Proc.# 2013.007

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| Procedure | Oxidase |

Principle: The oxidase test is qualitative procedure for determining the presence of cytochrome C oxidase activity in bacteria. This activity is dependent on the presence of an intracellular cytochrome oxidase system that catalyzes the oxidation of cytochrome D by molecular oxygen, which then serves as the terminal electron acceptor in the organism's electron transport system.

 N,N,N',N'-tetramethyl-p-phenylenediamine dihydrochloride is an aromatic amine that is dimethylated at its two amino groups. The reagent is colorless in the reduced state and purple in the oxidized state. In the oxidase test, cytochrome oxidase produced by the bacteria does not directly oxidize the p-phenylenediamine reagent, but rather oxidizes cytochrome C, which in turn oxidizes the reagent to form a purple colored compound. In essence, the oxidase test determines the presence or absence of cytochrome C.

 Organisms containing cytochrome C as part of their respiratory chain are oxidase positive; organisms lacking cytochrome C as part of their respiratory chain do not oxidize the reagent, leaving it colorless and are oxidase negative.

Specimen:

Patient Preparation: N/A

Type of Specimen: Bacteria colonies from primary plate or isolates from subculture.

Specimen Handling: Standard operating procedures for working with biohazardous materials are followed.

Equipment and Materials:

Equipment: N/A

Materials: Oxidase reagent dropper, filter paper, plastic or metal inoculation loop, & quality control organisms.

Materials Preparation: Oxidase reagent is ready as is.

Performance Parameters: Quality control organisms perform as expected.

Storage Requirements: Oxidase reagent droppers are stored at 15-30°C

Calibration: N/A

Quality Control: Quality control is completed each day of use. The following organisms are used:

 Positive: Pseudomonas aeruginosa ATCC 27853

 Negative: Escherichia coli ATCC 25922

Results of quality control run are documented on the Bacteriology QC spreadsheet.

Procedure:

Hold reagent dropper upright and point tip away from yourself, Grasp the middle with thumb and forefinger and squeeze gently to crush ampule inside the dropper. Caution: Break ampule close to its center one time only. Do not manipulate dropper any further, as the plastic may puncture and injury may occur.

Tap bottom on tabletop a few times. Then invert for convenient drop-by-drop dispensing of reagent.

Add a few drops of oxidase test reagent to a strip of filter paper (Whatman No. 1 or equivalent). Streak a loopful of bacteria on the reagent-saturated paper with a platinum loop or plastic loop. Do not add exces reagent, as it may cause the reaction to fade on oxidase-positive organisms.

Positive reactions turn the bacteria violet to purple immediately or within 10 to 30 seconds. Delayed reactions should be ignored.

Calculations: N/A

Reporting Results: Results are reported as positive or negative and may or may not appear on the bacteriology report.

Medical Alert Values: N/A

Notes:

Reference Ranges: N/A

Abnormal Results: N/A

Reporting Format: Positive or Negative

Limitations:

One should only allow 10 – 30 seconds for a positive reaction. Any delayed reaction should be considered negative.

Wire loops containing iron may give a false-positive reaction.

Perform the oxidase test on gram-negative bacilli only on colonies from non-selective and/or non-differential media to ensure valid results.

Colonies to be tested must be isolated from other colonies. The use of 24 hour cultures is recommended. Do use refrigerated cultures without allowing them to reach room temperature.

Viscid colonies may be negative due to poor penetration of the reagent.

Reactions from weak oxidase-positive organisms, e.g., Pasteurella species, may be inaccurate.

Results inconsistent with other biochemical reactions or with the organisms should be repeated.

Media containing high levels of glucose may inhibit oxidase activity, causing false negative oxidase reactions.

References:

Package insert from BBL Oxidase Reagent Droppers, M7-8513-1, Revised: August 1985

Lennette, E.H. et el, 1985. Manual of Clinical Microbiology, 4th Ed., ASM, Washington, D.C.

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