K9145B ALN

PRINCIPLE:
ALN detects the enzyme L-alanyl-L-alanyl aminopeptidase in bacterial cells. It is useful to rapidly separate *Fusobacterium* species (negative) from most *Bacteroides* species (positive). (See LIMITATIONS for *B. ureolyticus*.)
Hydrolysis of L-alanyl-L-alanyl ß-Naphthylamide releases pure naphthylamide which is red after adding PEP (cinnamaldehyde) reagent.

MSDS:
ALN Discs should be used only by trained individuals. Unbound naphthylamide in considered hazardous: the disc contains the bound form and unbound napthylamide is present only in a positive test. Do not handle the used test. Discard in a manner appropriate for bio-hazardous materials.
PEP reagent is a 0.1% solution of p-dimethylaminocinnamaldehyde in weak hydrochloric acid. Ir is mildly corrosive and stains clothing and hands.

STORAGE:
Store discs at 2-8 degrees C. Do not use beyond the expiration date. The discs should be white to cream colored. If discs have changed colors do not use them.

MATERIALS REQUIRED:
Key ALN discs are sold in packs of 50 discs with PEP reagent provided. The tests require fresh growth on media appropriate for the specimen .For best results use fresh cultures less than 48 hours old.

A sterile loop or stick for harvesting, a slide, and distilled water are required but not provided.

PROCEDURE:
1. Place a disc onto a clean slide and moisten slightly.
2. Using a sterile stick or loop, smear the disc with a visible paste of the suspected isolate. False negatives may result from insufficient inoculum.
3. Incubate at room temperature for 5 minutes.
4. Add 1 drop of PEP reagent and wait 2 minutes to observe color.

INTERPRETATION:
After adding reagent, a positive test will be a deep pink to deep purple while a negative test will be colorless, yellow, or blue green. The blue green color indicates a negative ALN and positive indole.

QUALITY CONTROL:
Each lot of discs should be tested with organisms of known reactivity prior to use. Suggested organisms are: Positive - *B. fragilis* ATCC 25285 and negative - *Fusobacterium nucleatum* ATCC 10953.

LIMITATIONS:
B. ureolyticus, which is ALN negative, can be differentiated from *Fusobacterium* by its negative reaction to spot indole and PYR.
Capnocytophaga species are ALN positive and can cause confusion with anaerobic gram negative bacilli. The colonial morphology, ability to grow at 35C in 5% carbon dioxide, and PRO reaction are helpful in eliminating these organisms.
PYR, spot indole, and PRO are all available from KEY Scientific. Published tables are available which list tests useful for definitive identification of this group of organisms.
It should be emphasized that this test is only one of a battery of tests for identifying anaerobic gram-negative bacilli. Further biochemical characterization may be necessary for specific identification.

REFERENCES:
1. Lennette, E.H., *et al,* 1985 Manual of Clinical Microbiology, 4th Ed., ASM, Washington,D.C.
2. Baranowski, J, et al. Separation of Anaerobic Gram Negative and Gram Positive Organisms using L-Alanyl-L-alanyl-ß-naphthylamide. Abstr. Ann Meet. ASM, 1984 C:140:260.
3. Finegold, S.M. et al, 1986. Diagnostic Microbiology 7th ed. C.V. Mosby Co. St. Louis, MO
4. Sutter, V.I. and W. T. Carter. 1972 Evaluation of media and reagents for indol-spot tests in anaerobic bacteriology. Am. J. Clin. Pathol. 58:335-338

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K9145B-0805

K9145B ALN

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MSDS:
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PEP reagent is a 0.1% solution of p-dimethylaminocinnamaldehyde in weak hydrochloric acid. Ir is mildly corrosive and stains clothing and hands.

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