K1378B LAP DISC

PRINCIPLE:

The LAP Disc is used to detect the enzyme leucine aminopeptidase, one of the tests in the definitive identification of catalase negative gram positive cocci. (1,2). Specifically it is used to differentiate *Aerococcus* and *Leuconostoc* from *Streptococcus, Enterococcus, Lactococcus*, and *Pediococcus.* The former are leucine aminopeptidase negative and the latter are positive. *Aerococcus* and *Leuconostoc* are easily differentiated by PYR and other tests (2). Hydrolysis of Leucine ß-Naphthylamide releases pure naphthylamide which is red after adding PEP reagent.

MATERIAL SAFETY:

LAP discs contain L-Leucine ß-naphthylamide in methanol. Unbound naphthylamide may be hazardous if inhaled, ingested or absorbed through the skin. The amount used in the disc does not present a hazard when used as directed. PEP reagent contains p-dimethyl-amino-cinnemaldehyde in a weak hydrochloric acid solution. The reagent is mildly corrosive and stains hands and clothing.

STORAGE:

Store at 2-8 degrees C.

MATERIALS REQUIRED:

Key LAP discs are provided in packs of 50 discs with PEP reagent. The tests require fresh growth on media appropriate for the specimen. A sterile loop or stick for harvesting, a slide, and purified water (pH 6.5-7.5) are required but not provided.

PROCEDURE

The discs should be white to cream colored. If discs have changed colors do not use them. For best results use fresh cultures less than 48 hours old.

1. Place a disc onto a clean slide and moisten slightly.
2. Using a sterile stick or loop, smear the disc with the suspected isolate.
3. Incuabtion is not required. Wait 5 minutes.
4. Add 1 drop of PEP reagent and wait 2 minutes to observe color.

INTERPRETATION:

A positive test after adding reagent will be a deep red to reddish purple while a negative test will be colorless, yellow, or green.

Aerococcus and *Leuconosttoc* are LAP negative, whereas other organisms in the group (*Streptococcus, Enterococcus, Lactococcus,* and *Pediococcus)* are almost always positive. Published tables are available which list all of the tests which are useful for definitive identification of this group of organisms. (1,2)

QUALITY CONTROL:

The LAP disc is for invitro diagnostic use only. Observe aseptic techniques when working with clinical specimens and microbiological cultures. Discard used tests in a manner appropriate for biohazardous materials. Each lot# of LAP discs should be tested with organisms of known reactivity prior to use. Suggested organisms are: E. faecalis ATCC 29212 and Aerococcus viridans, ATCC 11563.

LIMITATIONS:

Leuconostoc species exhibit a pale pink color in reaction to the PEP reagent. This pale color should not be confused with a positive test, which is bright pink. If you are unsure, observe Leuconostoc with PEP reagent on plain filter paper.

It should be emphasized that this test is only one of a battery of useful tests for identifying catalase negative gram positive cocci. Further biochemical characterization and serological grouping may be necessary for specific identification. False negatives may result from insufficient inoculum.

REFERENCES:

1. Colman, G. and L.C. Ball. 1984. Identification of Streptococci in the Medical Laboratory. J. Appl. Microbiol. 57:1-14
2. Facklam, R.R. and J.A. Washington, 1990. Streptococci and Related Catalase Negative Gram-positive Cocci. Manual of Clinical Microbiology, 5th Ed. ASM. Washington, D.C.
3. Lennette, E.H., et al 1985. Manual of Clinical Microbiology, 4th edition. ASM

KEY SCIENTIFIC PRODUCTS, INC
1113 EAST REYNOLDS STREET
STAMFORD, TEXAS 79553
VOICE 800-843-1539
FAX 888-440-4208
WWW.KEYSCIENTIFIC.COM

K1378-0805

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MATERIAL SAFETY:

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STORAGE:

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