National Department of Health

Title: Oxidase Test

ID: G_90_T_8_A

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Changes to the last authorized version:

Version	Date issued	Changes

1. Purpose and Scope

This document describes the Oxidase test procedure used to differentiate Gram negative bacilli as well as other bacteria.

The oxidase test is used to determine if an organism possesses the cytochrome oxidase enzyme. The test is used as an aid for the differentiation of Gram negative bacteria including Aeromonas, Neisseria, Moraxella, Campylobacter and Pasteurella species (oxidase positive).

2. Principle/Clinical application

In the presence of oxygen and cytochrome C the bacteria that possess the enzyme cytochrome oxidase, oxidize the phenylendiamine reagent and form a purple compound, indophenol. Organisms lacking cytochrome c as part of their respiratory chain do not oxidize the reagent, leaving it colorless and are oxidase negative.

3. Responsibilities

- Staff performing Oxidase test require specific training and demonstrated competency
- Staff performing Oxidase test are responsible for the setup, reading and recording of the Oxidase result
- Staff are required to test and record Oxidase positive and negative controls daily

4. Specimen

- Test a fresh 18-24 hr old culture with pure isolated colonies on Blood agar or Chocolate agar

5. Safety

For safety aspects, please review this document G_10_Info_3 Laboratory Biosafety

6. Equipment and Materials

- Prepared oxidase reagent ampules containing prepared N,N,N',N'-Tetramethyl-p-phenyl-enediamine dihydrochloride solution e.g. BBL- BD Diagnostic Oxidase reagent dropper Catalogue No: 261181 or N,N,N',N'-Tetramethyl-p-phenyl-enediamine dihydrochloride powder.
- If making fresh reagent from powder, 2ml sterile DI water in conical tube or other 5mL sterile container and sterile dry cotton tipped swab.
- Small pieces of filter paper
- Wooden sticks
- Glass slide or petri dish
- Contaminated waste bin
- Control organisms: Ps aeruginosa ATCC 27853 & E coli ATCC 25922
- Gloves

7. Procedures

Using prepared reagent ampules

7.1 Hold reagent dropper upright and POINT TIP AWAY FROM YOURSELF. Grasp the middle with thumb and forefinger and squeeze gently to crush ampule inside the dropper.

7.2 Tap bottom on tabletop a few times. Then invert for convenient drop-by-drop dispensing of reagent.

7.3 Add a drop of Oxidase test reagent to a strip of filter paper on a glass slide or petri dish.

7.4 Select a loopful of bacteria, with a platinum loop or wooden applicator stick. onto the reagent-saturated paper.

Always take colonies from Blood agar or Chocolate agar. Selective or differential media can carry over the indicator to the filter paper and cause false-negative reactions. Do not perform oxidase test on colonies from MacConkey or TCBS agar.

7.5 Observe for the appearance of a purple colour within 10 seconds. (POSITIVE oxidase).

7.6 ATCC QC organisms should be tested with the test organism daily.

7.7 Dispose of stick and filter paper in the contaminated waste bin.

Alternate procedure using powder

7.8 Remove a small amount of powder from the stock bottle into a sterile secondary container.

7.9 Mark the secondary container with the lot number and expiration date of the stock powder and the date. Do not allow water to come in contact with the stock powder. Store powder in the secondary container in the refrigerator. Store in the dark or wrap secondary container in foil.

7.10 Take dry sterile cotton tipped swab and dip into the secondary container containing powder.

7.11 A small amount of powder should adhere to the cotton swab.

7.12 Dip the swab into the 5mL tube containing sterile DI water.

7.13 Proceed with testing as for the ampule solution above starting at step 2.

Positive Oxidase

Negative Oxidase

8. Results Recording

- Record all results onto paper worksheet with registered lab number and patient identification.
- Record results into LIMS

9. Interpretation

- A Positive oxidase result will be demonstrated by the development of a strong purple colour within 10 seconds.
- Any delayed reaction should be considered negative.
- No colour change or delayed reaction indicates a Negative oxidase result.

Limitations

- Use of steel or nichrome loops may cause false-positive reactions.
- Some organisms give a weak oxidase reaction: Pasteurella, Burkholderia cepacian.
- False negative results occur in mixed cultures of Pseudomonas spp. & Neisseria spp. An inhibitory substance is produced by Pseudomonas spp. that interferes with oxidase in Neisseria spp.
- Never allow water to come into contact with the stock powder, or secondary container of powder.
- Always use Sterile DI water to make Oxidase reagent.
- Always use a secondary container to store a small amount of powder to prevent moisture from getting into the primary container.

10. Quality Control

- Pseudomonas aeruginosa ATCC 27853 - Oxidase Positive
- E coli ATCC 25922 – Oxidase Negative
- Record the QC results on the Bench Reagent QC Worksheet G_90_WS_1

11. Related Documents

- Bench Reagent QC Worksheet G _90_WS-1
- Laboratory Biosafety Info Sheet G_10_Info_3

12. References

- DMDP Oxidase SOP- Joanne Letchford 2014
- UK Standards for Microbiology Investigations https://www.gov.uk/uk-standards-for-microbiology-investigations-smi-quality-and-consistency-in-clinical-laboratories
- ASM Microbe Library, Oxidase Test Protocol
- BBL Diagnostic Oxidase Reagent Package insert