Leucine Aminopeptidase (LAP) Test

I. Principle

The LAP Test is a rapid assay for the detection of the enzyme leucine aminopeptidase in bacteria cultured on laboratory media. It is used as one of the tests for the presumptive identification of catalase-negative gram positive cocci. Leucine-β-naphthylamide impregnated discs serve as a substrate for the detection of leucine aminopeptidase. Following hydrolysis of the substrate by the enzyme, the resulting β-naphthylamine produces a red color upon the addition of cinnamaldehyde reagent.

II. Reagents and Materials

A. LAP discs: reagent discs impregnated with a solution of leucine-β-naphthylamide (0.7%) then dried (Murex). Store at 2-8°C until stated expiration date.
B. Color developer: 0.01% p-dimethylamino-cinnamaldehyde
C. Sterile deionized water
D. Wooden applicator stick or inoculating loop
E. Forceps

III. Quality Control

A. Quality control is performed upon receipt of each new lot or shipment. The control organisms and their expected reactions are as follows:
    1. *Enterococcus faecalis* ATCC 29212 = positive (pink/red color development)
    2. *Aerococcus viridans* ATCC 11563 = negative (no color/slight yellow color development)
B. If controls do not display expected results, quality control must be repeated. Notify the supervisor.

IV. Procedure

A. Using forceps, place a LAP disc on a glass slide or in a petri dish.
B Moisten the LAP disc with 10 μL of sterile deionized water.
C Select 5-10 pure or isolated colonies of catalase-negative, gram positive cocci with a loop or stick and rub onto the disc.
D Incubate at room temperature for 5 min.
E. Add one drop of the color developer, and examine for up to 1 min for pink to red color development.

V. Interpretation

A. Pink/red = positive
B. No color change/slight yellow = negative

VI. Limitations

A. The LAP Test is only part of the overall scheme for identifying catalase-negative, gram positive cocci. Further biochemical characterization and serological grouping may be necessary for specific identification. False negatives may result from using too small an inoculum.

B. When Gram stains are prepared from agar growth, some *Leuconostoc* and *Streptococcus* strains may appear coccobacillary or even rod-shaped and may be confused with members of the genus *Lactobacillus*. The most consistent Gram stains are prepared from broth media.

VII. References

A. Coleman, G., and L.C. Ball. 1984. Identification of Streptococci in the Medical Laboratory, J. Appl. Microbiol. 57:1-14.

B. Facklam, R.R. and J.A. Washington. 1990. Streptococci and Related Catalase Negative Gram-Positive Cocci, Manual of Clinical Microbiology, 5th Ed., ASM, Washington, DC.

C. Lennette, E.H., et al. 1985. Manual of Clinical Microbiology, 4th Ed., ASM, Washington, DC.

D. Murex Diagnostics LAP package insert. Sept 1993.

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Updates and Revisions: