Clinical Laboratory Department POLICY AND PROCEDURE

POLICY NUMBER: 1217
VERSION: 3

SUBJECT: Oxidase Test

Principle:
The cytochrome oxidase enzyme is able to oxidize the substrate tetra methyl-p-phenylenediamine dihydrochloride, forming a colored end product. The dark purple end product will be visible if a small amount of growth from a strain that produces the enzyme is rubbed on substrate impregnated filter paper.

Reagents and Storage:
Oxidase dropper containing 0.5 ml of a 1% aqueous solution of tetra methyl-p-phenylenediamine dihydrochloride.

Materials:
Filter paper
Petri dish
Inoculating loop, needle or stick
Oxidase reagent dropper

Specimen:
A well isolated colony of an organism, taken from BAP or Chocolate plate.

Quality Controls:
The oxidase reagent must be tested with positive and negative control organisms each day of use and reactions found to be as expected before unknown bacteria test results are reported.
Positive control organism = Pseudomonas aeruginosa ATCC 27853 QC organism #7
Negative control organism = E. coli ATCC 25922 QC organism #1

Procedure:
1. Hold dropper upright and point tip away from self.
2. Grasp the middle with thumb and forefinger and squeeze gently to crush ampule inside the dropper.
3. Invert dropper for convenient drop by drop dispensing of reagent to filter paper in petri dish.
4. Add a drop of Oxidase reagent to a piece of filter paper.
5. Streak a portion of culture on reagent saturated paper with platinum loop. Iron containing wire may give a false positive reaction.
6. Positive reactions turn purple within 10-30 seconds and then black. Delayed reactions should be ignored.

Limitations:
Viscid colonies may be falsely negative due to poor penetration of the reagent.
Perform the oxidase test on gram-negative bacilli only on colonies from non-selective and/or nondifferential media to ensure valid results.

References:
Difco Oxidase Reagent Droppers, product insert L-001133, rev. 7/99. Becton Dickinson and Company, Sparks, MD.

Approved By: Brian Yee (PHYS SPEC PATHOLOGY)	
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