

## Oxidase Test

### I. Principle

The oxidase test is based on the production of the enzyme indophenol oxidase by organisms containing cytochrome C. Indophenol oxidase, in the presence of atmospheric oxygen, oxidizes a redox dye (N,N-dimethyl-p-phenylenediamine dihydrochloride) to form a purple or blue indophenol compound.

### II. Reagents and Materials

- A. Oxidase reagent containing 1% aqueous solution of N,N-dimethyl-p-phenylenediamine dihydrochloride (Gordon and McLeod's reagent)
  - 1. Store oxidase reagent in the refrigerator.
  - 2. Protect reagent from light.
  - 3. Do not use reagent beyond expiration date.
- B. Filter paper
- C. Petri dish or glass slide
- D. Inoculating loop or applicator stick

### III. Quality Control

- A. Quality control is performed on each batch or lot and weekly thereafter. Quality control organisms and their expected reactions are:
  - 1. *Pseudomonas aeruginosa* ATCC 27853 = positive oxidase in <5 sec.
  - 2. *Escherichia coli* ATCC 25922 = negative oxidase at > 30 sec.
- B. If controls do not display expected reactions, quality control must be repeated. Notify the supervisor.

### IV. Safety

- A. Avoid contact with skin. Rinse thoroughly with water if reagent comes into contact with the skin.

### V. Procedure

- A. Place filter paper in bottom of petri dish or on a glass slide.
- B. Dispense reagent onto filter paper until moistened.
- C. Smear bacteria from a non-selective plate (BAP, CHOC) onto the paper with a loop or stick.
- D. Examine the inoculated filter paper for a maximum of 30 sec for the development of a blue color (positive reaction). No color development indicates a negative result.

### VI. Results

- A. Patient oxidase results should be compared to weekly QC reaction times. Any reaction delayed beyond the positive control times should be considered negative.

- B. Viscous colonies may be falsely negative due to poor penetration of reagent.

## **VII. Limitations**

- A. Wire loops containing iron may give a false-positive reaction.
- B. Do not use refrigerated cultures without allowing them to reach room temperature.
- C. Reactions from weak oxidase-positive organisms may be inaccurate. Results inconsistent with other biochemical reactions or with the organism should be repeated.
- D. Do not add excess reagent, as it may cause the reaction to fade for oxidase-positive organisms.
- E. The use of 24 h cultures is recommended.
- F. Colonies growing on selective media or media containing glucose cannot be used for oxidase determination since fermentation inhibits indophenol oxidase activity resulting in false negative results.

## **VII. References**

1. Blazevic, D.J. and Ederer, G.M. 1975. *Principles of Biochemical Tests in Diagnostic Microbiology*. New York, John Wiley and Sons.
2. MacFaddin, Jean F. 1976. *Biochemical Tests for Identification of Medical Bacteria*. Baltimore, Williams and Wilkins.

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Updates and Revisions: