

## The Rapid CAMP Test for Identification of *Streptococcus agalactiae* Using Alpha Toxin

### Short Communicatuin

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### Summary

Alpha toxin derived from *Clostridium perfringens* was used for diagnosis of *Streptococcus agalactiae* (*St.agalactiae*) by rapid and spot CAMP tests. The selected *Cl.perfringens* strain was cultured in optimized conditions; the supernatant of culture was concentrated by ultra filter and purified by DEAE cellulose chromatography. The efficacy of both purified and crude  $\alpha$ -toxins were examined by rapid and spot CAMP tests. The result of this study indicates that there is no significant difference between the purified and crude enzyme in identification of *St.agalactiae*. The specificity of the enzyme was confirmed by testing of different *Streptococcus* spp. The run time require for spot and rapid CAMP tests using  $\alpha$ -toxin were between 10-90 minutes and 4-6 hours, respectively, whereas the traditional test needs 18-24 hours for running.

**Key words:** *Streptococcus agalactiae*, *Clostridium perfringens*,  $\alpha$ -toxin, CAMP

### Introduction

*Streptococcus agalactiae* (*St.agalactiae*) is associated with abroad spectrum of clinical syndromes ranging from simple colonization to acute form in both the adults and neonates such as septicemia and meningitis. This bacterium is one of the causative agents of mastitis in cow (Joklik 1992). Varieties of methods are currently available to identify *St.agalactiae* including precipitation with grouping antiserum, agglutination reaction, fluorescent microscopy, and biochemical tests (Joklik 1992). The CAMP (Christic, Atkins, and Munch-Peterson) test has also been used as a

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simple and inexpensive test for diagnosis in which approximately 98% of the strains produce CAMP factor protein (Darling 1957, Jewes 1986, Philips 1980). Wilkinson *et al* (1977) and Diperso *et al* (1985) described the interactions between CAMP factor and Staphylococcal Beta-hemolysin. Some studies showed that *Clostridium perfringens* (*Cl.perfringens*) can use instead of *Staphylococcus aureus* (*S.aureus*) in CAMP test (Gubach 1978, Holth-Haug 1981) or in a reverse CAMP test, *St.agalactiae* could use for diagnosis of *Cl.perfringens* (Buchanan 1982). *Cl.perfringens* produce phospholipase C ( $\alpha$ -toxin), with different molecular weight from 43-51kD, has similar activity to  $\beta$ -hemolysin, which produce by *S.aureus* (Dine 1970, Pastan 1968). The enzyme catalyses the hydrolysis of lecithin into phosphorylcholine and 1, 2-diglyceride, which cause turbidity in egg yolk agar (Diner1970). The aim of this study was introduced a simple and rapid CAMP test to identify *St.agalactiae* instead of the traditional test.

### **Materials and Methods**

**Preparation of crude  $\alpha$ -toxin and purification procedure.** *Cl.perfringens* (Type A) obtained from Anaerobic Bacterial Vaccines Production Dept., Razi Institute was grown at 37°C for 6h in 20 liters of synthetic medium including peptone 45g,  $N_2H_4PO_4$  ( $12H_2O$ ) 14.32g,  $K_2HPO_4$  1.36g, yeast extract 0.5g, beef extract 5g,  $MgSO_4$  ( $7H_2O$ ) 0.2g,  $ZnSO_4$  ( $7H_2O$ ) 5.75mg,  $MnCl_2$  ( $4H_2O$ ) 3.94mg,  $FeSO_4$  ( $H_2O$ ) 7mg,  $KHCO_3$  1g, thioglycolic acid 0.01mg, glucose 5g, L-cysteine 0.5g to final volume of 1 liter, pH7.6. The bacterial suspension was centrifuged and the supernatant fluid was passed through a 0.45 $\mu$ m cellulose acetate filter. PH of the filtrate was adjusted to 7.5 and then was saturated with ultra filter. For purification of the saturated enzyme DEAE-cellulose chromatography was performed. It was applied to a DEAE-Cellulose column (1ml of DEAE-cellulose/mg protein) equilibrated with 0.01M Tris-HCl, pH7.5. After washing of the column, a linear gradient of NaCl in 0.01M Tris-HCl (pH7.5) was applied. The presence of protein was confirmed by

absorbency at 280nm, making lysates in sheep blood agar, and producing turbidity in 10% egg yolk agar. Fractions with high activity were electrophoresed with SDS-PAGE electrophoresis method.

**Spot CAMP test.** Fresh cow milk and neonatal blood samples were streaked on blood agar plates and incubated aerobically at 37°C. A drop of  $\alpha$ -toxin was placed adjacent to an individual colony growing on a primary sheep blood agar plate, which was suspected to be the genus *Streptococcus*. The plate was incubated aerobically at 37°C and examined for evidence of synergistic hemolysis at 10-min intervals for up to one hour. An increase of >20mm in the radius of hemolysis zone following application of this enzyme was considered a positive reaction.

**Rapid CAMP test.** *St.agalactiae* was streaked across a sheep blood agar plate. A papered stripe conjugated with 5 $\mu$ l of the enzyme was placed at right angles but not touching the bacterial streak. *S.aureus* was included as a positive control. The plate was examined in 50-min intervals for up to 18-24h following incubation at 37°C. A positive CAMP test was indicated by a distinct arrow head-shaped area of enhanced hemolysis at the junction between the streptococcal and enzyme lines.

**Specificity of  $\alpha$ -toxin.** The specificity of the enzyme was checked by testing of different *Streptococcus* spp. including *St.agalactiae* (14 strains), *St.pyogenes* (6 strains), *St.pneumonia* (6 strains), *St.hemolyticus* (6 strains), *St.fecalis* (10 strains). All of them were obtained from the Microbiology department of Razi Institute, Iran.

**Effect of purity and storage condition of  $\alpha$ -toxin.** The required time for positive reaction in spot and rapid CAMP test were examined by both crude and pure  $\alpha$ -toxin. Storage of the toxin in different conditions; lyophilized, -20°C and +4°C was examined in both CAMP tests every month for up to two years.

### **Results and Discussion**

Results of spectrophotometry at 280nm turbidity in 10% Egg yolk agar reveal that the toxin was purified. Electrophoresis of the purified toxin was shown different

fractions with 47-51kD molecular weight. Both crude and purified toxins had near required time for showing the positive reaction. In spot CAMP test the time for crude and purified  $\alpha$ -toxin was 90min and 80min, respectively and in rapid test this time was 6h, and, 5h and 45min, respectively. There is not any noticeable difference between use of crude and purified enzyme based on the required time for positive reaction so, the use of crude enzyme without any purification is suggested. Moreover, using of  $\alpha$ -toxin reduces the required time from 36-48h with traditional CAMP test to 24-30h and 19-25h in rapid and spot CAMP tests, respectively. The positive reaction in both CAMP tests is shown in figures 1 and 2.

Figure 1. Spot CAMP on blood agar plate test for diagnosis of *St.agalactiae* streak using  $\alpha$ -toxin of *Cl.perfringenes*

Figure 2. Rapid CAMP test on blood agar plate for diagnosis of *St.agalactiae* colonies using  $\alpha$ -toxin of *Cl.perfringenes*

*Specificity of  $\alpha$ -toxin.* This enzyme showed positive reaction with all of *St.agalactiae* strains and negative reaction with all of the other species of *Streptococcus* genus. No false positive or false negative reactions were shown. The same results were taken from *S.aureus*. Use of  $\alpha$ -toxin instead of *S.aureus* cultural streak for performance of CAMP test has been suggested (Hansen 1980, Holth-Haug 1981, Gubash 1978). This study approved the result of other studies that phospholipase c ( $\alpha$ -toxin), can identify the *St.agalactiae* with the same sensitivity and specificity of *S.aureus* streak.

*Stability test.* The stability of the enzyme in different conditions was evaluated. It was stable in lyophilized condition at least for 2 years, at -20°C for 21 months and at +4°C for 18 months.

In conclusion, use of  $\alpha$ -toxin for diagnosis of *St.agalactiae* by CAMP test is more appropriate and useful than *S.aureus* streak. It is more powerful and more stable than streak of bacteria, which cannot produce  $\beta$ -hemolysin after some subcultures (Gubash 1978). Preservatives are not necessary for storage of  $\alpha$ -toxin at least for 18 months at +4°C; therefore, the storage of the enzyme is easier than *S.aureus* streak.

### References

- Buchanan, A.G. (1982). Clinical laboratory evaluation of a reverse CAMP test for presumptive identification of *Clostridium perfringenes*. *Journal of Clinical Microbiology* 16:761-762.
- Darling, C.L. (1975). Standardization and evaluation of the CAMP reaction for the prompt presumptive identification of *Streptococcus agalactiae* (Lance field group B) in clinical material. *Journal of Clinical Microbiology* 1:171-174.
- Diner, B.A. (1970). Purification and properties of *Clostridium welchii* phospholipase C. *Biochemica et Biophysica Acta* 198:514-522.
- Dipersio, J.R., Barrett, J.E. and Kaplen, R.L. (1985). Evaluation of the spot CAMP test for the rapid presumptive identification of Group B *Streptococci*. *American Journal of Clinical Pathology* 84:216-219.
- Gubash, S.M. (1978). Synergistic hemolysis phenomenon shown by an alpha toxin producing *Clostridium perfringens* and streptococcal CAMP factor in presumptive streptococcal grouping. *Journal of Clinical Microbiology* 8:480-488.
- Hansen, M.V., Elliott, L.P. (1980). New presumptive identification test for *Clostridium perfringens* reverse CAMP test. *Journal of Clinical Microbiology* 12:617-619.

- Holt-Haug, R., Olsvik, O. (1981). The CAMP test performed by using *Staphylococcus aureus* sphingomyelinase (Beta hemolysin) and *Clostridium perfringens* Lecitinase. *Niph Annual* 4:43-48.
- Jewes, L.A., Jones, D. (1968). Rapid method for detection of group B *Streptococci* human sources. *Journal of Applied Bacteriology* 61:219-223.
- Joklik, W.K., Willett, H.P., Amos, D.B. and Wilfert, C.M. (1992). *Zinsser Microbiology*. (20th edn.), Pp:466-467. Appleton & Lange.
- Pastan, I.R.A., Macchia, V. and Katzen, R. (1968). A phospholipase specific for sphingomyelin from *Clostridium perfringens*. *Journal of Biological Chemistry* 243:3750-3755.
- Philips, E.A., Tapsal, J.W. and Smith, D.D. (1980). Rapid tubes CAMP test for identification of *Streptococcus agalactiae* (Lancfield group B). *Journal of Clinical Microbiology* 12:135-137.
- Wilkinson, H.W. (1977). CAMP-disk test for presumptive identification of group B *Streptococci*. *Journal of Clinical Microbiology* 6:42-45.