Gaby-Hadley Reagent A

R027

Intended use

Gaby-Hadley Reagent A is used for detection of oxidase activity of a bacterial culture along with Gaby-Hadley reagent B (R028).

Composition**

Ingredients

a-Naphthol	1.0 gm
Ethanol (98%)	100.0 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 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 colourless reagent becomes an oxidized coloured 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 colourless to a dark blue or purple compound, indophenol blue.

Type of specimen

1.The specimen is any isolated colony on primary or subculture plates.

Specimen Collection and Handling

1. For clinical samples follow appropriate techniques for handling specimens as per established guidelines.
2. For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines.
3. For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.

After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions

In Vitro diagnostic use only. Read the label before opening the container. Wear protective gloves/protective clothing/ eye protection/face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations

1. The reagents used in the oxidase test have been shown to auto-oxidize, so it is very important to use fresh reagents, no older than 1 week.
2. Both bacteria and yeast grown on media containing high concentrations of glucose show inhibited oxidase activity, so it is recommended to test colonies grown on media without excess sugar, such as nutrient agar. Tryptic soy agar is also an excellent media.
3. Bacteria grown on media containing dyes may give aberrant results.
4. The test reagents will effectively kill the microorganisms, so sub-culturing should be done prior to adding any reagent to an active culture.
5. The oxidase test can be used in the presumptive identification of Neisseria and in the differentiation and identification of gram-negative bacilli. Oxidase-positive organisms should be examined by gram stain to determine morphology and gram reaction. Additional biochemical tests are recommended for complete identification.
6. Use of a nichrome or other iron containing loop may yield false-positive reactions. Platinum loops are recommended.
7. Most Hemophilus are oxidase-positive. Less sensitive strips or reagents may yield false-negative results.
8. Oxidase reactions of gram-negative bacilli should be determined on non-selective and non-differential media to ensure valid results. Also, colonies taken from media containing high levels of glucose may give false-negative reactions.
9. It is recommended to use colonies that are 18-24 hours old. Older colonies will produce weaker reactions.
10. Any color changes appearing after 20 seconds should be disregarded.

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature

Quality Control

- **Appearance :** Colourless to reddish brown coloured solution.
- **Clarity :** Clear solution without any precipitate.

Cultural Response

Organism	Oxidase Reaction
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 24-48 hours old isolated colony.
Neisseria gonorrhoeae ATCC 19424	Positive (development of purple-blue colour)
Pseudomonas aeruginosa ATCC 27853 (WDCM 00025)	Positive (development of purple-blue colour)

Organism	Oxidase Reaction
Staphylococcus aureus subsp. *aureus* ATCC 25923 (WDCM 00034)	Negative (No change in colour)

Storage and Shelf Life

Store between 10-30°C in tightly closed container and away from bright light. Use before expiry date on label. On opening, product should be properly stored in dry ventilated area protected from extremes of temperature and sources of ignition. Seal the container tightly after use.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques.

Reference

1. Gaby, W. L., and L. Free. 1958. Differential diagnosis of pseudomonas-like microorganisms in the clinical laboratory. J. Bateriol. 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.
4. Rice E.W., Baird, R.B., Eaton A. D., Clesceri L. S. (Eds.), 2012, Standard Methods for the Examination of Water and Wastewater, 22nd ed., APHA, Washington, D.C
5. Wehr H. M. and Frank J. H., 2004, Standard Methods for the Microbiological Examination of Dairy Products, 17th Ed., APHA Inc., Washington, D.C.
6. Lapage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.
7. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore

Storage temperature


Do not use if package is damaged

In vitro diagnostic medical device

CE Marking


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