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MICROGEN BIOPRODUCTS

OXIDASE DETECTION STRIPS (MID61g)

INTRODUCTION

The oxidase test (indophenol cytochrome oxidase) was first described by Kovac (1) as a useful test for the differentiation of ***Pseudomonas*** spp. and the Enterobacteriaceae. The test is based on the production of an intracellular cytochrome oxidase enzyme which activates the oxidation of reduced cytochrome by oxygen. The oxidised cytochrome then acts as an electron acceptor in the terminal stages of the electron transfer system. This system being an integral part of energy production within the bacterial cell.

Reduced cytochrome C + H_2 + ½ O2 $\Rightarrow$ Oxidised Cytochrome C + H_2O

Artificial substrates such as phenylenediamine may be substituted for the natural electron acceptors producing a coloured end product in its oxidised state.

Reduced Cytochrome C + H_2 + ½ O2 $\Rightarrow$ Oxidised Cytochrome C + H_2O

Oxidised Cytochrome C + Artificial Substrate $\Rightarrow$ Oxidised Artificial Substrate
(Colourless) (Coloured)

Although a simple test to perform, care must be taken to ensure that a correct result is achieved. To minimise the potential errors associated with the performance of this test, Microgen Bioproducts has available a standardised and stable oxidase strip (MID61g) for the detection of cytochrome oxidase in bacteria including gram negative bacilli and ***Neisseria*** spp.

METHOD

1. Media
   Ideally the test should be performed from fresh colonies grown on a basal nutrient medium. Although often not possible, the test should not be performed directly on colonies from media containing blood or fermentable carbohydrates eg glucose, lactose etc or from a medium containing nitrate (2, 3, 4). If doubt edxists as to the true oxidase reaction of an organism, it should be subcultured onto nutrient agar and incubated for 24 hours after which the test should be repeated.

2. Performing the test
   Whenever possible the test should be performed by removing a single colony from the culture media using either a platinum wire, glass rod or wooden applicator. Care should be taken not to carry over any of the culture media. The growth removed is applied to a fresh oxidase strip.
   A positive result is indicated by development of a dark purple colour within 5 seconds. If the test is left too long before reading, many oxidase negative organisms will produce positive results. Some organisms do possess a small amount of cytochrome oxidase and may produce a weak or delayed positive reaction (10 – 15 seconds)

PRECAUTIONS

1. Colonies must not be removed from media using nichrome wire loops. Nichrome contains iron which may may catalyse the oxidation of the oxidase reagent (4).
2. Care must be taken when performing the oxidase test on colonies selected from blood containing media. Red blood cells contain cytochrome oxidase which may give false positive results (4).
3. Oxidase tests should not be performed on colonies taken directly from carbohydrate containing media. The low pH of the test colony and the surrounding media could result in false negative results (2, 3).

REFERENCES

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