



Gaby-Hadley Reagent B

R028

The reagent is used for detection of oxidase activity of a bacterial culture along with Gaby-Hadley reagent A (R027).

Composition**

Ingredients

p-aminodimethyl hydrochloride (oxalate)	0.100 gm
Distilled water	100.000 ml

**Formula adjusted, standardized to suit performance parameters

Directions

1. Isolate the bacteria under test on Nutrient agar plate to get 18-24 hours culture by streak plate method.
2. Add 0.2 ml of Gaby Hadley reagent A(R027) and then add 0.3 ml of Gaby Hadley reagent B(R028) on isolated colony.
3. Observe for color changes.
4. Microorganisms are oxidase positive when the color changes to deep purple to blue within 15 to 30 seconds.
Microorganisms are delayed oxidase positive when the color changes to purple within 2 to 3 minutes. Microorganisms are oxidase negative if the color does not change.

Principle And Interpretation

The oxidase test is a biochemical reaction that assays for the presence of cytochrome oxidase, an enzyme sometimes called indophenol oxidase. In the presence of an organism that contains the cytochrome oxidase enzyme, the reduced colorless reagent becomes an oxidized colored product. The final stage of bacterial respiration involves a series of membrane-embedded components collectively known as the electron transport chain. The final step in the chain may involve the use of the enzyme cytochrome oxidase, which catalyzes the oxidation of cytochrome c while reducing oxygen to form water. The oxidase test often uses a reagent, tetra-methyl-p-phenylenediamine dihydrochloride, as an artificial electron donor for cytochrome c. When the reagent is oxidized by cytochrome c, it changes from colorless to a dark blue or purple compound, indophenol blue.

Quality Control

Appearance

Blackish purple coloured solution.

Clarity

Clear without any precipitate.

Cultural Response

Biochemical identification was carried out by pouring 0.2ml of Gaby-Hadley Reagent A (R027) and 0.3ml of Gaby-Hadley Reagent B (R028) on to the Nutrient Agar (M001) plate containing 18-24 hours old isolated colony.

Organism	Colour of Colony	Oxidase Reaction
<i>Neisseria gonorrhoeae</i> ATCC 19424	Deep purple to blue	Positive
<i>Pseudomonas aeruginosa</i> ATCC 27853	Deep purple to blue	Positive
<i>Staphylococcus aureus</i> ATCC 25923	No colour change	Negative

Storage and Shelf Life

Store below 30°C in tightly closed container and away from bright light. Use before expiry date on label.

Reference

- 1) Gaby, W. L., and L. Free. 1958. Differential diagnosis of pseudomonas-like microorganisms in the clinical laboratory. J. Bacteriol. 76:442–444.
- 2) Gaby, W. L., and C. Hadley. 1957. Practical laboratory test for the identification of Pseudomonas aeruginosa. J. Bacteriol. 74:356–358.
- 3) MacFaddin, J. 1972. Biochemical tests for the identification of medical bacteria. Williams and Wilkins Company, Baltimore, MD.

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