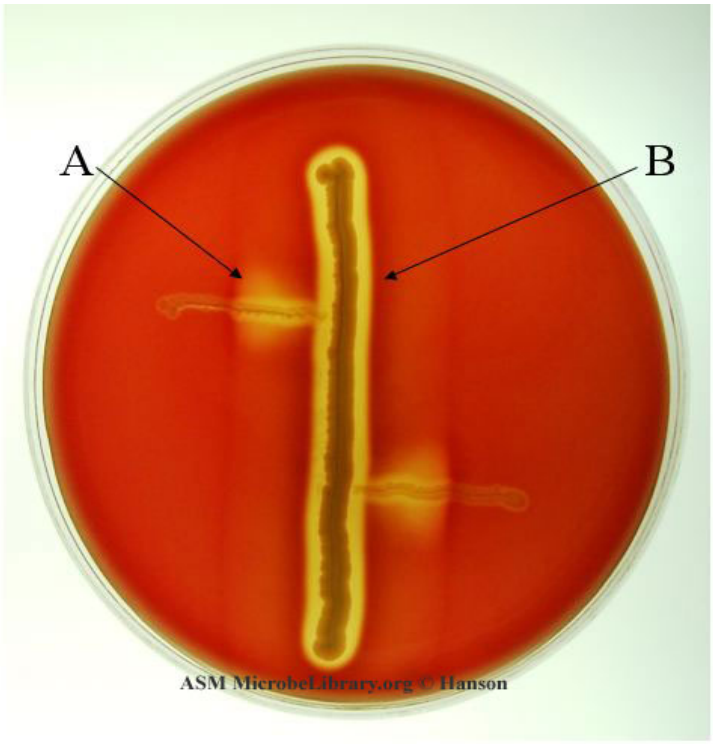

FIG. 3. CAMP test for the identification of *Listeria monocytogenes*. (A) *Listeria monocytogenes*shows a positive CAMP reaction when streaked at a right angle to (B) *Staphylococcus aureus*.

Reverse CAMP test for the identification of *Clostridium perfringens*

Gubash first reported a synergistic effect between S*treptococcus agalactiae* (group B) and hemolytic*Clostridium perfringens*. The synergistic relationship was studied to determine if using *Clostridium perfringens* in place of *Staphylococcus aureus* would decrease the number of false positives observed using the traditional CAMP test. An arrowhead-shaped zone of hemolysis was observed when the two organisms grew at right angles on the sheep blood agar plate (6).

Hansen used the synergistic relationship between these two microbes to develop a test, known as the reverse CAMP test, using *Streptococcus agalactiae* (group B) for the identification of *Clostridium perfringens*. The

reverse CAMP is performed by streaking *Clostridium perfringens* down the center of a sheep blood agar plate (in place of *S. aureus* in the traditional CAMP). *Streptococcus agalactiae*(group B) is streaked at a right angle to *Clostridium perfringens* so that the streaks are not touching but are separated by 1 to 2 mm. Plates are incubated anaerobically to allow for the growth of anaerobic *Clostridium perfringens*. A positive reverse CAMP results in an arrow-shaped zone of hemolysis between the streaks of reverse CAMP-positive *Clostridium perfringens* and *Streptococcus agalactiae* (group B) (Fig. 4) (7, 12).

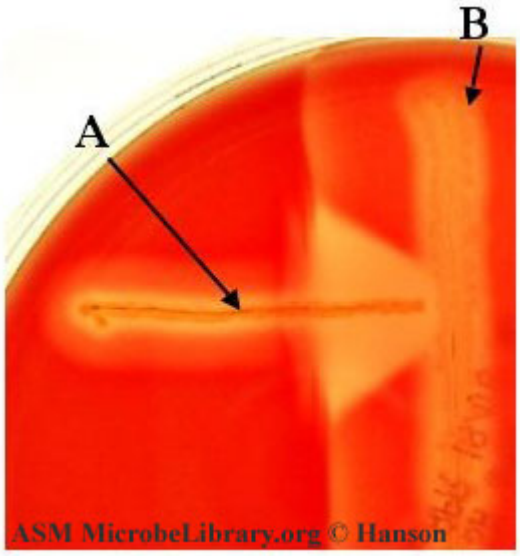

FIG. 4. The reverse CAMP test for the identification of *Clostridium perfringens*. (A) *Streptococcus agalactiae* (group B) is streaked at a right angle to (B) reverse CAMP-positive *Clostridium perfringens*.The reverse CAMP test can also be done by streaking *Streptococcus agalactiae* (group B) down the center of a sheep blood agar plate. The test organism, *Clostridium sp.,* is streaked at a right angle to and within 1 to 2 mm of the *Streptococcus agalactiae* (group B) inoculum. Plates are incubated anaerobically to allow for the growth of anaerobic *Clostridium perfringens*. A positive reverse CAMP result, shown by *Clostridium perfringens*, is a "bow tie" or reversed arrow zone of enhanced hemolysis at the junction of the two cultures (Fig. 5) (1, 8). Atlas Images CAMP Test Atlas Images- Reverse Camp test for the Identification of *Clostridium perfringens*

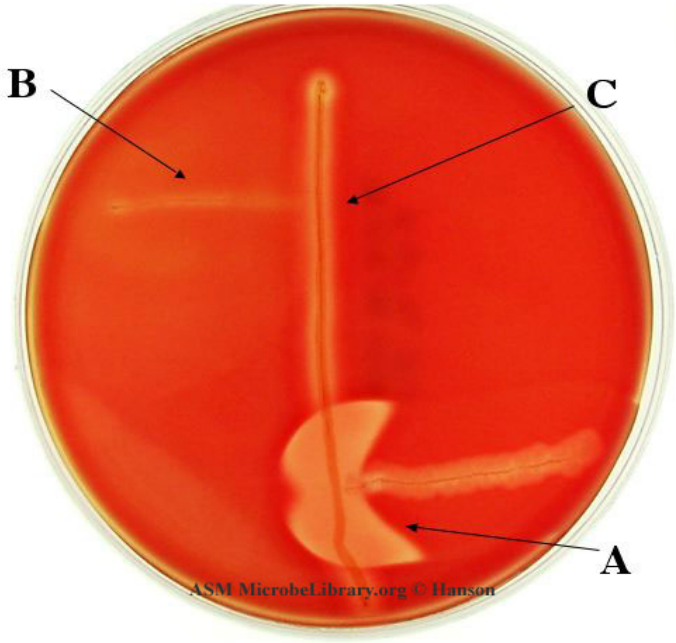

FIG. 5. The reverse CAMP test for the identification of *Clostridium perfringens*. (A) Reverse CAMP-positive *Clostridium perfringens* and (B) reverse CAMP-negative *Clostridium septicum*streaked at right angles to (C) *Streptococcus agalactiae* (group B).

SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the ASM Curriculum Recommendations: Introductory Course in Microbiology and the Guidelines for Biosafety in Teaching Laboratories.

COMMENTS AND TIPS

This section is to evolve as feedback on the protocol is discussed at ASMCUE. Please contact the project manager for further information.

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