

# K915, K915B GRAM REACTION (APNA)

## PRINCIPLE/DISCUSSION:

APNA Discs are used in confirming the gram staining reaction of Gram-negative and Gram-positive aerobic and facultatively anaerobic bacteria. The presence of cell wall aminopeptidase is detected by the hydrolysis of L-alanine p-nitroanilide indicating a Gram-negative reaction.(2)

## STORAGE:

Store tightly sealed at 2-8°C with desiccant provided.

## MATERIALS REQUIRED:

Key APNA discs are sold in packs of 15 or 30 discs. The tests require fresh growth on plated media appropriate for the specimen. A sterile loop or swab for harvesting, distilled water (neutral pH) and small test tubes 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 2-3 drops of distilled water into a small test tube.
- (2) Add sufficient organism being examined to produce a milky suspension.
- (3) Add 1 APNA disc to the suspension and incubate aerobically at 35-37°C for 5-20 minutes.

## INTERPRETATION:

The development of a yellow color is a positive test, confirming that the organism is Gram-negative. No color change indicates a negative test and the organism is Gram-positive.

**Optional:** Add 1 drop of spot indole reagent (sold separately) to confirm the reaction. A blue/purple color indicates a positive test, whereas red or yellow indicates a negative Test.

## LIMITATIONS:

APNA discs will not give predictable reactions for anaerobic organisms. Also, some Streptococci (members of groups A, C, F, and G) may give false positive reaction.

**NOTE:** Using an inoculating loop or needle, pull the disc up the side of the tube away from the inoculum to verify the color of the disc for reaction results. Push disc back into inoculum for performing the optional confirmatory test.

## QUALITY CONTROL:

The APNA disc is for invitro diagnostic use only. Observe aseptic techniques when working with clinical specimens and microbiological cultures. Discard used test in a manner appropriate for biohazardous materials. Each lot of APNA discs should be tested prior to use with known Gram reaction organisms. Suggested organisms are:

- Gram-positive: *Staphylococcus aureus*-ATCC 25923
- Gram-negative: *Escherichia coli*- ATCC 25922

## REFERENCES:

- (1) Murray, Patrick R. et al, Manual of Clinical Microbiology, 7th Edition, 1999. American Society for Microbiology.
- (2) Carlone, G.M.,M.J. Baldez, and M.J. Pickett. Method for Distinguishing Gram-positive Bacteria from Gram-negative Bacteria. Journal of Clinical Microbiology 1983. p.1157-1159.



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