| KCMC Biotechnology Laboratory, Microbiology | STANDARD OPERATING PROCEDURE | Effective Date 18 April 07 | SOP-Number MIC.043 |
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**Title: OXIDASE TEST**

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**This SOP has been read and understood by:**

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| Annual Review | |
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**Title: OXIDASE TEST**

**Document History:**

| Version Number | Reason for Changes | Date |
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**Title: OXIDASE TEST**

**PURPOSE**
For the detection of cytochrome c oxidase activity in a variety of Gram negative organisms.

**PRINCIPLE**
A drop of oxidase reagent (N,N,N,N-tetramethyl-p-phenylenediamine dihydrochloride) is placed on a small piece of filter paper. A few colonies of test organism from a non-selective medium (e.g. Blood or Chocolate Agar) are rubbed into the reagent on the filter paper. If cytochrome oxidase is produced by the organism it oxidizes cytochrome c which in turn oxidizes the reagent to form a purple compound.

**SCOPE**
This Standard Operating Procedure applies to the testing for cytochrome oxidase by technical staff in the microbiology laboratory who have been trained and are competent in the use and performance of this test.

**SPECIMEN COLLECTION – NA**

**EQUIPMENT/MATERIALS**

Oxidase reagent (BD) Store at 15-30 C
Filter paper (small piece)
Glass slide
Inoculating loop or wooden applicator stick.
Fresh culture (24-48 hrs) of test organism on non-selective media (e.g. Blood or Chocolate Agar)

**QUALITY CONTROL**

**Frequency:** Each shipment of each lot of reagent

**Controls/Acceptable Results:**

*P. aeruginosa* ATCC27853 - Oxidase Positive/purple
*E. coli* ATCC 25922 - Oxidase Negative/no color change

**Corrective Actions for Unacceptable QC Results**:

1. Repeat test using fresh subcultures of control organisms.
2. If still unsatisfactory, do not use for identification of isolates.
3. Arrange for replacement.

**Documentation:**

1. Record QC results on OXIDASE QC sheet.
2. Record unacceptable results and corrective actions on a QC DEVIATION form and submit to supervisor for review..
3. Supervisor will review QC monthly.

**Title: OXIDASE TEST**

**TEST PROCEDURE**

1. Place a small piece of filter paper on a glass slide.
2. Add a drop of oxidase reagent to filter paper.
3. Using an inoculating loop or wooden applicator stick remove the colonies to be tested.
4. Smear the organism directly on to the reagent on the filter paper.

**TEST READING**

1. Examine the reaction area for development of purple color. Color will develop in 20 seconds.
2. Some organisms may produce a very light purple color or reaction may take longer than 20 sec. to develop (e.g. *Pasteurella* spp., mucoid *P. aeruginosa*).
3. A slight gray discoloration may develop with organisms that are oxidase negative – disregard.

**INTERPRETATION**

**Positive** – purple color development
**Negative** – no color change

**RECORDING RESULTS**

Enter results on POSITIVE CULTURE Form.

**REFERENCE**


Package Insert, BBL/BD DrySlide Oxidase, 2003. BD, Sparks, MD.

Kimberle, CC, Lauderdale, T. 2003. Reagents, Stains and Media. In: Manual of Clinical Microbiology, 8th Ed., ASM Press, Washington, DC.


**APPENDIX A – Oxidase Test QC sheet**

**Title: OXIDASE TEST**

**APPENDIX A**

**KCMC Biotechnology Laboratory** **Quality Control**
**Microbiology** **OXIDASE TEST**

**CONTROL ORGANISMS/ACCEPTABLE RESULTS:**

*Pseudomonas aeruginosa* - Positive
*E. coli* - Negative

| QC DATE | QC BY | LOT# | *P. aeruginosa* (+) | *E. coli* (-) | A/NA* |
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***A/NA = Acceptable/Not acceptable** **DOCUMENT ALL CORRECTIVE ACTION ON QC DEVIATION FORM**

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| **Supervisor Review :** | | | | | | |
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**OXIDASE QC/QC SHEETS**