PURPOSE: The oxidase test is used to detect cytochrome oxidase in bacteria. This enzyme, in the presence of atmospheric oxygen, oxidizes tetramethyl-*p*-phenylenediamine to form a purple coloured compound.

SAMPLE INFORMATION:

Type	One, well isolated colony that is 18 to 24 hours old

REAGENTS and/or MEDIA:

Type	Pro-Lab TestOxidase Reagent
Stability and Storage Requirements	• Store reagent at room temperature (15°C to 30°C) in original container. • Protect from light. • Do not freeze or overheat. • Keep the screw cap tightly closed. • Do not use if the reagent is purple.

SUPPLIES:

- Wooden sticks
- Disposable loops
- Sterile swabs
- Filter paper

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potentially infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure to splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

- Quality control is performed daily:
  - Positive: *Pseudomonas aeruginosa* ATCC 27853
  - Negative: *Escherichia coli* ATCC 25922
- A TQC order is automatically generated daily to record the QC results.

PROCEDURE INSTRUCTIONS:

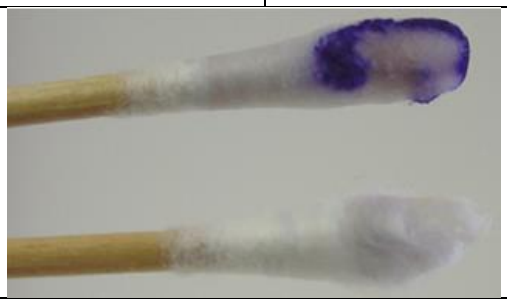
Step	Action
Performing the oxidase test – filter paper method	
1	Add 1 to 2 drops of TestOxidase regent to a piece of filter paper. Wait 1 to 2 minutes for the proper reagent distribution.
2	Using a wooden stick or disposable loop, remove a medium size colony from the surface of the agar and rub onto the reagent-saturated area of the filter paper.
3	Observe the filter paper for colour change within 30 seconds.

Step	Action
Performing the oxidase test – swab method	
1	Using a sterile swab, remove a medium sized colony from the surface of the agar.
2	Add 1 to 2 drops of TestOxidase reagent onto the culture on the swab.
3	Observe the swab for a colour change within 30 seconds.

Step	Action
Performing the oxidase test –direct colony method	
1	Add 1 drop of TestOxidase reagent to a well-isolated colony on the surface of the agar.
2	Observe the colony for a colour change within 30 seconds. If the isolate produces excessively mucoid or slimy colonies, allow up to 1 minute for colour development.

INTERPRETATION OF RESULTS:

IF	THEN
Distinct blue or purple colour within 30 seconds	**Oxidase = Positive**
Colourless or light pink colour within 30 seconds	**Oxidase = Negative**



LIMITATIONS/PRECAUTIONS:

1. A Gram-negative bacillus with a delayed oxidase reaction probably is not a member of the family Enterobacteriaceae.
2. Growth from MacConkey agar or other differential media is not suitable for testing. The indicators in the media may cause false-negative reactions.
3. Timing is critical for interpretation of test results.
4. False-negative results may occur with mixed cultures containing the two genera *Pseudomonas* and *Neisseria*. *Pseudomonas* species that elaborate oxidase also produce an inhibitory substance that interferes with the production of oxidase by *Neisseria* species.
5. Weak oxidase producers, e.g. *Pasteurella*, may appear negative within the time limits of the test.
6. Colonies to which the oxidase reagent has been directly applied become nonviable within the time limits of the test.
7. Media containing high levels of glucose may inhibit oxidase activity resulting in false negative reactions.
8. Avoid contact with skin, eyes and clothing. Rinse thoroughly with water if spilled.

REFERENCES:

- PRO-LAB, Test Oxidase Reagent package insert, 2017 10
- Clinical Microbiology Procedures Handbook, 4th edition, ASM Press, 2016

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	5 APR 19	Initial Release	L. Steven