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Pulse LAP Disc Test

INTENDED USE

The Pulse LAP Disc Test is used to detect the enzyme leucine aminopeptidase(LAP), one of the tests considered as a definitive identification test for catalase negative gram positive cocci (1,2). Specifically it is used to differentiate Aerococcus and Leuconostoc from Streptococcus, Enterococcus, Lactococcus and Pediococcus.

SUMMARY & PRINCIPLES

The Pulse Lap Disc Test is used to detect the enzyme leucine aminopeptidase, one of the tests considered as a definitive identification test for catalase negative gram positive cocci (1,2). Aerococcus and Leuconostoc both do not contain LAP and will yield negative results. Streptococcus, Enterococcus, Lactococcus and Pedicoccus are LAP positive. Aerococcus and Leuconostoc can be easily differentiated by the PYR and other tests (2).

Hydrolysis of leucine p-Naphthylamide releases pure naphthylamide which is red after adding the PEP Reagent.

MATERIALS SUPPLIED

LAP Discs in vials
PEP Reagent

Additional Items Required:

Sterile loop or stick to harvest culture
distilled water at neutral pH

STORAGE & STABILITY

The components should be stored at 2-8 °C.

PRECAUTIONS

This product is for in-vitro diagnostic use by trained, qualified personnel. Established laboratory procedures must be followed in the handling and disposal of all infectious bacteria. This product is only one integral part of the overall plan for the collection, transportation, isolation, identification, and antimicrobial agent susceptibility testing of disease-producing microorganisms. Consult appropriate references for additional methods and procedures.

LAP Discs contain leucine p-Naphthylamide in Tris Buffer. 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.

LAP Discs should be white to cream color. If discs have changed colors, do not use them. For best results, use fresh cultures that are less than 48 hours old.

PEP reagent contains p-aminodimethyl-cinnamaldehyde in a weak hydrochloric acid solution. The reagent is mildly corrosive and stains hands and clothing.

PROCEDURE

1. Add 100uL of distilled water to the disc inside the tube.

- Using a sterile loop or stick, harvest sufficient suspected organisms and smear onto the moistened disc. For best results, use fresh cultures less than 48 hours old.
- Incubate at room temperature for 5 minutes.
- Add 1 drop of PEP Reagent and wait 2 minutes to observe for color.
- Record results.

INTERPRETATION OF RESULTS

Positive : Disc will show a deep red to reddish purple after the addition of the PEP Reagent.

Negative: Disc will show Colorless or Yellow color after the addition of the PEP Reagent.

Aerococcus and Leuconostoc 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 in vitro diagnostics use only. Observe aseptic techniques when working with clinical specimens and microbiological cultures. 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 viridians ATCC 11563.

LIMITATIONS

It should be emphasized that there are many tests used in the identification of catalase negative gram positive cocci. The LAP Disc Test is just one of these tests. Therefore, further biochemical characterization and serological grouping may be necessary for specific identification. False negative results may result from insufficient inoculum used.

Leuconostoc species exhibit a pale pink color in reaction to the PEP reagent. This pale pink color SHOULD NOT be confused as a positive test, which should be bright pink. If in doubt, observe the color by smearing Leuconostoc with PEP reagent on plain filter paper for color comparison.

REFERENCES

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- 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.
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