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Oxidase Test: A Biochemical Methods in Bacterial Identification

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SUMMARY

The oxidase test is done to find out the presence of a cytochrome oxidase enzyme which will catalyse and transport electrons between electron donors in the bacteria and a redox dye- tetramethyl-p-phenylene-diamine. At the end dye colour changes to a deep purple colour. This test is used in the identification of Pseudomonas, Neisseria, Alcaligens, Aeromonas, based on the production of cytochrome oxidase enzyme.

INTRODUCTION

Principle of Oxidase Test: Mainly the organisms that contain cytochrome-c produces an intracellular enzyme called oxidase enzyme. these organisms mainly show oxidase-positive reaction and turn the reagent blue/purple and oxidase-negative organisms lacking cytochrome c as part of their respiratory chain and not able to oxidize the reagent, this oxidase enzyme catalyses the oxidation of cytochrome c. Oxidase positive bacteria contain cytochrome oxidase or indophenol oxidase from donor compounds i.e. NADH These catalyse the transport of electrons to electron acceptors which is usually oxygen. The oxidised reagent product forms are coloured compound indophenol blue. The aerobic organisms that contain the cytochrome system are capable of utilising oxygen as the final hydrogen acceptor. The final product is either water or hydrogen peroxide.

Reagents used for oxidase test are given below:

- **Kovacs Oxidase Reagent:** 1% tetra-methyl-*p*-phenylenediamine dihydrochloride, in water
- **Gordon and McLeod's Reagent:** 1% dimethyl-*p*-phenylenediamine dihydrochloride, in water
- **Gaby and Hadley (indophenol oxidase) Reagent:**1% α-naphthol in 95% ethano, 1% *p*-aminodimethylaniline HCL

Procedure of Oxidase Test: There are many methods available to check out oxidase tests. Some of the important are, Filter paper test, Direct plate method, Swab method, Impregnated oxidase test strip method and test tube method.

Application of oxidase Test: It is done to find out the presence of cytochrome oxidase enzymes and separation of *Neisseria, Moraxella, Campylobacter*. It is also used to separate *pseudomonas* from related species

Dry Filter Paper Method: Whatman's No. 1 filter paper is taken and dipped in a freshly prepared solution of 1% of tetramethyl-p-phenylene-diamine dihydrochloride then drained for about 30 seconds, the strips are later freeze dried and finally stored in a dark bottle tightly sealed with a screw cap. While testing, the strip is removed, and are kept in a petri dish and sprayed with distilled water. A platinum loop is used to pick up the colony and smear over the moist area. intense deep-purple hue, appearing within 5-10 seconds, indicates its positive reaction, absence of colouration or by colouration later than 60 seconds is a negative reaction. The oxidase reagents are actually unstable and need to be freshly prepared for use, so this method was found to be convenient.

Fig.1: Oxidase-positive *Pseudomonas aeruginosa* (left) and oxidase-negative *Escherichia coli* (right). source microbes online.com

Wet Filter Paper Method: Filter paper strips are soaked in a freshly made 1% solution of the reagent. The platinum loop is rubbed on the culture. Intense deep-purple hue, appearing within 5-10 seconds is indication of positive reaction and absence of colouration is a negative reaction.

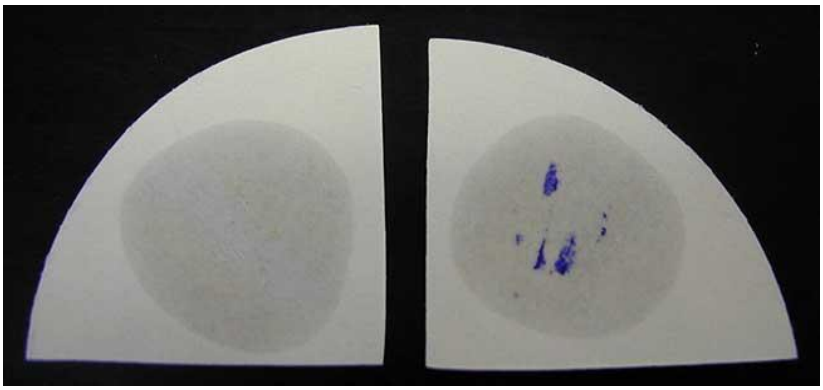

Fig.2: Oxidase test. The filter paper on the left-hand is negative while the filter paper on the right-hand side is positive as indicated by the blue colour. *source:* https://www.microbiologyclass.com

Swab Method: Swab is dip in a reagent and then rubbed in an isolated suspect colony there will be a change in colour within 10 seconds.

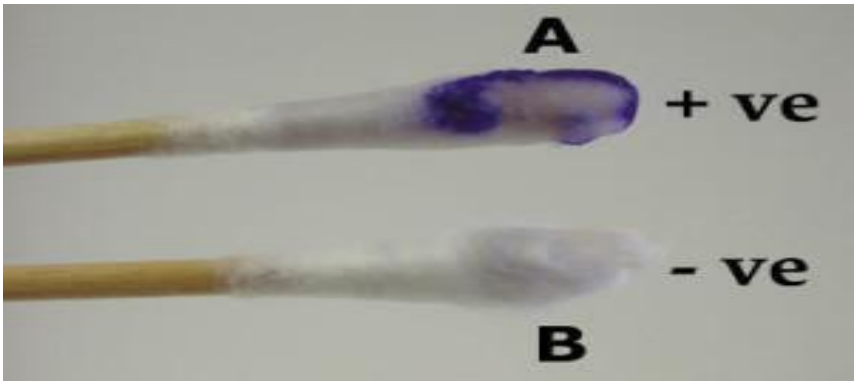

fig. 3: swab method (Source: Microbiology info.com)

Result Interpretation of Oxidase Test: Intense deep purple-blue/blue colour indicates oxidase production within 5-10 seconds and indicates positive result whereas no colour change indicates it as negative result**.**

Examples: Oxidase Positive Organisms: *Pseudomonas, Neisseria, Alcaligens, Aeromonas, Campylobacter, Vibrio, Brucella.* **Oxidase Negative Organisms:** Enterobacteriaceae (e.g. *E. coli*)

Quality Control for Oxidase Test: Positive Control: *Pseudomonas aeruginosa* ATCC 27853, **Negative Control:** *Escherichia coli* ATCC 25922

Limitations of Oxidase Test

- Fresh reagents need to be prepared all the time because the reagents used in the oxidase test get auto-oxidize so it is recommended to use colonies that are not more then 18-24 hours old as older colonies will produce weaker reactions
- Media containing high concentrations of glucose and organisms like bacteria and yeast growing on such media show inhibited oxidase activity, so in such cases media should be without excess sugar, such as nutrient agar.
- Sub-culturing should be done before adding any reagent to an active culture. since the test reagents will effectively kill the microorganisms.
- The oxidase test can be done in the identification of *Neisseria* and in the separation and identification of gram-negative bacilli.
- Less sensitive strips or reagents may yield false-negative results.

- for a gram-negative bacilli oxidase reaction should be done on non-selective and non-differential media to get valid results.

CONCLUSION

Result obtained by change in colour. When the colour changes to blue within 15 to 30 seconds these microbes are oxidase positive. and when the change in colour is delayed that is when the colour changes to purple within 2 to 3 minutes then its delayed oxidase positive. and oxidase negative if the colour does not change**.**

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