Title: MIC50400-Oxidase Test

Type: Laboratory Services Program SOP

Issuing Authority: Director of Health Services

Next Review Date:

Date Approved:

PROGRAM Standard Operating Procedure – Laboratory Services		
Title: MIC50400 – Oxidase Test Policy Number:		
Program Name: Laboratory Services		
Applicable Domain: Lab, DI and Pharmacy Services		
Additional Domain(s):		
Effective Date:	Next Review Date:	
Issuing Authority:	ing Authority: Date Approved:	
Director of Health Services		
Accreditation Canada Applicable Standard: N/A		

GUIDING PRINCIPLE:

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.

PURPOSE/RATIONALE:

This standard operating procedure describes how to perform the oxidase test.

SCOPE/APPLICABILITY:

This procedure applies to Medical Laboratory Technologists (MLTs) performing the oxidase test.

SAMPLE INFORMATION:

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

REAGENTS and/or MEDIA:

Туре	Pro-Lab Test Oxidase Reagent	
Chalailina	Store reagent at room temperature (15°C to 30°C)	
Stability	Protect from light	
and Storage	Do not freeze or overheat	
Requirements	Keep the screw cap tightly closed	
	Do not use if the reagent is purple	

SUPPLIES:

- Wooden sticks
- Disposable loops

- Sterile swabs
- Filter paper

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SPECIAL SAFETY PRECAUTIONS:

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

- Ensure that appropriate hand hygiene practices be used.
- 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 of 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. Routine Practices 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
Perfo	rming the oxidase test – filter paper method
1	Add 1 to 2 drops of oxidase reagent 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.
3	Observe the filter paper for colour change within 30 seconds.
Perfo	rming 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 oxidase reagent onto the culture on the swab.
3	Observe the swab for a colour change within 30 seconds.
Perfo	rming the oxidase test - direct colony method
1	Add 1 drop of oxidase 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.

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INTERPRETATION OF RESULTS:

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

LIMITATIONS:

- 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:

1. PRO-LAB. (2017-10). Test Oxidase Reagent package insert

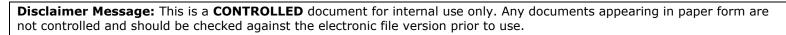
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APPROVAL:		
Date		

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	05 Apr 19	Initial Release	L. Steven
2.0	30 Jun 21	Procedure reviewed and added to NTHSSA policy template	L. Steven



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