PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

Oxidase Test Procedure

Department of Microbiology

Effective date: 01/24/2006

Last Revision: 01/12/2016

Last reviewed: 01/12/2016

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1.0 Clinical Significance

This test is useful in the initial characterization of Gram-negative bacteria.

2.0 Principle

In the presence of atmospheric oxygen, a bacterium's intracellular cytochrome oxidase enzymes oxidize the phenylenediamine reagent (an electron acceptor) to form a deep purple compound, indol phenol.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained to perform and interpret testing. Testing includes but is not limited to: basic troubleshooting, QC checks, and technical proficiency in accordance with the department SOP.

4.0 Safety - Personal Protective Equipment

Performance of this procedure may expose testing personnel to biohazardous material. All specimens must be handled as potentially infectious material as outlined in the Providence Sacred Heart Microbiology Safety Guidelines.

The reagents used in this procedure may be hazardous to your health if handled incorrectly. This reagent may cause irritation to skin, eyes, and mucous membranes. Avoid contact with skin, eyes, and clothing. More extensive information concerning the safe handling of the reagents and/or chemicals used in this procedure, as well as other important safety information may be obtained by consulting the Safety Data Sheet (SDS). Before performing any part of this procedure, the technologist must take any and all precautions and adhere to all prescribed policies.

This procedure may expose you to:

- Enteric pathogens
- Hazardous reagents

To perform this procedure, you must use:

- Laboratory Coat – must be worn when handling cultures and reagents.

Disinfectant following procedure:

- Bleach dilution sprayers can be used for on demand disinfectant.

Reference for spill/decontamination:

- MSDS
- Chemical hygiene plan

5.0 Specimen Requirements

Appropriate test isolates include aerobic, facultatively anaerobic, or microaerobic Gram-negative rods and cocci from blood, Mueller-Hinton agar, BHI or chocolate agar. Do not test isolates from MacConkey agar. Do not test strictly anaerobic organisms. Do not test organisms growing on media that contain glucose. Test colonies should be in isolation from other organisms and 18 – 24 hours old, unless longer incubation is necessary to achieve sufficient growth.

6.0 Materials

6.1 Consumables

- Swabs (Oxichrome)
- Filter paper
- Petri dish or glass slide
- Wooden applicator

6.2 Reagents

- Oxichrome (Remel) – Reactive Ingredient: N,N,N,N-tetramethyl-ρ-phenylenediamine. Store product in its original container at room temperature until used. This product should not be used if (1) the color has changed from colorless or very light blue-grey, (2) particulate matter is observed, (3) the expiration date has passed, or (4) there are other signs of deterioration.
- Alternative oxidase reagent: 1% aqueous solution of N,N-dimethyl-p-phenylenediamine dihydrochloride (Gordon and McLeod's reagent) may be used. Store in the refrigerator when not in use. Protect the reagent from light by using amber bottles. Do not use reagent beyond expiration date (reagent is stable for 1 week after preparation).

7.0 Procedure

7.1 Oxichrome Reagent

1. Add one drop of Oxichrome Reagent to the end of a fiber-tipped applicator (swab).
2. Firmly touch the reagent-impregnated end of the applicator to a well-isolated colony.
3. Observe for a color change at the point of inoculation within 30 sec.

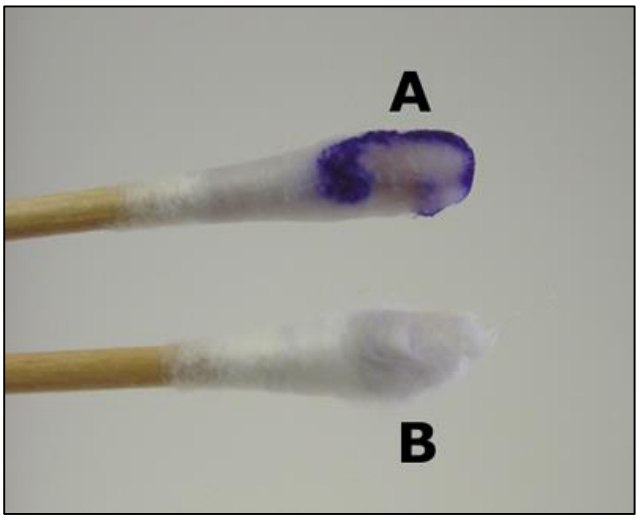
7.2 Gordon and McLeod's Reagent

1. Place filter paper in bottom of petri dish or on a glass slide.
2. Dispense reagent onto filter paper until moistened.
3. Select an isolated test colony using a wooden applicator and smear the organism onto the moistened filter paper.
4. Examine the inoculated filter paper for a maximum of 30 sec for the development of a blue-purple color (positive reaction).

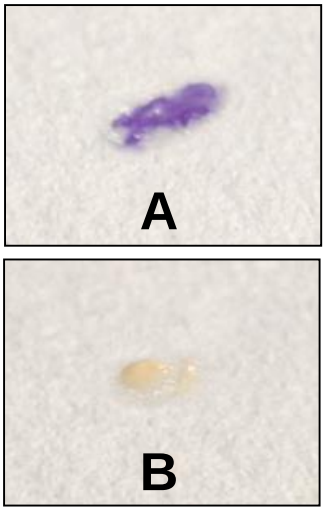
8.0 Interpretation of Results

Positive Test - Blue to purple color within 30 sec (examples A below)

Negative Test - No color change within 30 sec (examples B below)



Oxichrome Reagent
Swab Test



Gordon & McLeod's Reagent
Filter Paper Test

9.0 Quality Control & Quality Assurance

Quality Control testing must be performed on each new lot or shipment of Oxichrome reagent. Gordon & McLeod's reagent should be tested with each new batch or lot (prepared weekly). Results should be documented into LIS. If aberrhant QC results are noted, patient results should not be reported. Notify the supervisor or technical specialist.

- ***Pseudomonas aeruginosa* ATCC 27853**: Positive within 30 sec
- ***Eschericia coli* ATCC 25922**: Negative within 30 sec

10.0 Limitations

1. This test is only part of the overall scheme for identification of gram-negative bacilli. Additional testing is required for definitive identification of the test isolate.
2. Use only pure cultures that are 18-24 hours old. Older cultures or isolates grown on old or deteriorated media will have decreased levels of enzyme activity and may yield false-negative test results.
3. Select colonies for testing which have not been grown on selective media or media containing glucose, as selective agents and the end products of glucose fermentation may inhibit oxidase activity and cause false-negative results.
4. Viscous colonies may be falsely negative due to poor penetration of reagent.

11.0 Verification

Due to the lack of available reagent to prepare Gordon and McLeod's oxidase reagent, in 2016 commercially available reagents were evaluated. The evaluation included reagent from BD, Remel, and Hardy Diagnostics. The reagents were used according to the manufacturer's package insert. Test isolates included ATCC strains and commercial isolates of *Pseudomonas aeruginosa, Neisseria* species, *Campylobacter* species, and *Helicobacter pylori*. None of the commercial reagents performed as well as the Gordon & McLeod's reagent when using filter paper. However, the Remel Oxichrome Reagent produced the best results when using the swab method.

12.0 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.
3. Garcia L, Isenberg H. *Clinical Microbiology Procedures Handbook*. Washington, DC: ASM Press; 2010.
4. Remel Oxichrome Reagent Package Insert: Revised February 5, 2009.

13.0 Document Control History

Microbiology Director Approval: Dr. Ann Robinson 01/24/2006, 01/12/2016

Medical Director Approval: Dr. Joseph Schappert 03/10/2010

Microbiology Supervisor Reviews: Jerry Claridge 01/24/2006, 11/2006, 10/2007, 05/2008, 05/2009, 04/01/2011, 03/2013, Jason Ammons 05/2015, 01/12/2016.

Revisions & Updates: 01/12/2016 Instructions were added for using Oxichrome Reagent.