



Gordon-McLeod Reagent (Oxidase Reagent)

R026

Intended use

Gordon-McLeod Reagent (Oxidase reagent) is used to carry out the oxidase test, to determine the presence of oxidase enzymes.

Composition**

Ingredients

N,N-Dimethyl p-phenylenediaminehydrochloride	0.150 gm
Distilled water	10.000 ml

**Formula adjusted, standardized to suit performance parameters

Directions

Biochemical identification was carried out by using Gordon-McLeod Reagent (R026) and Soyabean Casein Digest Agar Plate (M290) containing 24-48 hours old culture. Place 2 to 3 drops of Gordon McLeod Reagent (R026) on to a filter paper in a petridish. With a platinum wire loop (not nichrome), plastic loop or glass rod, smear some of the growth on Soyabean Casein Digest Agar Plate (M290) on the prepared filter paper. Observe for appearance of deep blue purple colour within 10 seconds.

Principle And Interpretation

The oxidase test is based on bacterial production of an intracellular oxidase enzyme. This oxidase reaction is due to a cytochrome oxidase system that activates oxidation of reduced cytochrome by molecular oxygen, which in turn acts as an electron acceptor in the terminal stage of the electron transfer system. All Pseudomonas and Neisseria spp., produce an oxidase enzyme which, in the presence of atmospheric oxygen, cytochrome c, and an oxidase reagent, oxidize the reagent to a colored compound, indophenol.

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(1,2).
 2. For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (3,5).
 3. For water samples, follow appropriate techniques for sample collection, processing as per guidelines and local standards.(4)
- 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 *Haemophilus* 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 product is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Black coloured solution.

Clarity

Clear without any precipitate.

Cultural Response

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Organism

Neisseria gonorrhoeae ATCC 19424

Staphylococcus aureus ATCC 25923

Pseudomonas aeruginosa ATCC 27853

Oxidase Reaction

Positive(development of purple-blue colour)

Positive(development of purple-blue colour)

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 (6,7).

Reference

1. Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2nd Edition. Vol. 2.
2. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock, D.W. (2015)
3. Downes F. P. and Ito K. (Ed.), 2001, Compendium of Methods for the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.
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. Lepage S., Shelton J. and Mitchell T., 1970, Methods in Microbiology', Norris J. and Ribbons D., (Eds.), Vol. 3A, Academic Press, London.

