

M.V.Sc. (Veterinary Microbiology), Monsoon semester

Date 31.12.2020

VMC- 602 (Bacteriology II), Unit III, Practical class

CAMP Tests (Standard and Rapid) and Reverse CAMP test

Dr. Savita Kumari

Assistant Professor-cum-Jr. Scientist

Department of Veterinary Microbiology

Bihar Veterinary College, BASU, Patna

CAMP factor

- ❖ *S. agalactiae* contains the CAMP factor, only beta-hemolytic *Streptococcus* secretes
- ❖ Pore-forming toxin first identified in this bacterium
- ❖ CAMP reaction is based on the co-hemolytic activity of the CAMP factor
- ❖ Commonly used to identify *S. agalactiae*
- ❖ Closely related proteins present also in other Gram-positive pathogens
- ❖ *cfb* gene encodes CAMP factor

CAMP test

- ❖ CAMP reaction- consists in a zone of strong hemolysis that is observed when *S. agalactiae* is streaked next to *Staphylococcus aureus* on blood agar
- ❖ *S. aureus* secretes sphingomyelinase
- ❖ Sheep red blood cells - rich in sphingomyelin, and upon exposure to sphingomyelinase become greatly sensitized to CAMP factor, which then effects hemolysis
- ❖ Hemolysis most pronounced in the zone between the colonies of the two bacterial species
- ❖ Co-hemolytic phenomenon- presumptive identification of Group B *Streptococci* (*S. agalactiae*)

CAMP test

- ❖ First described by Christie, Atkins, and Munch –Petersen in 1944
- ❖ The protein was named CAMP factor for the initials of the authors of the article that first described the phenomenon
- ❖ Types:
 - ❖ Standard CAMP test
 - ❖ Rapid CAMP test (spot test)
- ❖ Standard camp test are time consuming and/or expensive compared to the CAMP spot test

Principle

CAMP test detects the production of diffusible, thermostable, extracellular protein known as CAMP factor, produced by Group B *Streptococcus*

- ❖ The CAMP factor acts synergistically with the beta lysin produced by *Staphylococcus aureus* to produce a zone of enhanced lysis of sheep or bovine erythrocytes
- ❖ The standard CAMP test depend on the elaboration of two toxins during growth to form a typical arrowhead or flame-shaped clearing at the junction of the two organisms when they are placed perpendicular to each other

Standard CAMP test

Depend on the elaboration of two toxins during growth to form a typical arrowhead or flame-shaped clearing at the junction of the two organisms when they are placed perpendicular to each other

Rapid test

- Utilizes an extract of *Staphylococcal* beta-lysin that acts directly with the CAMP factor previously diffused in the medium around the *S. agalactiae* colony
- A positive CAMP reaction is indicated by an enhanced hemolysis within 30 minutes to 1 hour of adding a drop of CAMP factor reagent

Reverse CAMP

- ❖ A reverse CAMP reaction is a reaction whereby hemolysis by the beta-hemolysin of *Staphylococcus*
- ❖ Inhibited through the production of phospholipase C or D by some organisms (e.g. *S. agalactiae*, *Listeria* sp., *Corynebacterium* spp., and *Clostridium perfringens*)
- ❖ An arrow of no hemolysis is formed at the junction of the organism being tested with the staphylococci if the reverse CAMP test is positive

Purpose of CAMP test

Useful in the identification of both *S. agalactiae* and many gram-positive rods, including *Listeria monocytogenes*

Procedure of CAMP Test

A. Standard CAMP test

- Using an inoculating loop, streak a beta-lysin-producing *Staphylococcus aureus* (ATCC25923) in a straight line across the centre of a sheep blood agar plate
- Streak test organism in a straight line perpendicular to the *S. aureus*, but avoid touching the previously streaked area
- Streak the positive control organism parallel to and approximately 1 in. from the unknown organism
- Incubate the plate overnight at 37 °C in a CO₂ incubator

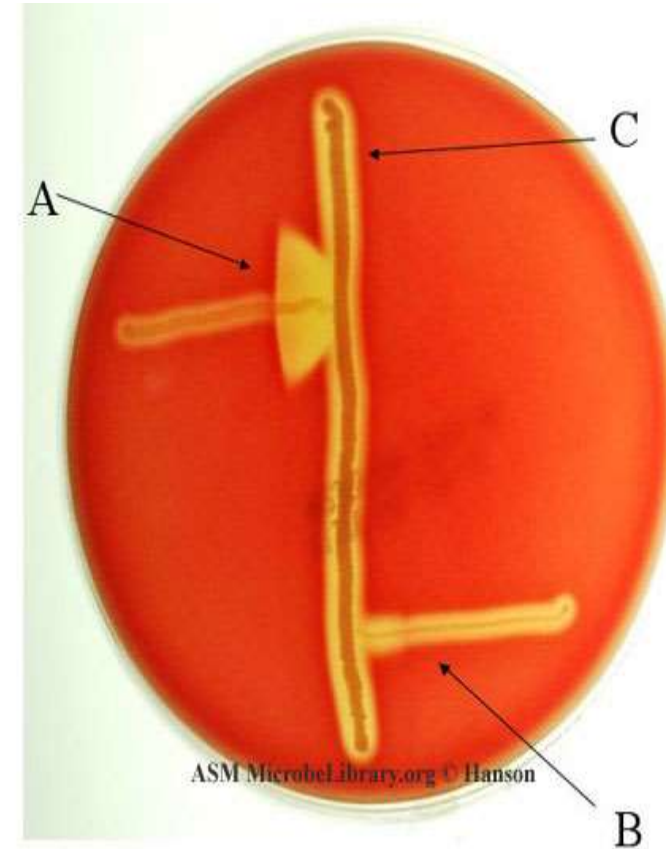


Image source- <https://www.asmscience.org>

B. Disk method

- Place disk containing beta-lysin of *S. aureus* on blood agar plate (BAP)
- Streak microorganism 2 to 3 mm from the edge of the disk
- Incubate the plate overnight at 35⁰ C in a CO₂ incubator

C. Spot rapid method

- Place 1 drop or a 10 µl loopful of CAMP Spot Test Reagent next to a presumptive *S. agalactiae* colony growing on BAP
- Incubate the plate right side up, for 20 min at 35⁰ C
- Examine with transmitted light for a zone of enhanced hemolysis next to the colony
- Re-incubate for up to 30 min if reaction is initially negative
- Use a hand lens if necessary for examining the plate
- Refrigeration may enhance reaction after incubation

INTERPRETATION

Positive standard assay

formation of a distinct arrowhead of hemolysis at the intersection of the staphylococcus and test organism streaks

Positive Reverse CAMP or phospholipase D

Distinct arrow of no hemolysis at the intersection of the two hemolytic organisms

Positive Disk test

Distinct crescent- or arc-shaped zone of complete hemolysis at intersection of disk of beta-lysin and isolate

Positive Rapid spot test

presence of clear enhanced hemolysis only where the diffused hemolysis overlaps

Negative test

Lack of enhanced hemolysis near the colony being tested

RESULTS

- ❖ A streptococcus giving positive CAMP test and morphologically and biochemically consistent (catalase-negative, Gram-positive cocci in pairs and chains) - *Streptococcus agalactiae*
- ❖ CAMP test separates *L. monocytogenes*, from most other *Listeria* species

Few CAMP test positive Gram-positive rods are :

Rhodococcus equi, *L. monocytogenes*, *Propionibacterium avidum/granulosum*, *Actinomyces neuii*, *Corynebacterium glucuronolyticum*, *Corynebacterium coyleae*, *Corynebacterium imitans*, and some strains of the *Corynebacterium striatum* and *Corynebacterium afermentans* group

Reverse CAMP positive

Corynebacterium pseudotuberculosis, *Corynebacterium ulcerans*, *Arcanobacterium haemolyticum*, *Mycoplasma hyorhinis*, *C. perfringens*

CAMP Test for the identification of *Listeria monocytogenes*

First used by Groves to identify *Listeria monocytogenes*

Pathogenic *Listeria monocytogenes* also positive for CAMP test

Listeria monocytogenes is streaked at right angle to the streak of beta-hemolytic *Staphylococcus aureus* on sheep blood agar plate, incubated at 37 °C for 24 hours

Positive CAMP: Enhanced zone of beta-hemolysis and a smaller less obvious rectangular zone of hemolysis



Image source- <https://www.asmscience.org>

L. ivanovii :

➤ only shows a positive CAMP reaction when using an alternative CAMP test method, in which *Rhodococcus equi* replaces *S. aureus*

➤ “arrowhead” hemolysis occurs appear between streaks of *Listeria ivanovii* and *Rhodococcus equi*

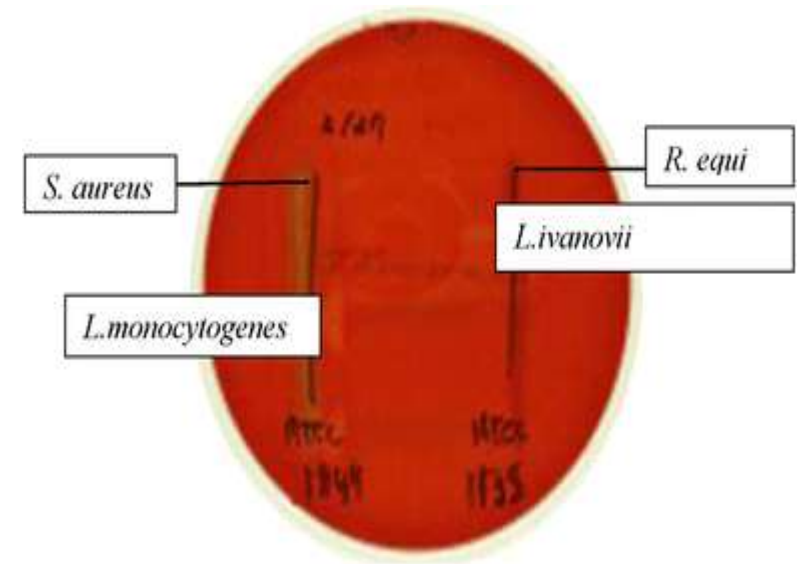


Image source- <https://media.springernature.com>

LIMITATIONS

- The CAMP test should not be used alone, but rather, in combination with the appropriate colony morphology, gram reaction, and other biochemical tests on colonies from pure culture for complete identification
- Extended incubation times or elevated incubation temperatures may give false-positive results
- Interpretation of the CAMP spot test can be affected by excessive agar depth
- Only colonies that have been growing for at least 18 hours should be tested with the spot test
- Colonies only 12 hours old can give false-negative results, presumably because the colony may not yet have produced adequate amounts of CAMP factor in the synergistic hemolysis

Reverse CAMP test

- ❖ *Clostridium perfringens*, *Corynebacterium pseudotuberculosis*, *Mycoplasma hyorhinis*
- ❖ Antagonistic interaction (reverse CAMP phenomenon)
- ❖ Attributed to phospholipase D (PLD) activity
- ❖ Beta-hemolysis of staphylococci inhibited, through the activity of a phospholipase D (PLD)
- ❖ Differentiation of *Clostridium perfringens* from other *Clostridium* species

- ❖ CAMP positive Group B *Streptococcus* is streaked in the centre of sheep blood agar and *Clostridium perfringens* is streaked perpendicular to it
- ❖ Following incubation at 37°C for 24-48 hours in anaerobic conditions, an “arrowhead” hemolysis is seen between growth of *C. perfringens* and Group B *Streptococcus*
- ❖ Alpha toxin produced by *C. perfringens* interacts with CAMP factor and produce synergistic hemolysis



Image source- <https://www.asmscience.org>

Thank You

The images for slides are taken from resources available on internet and used only for the purpose of teaching the students