

# K520 ENT OXIDASE TEST

## DISCUSSION:

Cytochrome oxidase is an enzyme which participates in the electron transport and nitrate metabolism mechanisms of some bacteria. The ENT oxidase test can be used to initially characterize gram negative bacilli. Both fermentative and nonfermentative categories of bacteria include both oxidase positive and oxidase negative species. Most fermentors are oxidase negative, with the exceptions occurring in the genera *Vibrio*, *Pasteurella*, *Aeromonas* and *Chromobacterium*. Most nonfermentors are oxidase positive. The oxidase test is also very useful for a presumptive identification of *Neisseria spp.*, as all *Neisseria spp.* are oxidase positive, though the enzyme involved is indophenol oxidase. Key ENT Oxidase Test Strips indicate oxidase positive organisms by the appearance of a deep blue to black color within 10-30 seconds when the colony is rubbed on a moistened strip.

## MATERIAL SAFETY DATA:

ENT Oxidase strips are impregnated with a solution of ascorbic acid and tetra methyl p-phenyl diamine HCl in distilled water. The tetra methyl p-phenyl diamine HCl may be hazardous if inhaled, ingested or absorbed through the skin. To the best of our knowledge, the chemical, physical and toxicological properties of this chemical have not been thoroughly investigated. No hazards are expected in the handling of the strips.

## INSTRUCTIONS:

- (1) Moisten a section of the strip by using a loop to place a drop of distilled water on the strip and rub it about lightly. For ease in handling, simply lay the strip in the moist lid of the petri dish and do not add additional water.
- (2) Transfer a small amount of bacteria to the strip and rub gently.
- (3) Observe the strip for 10-30 seconds. A positive result is the appearance of a deep blue color where the bacteria have been smeared. **CRITICAL:** Disregard color which appears after longer standing. Any test can appear positive after 30 seconds.

## OPTIONAL METHOD:

Hold the strip with forceps and lightly touch the colony to be tested or dip into the control well of diagnostic strips such as API. The test will turn immediately on the area touched. If using this method be sure not to pick up any of the media which could cause false positives. Only one test can be done on each end of the strip by this method.

## PRECAUTION \ LIMITATIONS:

- (1) Because a nichrome wire loop can cause a false positive result, a plastic or platinum loop or a wooden applicator stick should always be used to transfer the bacteria.
- (2) Care should be taken when testing pigmented organisms that the color of the culture is not confused with a positive test. In this situation, watch for a deepening to a blue-black color.

## MATERIALS REQUIRED:

Key ENT Oxidase Test Strips require preliminary growth on appropriate media. Consult a reference such as the Manual of Clinical Microbiology for recommendations. Key ENT Oxidase strips are provided 50 per bottle. The following materials are needed but not provided: distilled water, plastic or platinum loop or wood stick, forceps (if using optional method).

## QUALITY CONTROL:

Positive and Negative controls should be run daily. There is room on each strip for both positive and negative controls to be run with each test if desired. Key suggests *E. coli ATCC 25922* for the negative control, and *Pseudomonas aeruginosa ATCC 27853* for the positive control. Discard used strips in a manner appropriate for biohazardous material.

## STORAGE:

Store tightly covered in the **dark** at 2-8C. Exposure to light will affect the strips.

## REFERENCES:

- (1) Manual of Clinical Microbiology



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