

# K485B NITRATE DISCS

## PRINCIPLE/DISCUSSION:

Nitrates often serve as a source of nitrogen for bacteria, but they must be reduced. The first step in this is the removal of one oxygen to form nitrite. Some organisms have this ability while others (such as some of the non-fermentors) are capable of taking the next step and reducing the nitrite to nitrogen by replacing the remaining oxygens with hydrogens. The addition of reagents will produce a colored end product in the presence of nitrites.

## ACTIVE INGREDIENTS:

KEY NITRATE DISCS contain Sodium nitrate in desiccated form. Sodium nitrate is not known to be hazardous.

## STORAGE:

Store unused discs tightly sealed at 2-8°C.

## QUALITY CONTROL:

NITRATE DISCS should be tested prior to use with organisms which produce known reactions. Key recommends *Pseudomonas aeruginosa* ATCC 27853 as positive control and *Pseudomonas putida* ATCC 49128 as negative. Finished tests should be discarded in a manner appropriate for biohazardous materials.

## MATERIALS REQUIRED:

K485B Discs are provided 50 tests per vial. Nitrate tests require preliminary colony isolation on media appropriate for the specimen. Consult a manual such as the Manual for Clinical Microbiology for this information. These items are required but not provided:

- test tubes (optional)
- inoculating needle
- distilled water
- Nitrate A (K980480 or equivalent)
- Nitrate B (K980481 or equivalent)
- Zinc (K700 or equivalent).

## PROCEDURE:

### Tube Method:

- (1) Add 1 disc to a small tube and add 3 drops of water.
- (2) Inoculate heavily then incubate, uncovered, at 35-37°C for 2 hours.
- (3) Add 1 drop of Nitrate reagent A and 1 drop of Nitrate reagent B to tube and observe for color.

### Plate Method:

1. Place 1 disc onto an area of heavy growth on a plate of the suspected organism.
2. Incubate at 37°C for 2-4 hours (not longer).
3. Add 1 drop of Nitrate reagent A and 1 drop of Nitrate reagent B to the disc, rubbing gently with a loop to mix the reagents and moisten the disc.
4. A positive reaction will be pink to red in color.

## INTERPRETATION:

After the addition of the nitrate reagent, the formation of a pink to red color indicates nitrite formation. If the test appears to be negative by either method, add zinc reagent. If there has been no reduction of nitrates, the zinc will now reduce them and the red color will appear, indicating a negative test. No color change after adding zinc is a positive test indicating the organism has reduced the nitrate completely to nitrogen.

## REFERENCES:

- 1) Manual of Clinical Microbiology, Fifth Edition, Chapter 122, "Reagents and Stains"
- 2) Bailey and Scott's Diagnostic Microbiology, Seventh Edition, Chapters 9, 27 and 28.



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