

OXIDASE TEST

BIOCHEMICAL TEST FOR BACTERIAL IDENTIFICATION

MEDICAL MICROBIOLOGY

USMB502

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Class- TYMB

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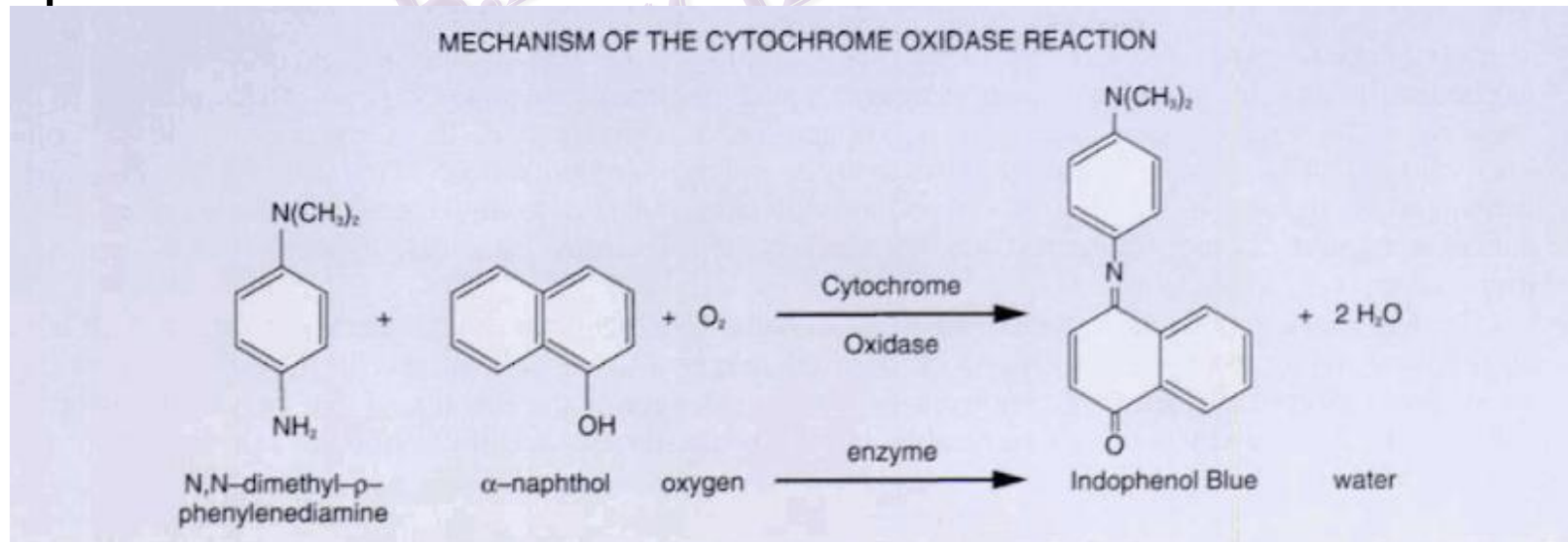
MULYE

AIM

- To perform Oxidase test in order to identify bacteria that produce cytochrome c oxidase enzyme.
- To screen colonies suspected of being one of the *Enterobacteriaceae* (all negative) and in identifying colonies suspected of belonging to other genera such as *Pseudomonas*, *Neisseria*, *Campylobacter*, *Aeromonas* (all positive).

PRINCIPLE

- Cytochromes- agent in aerobic respiration that transfer electrons to oxygen and forms water
- *p*-phenylenediamine dihydrochloride - redox dye used in the oxidase test
- Reduced condition- colourless
- Presence of cytochrome oxidase and atmospheric oxygen(oxidized)- deep purple colour



REQUIREMENTS

PARTICULARS	QUANTITY	VOLUME
A] REAGENTS		
1. <u>Kovac's oxidase reagent</u> : 1% Tetramethyl- <i>p</i> -phenylenediamine dihydrochloride	-	5mL
2. <u>Gordon and McLeod's reagent</u> : 1% Dimethyl- <i>p</i> -phenylenediamine dihydrochloride	-	5mL
B] SAMPLE:		
1. Provided bacterial culture plate	1	-
C] MISCELLANEOUS:		
1. Sterile discs impregnated with the oxidase reagent	-	-
2. Sterile toothpicks	-	-
3. Dropper	1	-

PROTOCOL

ISOLATE FROM THE BACTERIAL CULTURE PLATE

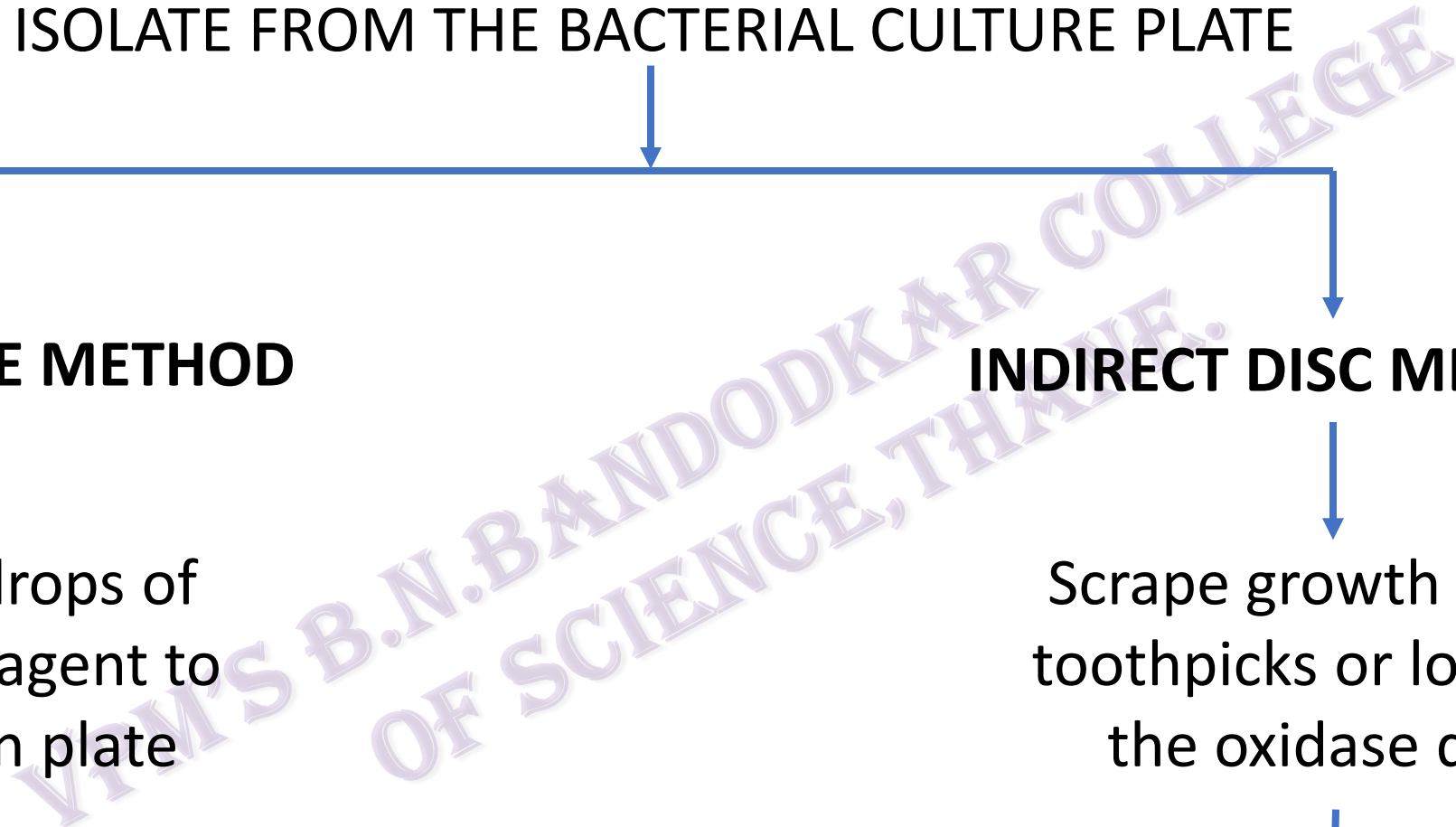
DIRECT PLATE METHOD

Add 2-3 drops of oxidase reagent to colony on plate

INDIRECT DISC METHOD

Scrape growth using toothpicks or loop on the oxidase disc

Observe after 10 seconds



OBSERVATION AND INTERPRETATION:

- Observe for colour change within 10 seconds.
- **Interpretation:**
- Positive Result: Development of a deep purple colour indicates oxidase production within 10 seconds.
- Negative Result: No colour change



EXAMPLES

- Oxidase positive organisms:

1. *Pseudomonas aeruginosa*

2. *Helicobacter pylori*

3. *Legionella pneumophila*

- Oxidase negative organisms:

1. Enterobacteriaceae (e.g. *Escherichia coli*, *Salmonella sp.*)

PRECAUTIONS

- Reagents- auto-oxidize
- Nichrome wire loop or iron containing loop
- Colour changes after 20 seconds- disregarded
- Older colonies- weaker results

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