Nutrient Agar for Oxidase

M1274

Nutrient Agar is used for confirmation of presence of oxidase in microorganisms in water.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	1.000
Meat extract	1.000
Sodium chloride	5.000
Agar	15.000
Final pH (at 25°C)	7.3±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 22 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Nutrient Agar is recommended by APHA (1) for differentiation of the coliform bacteria on the basis of presence of enzyme cytochrome oxidase. It is also recommended by ISO Committee (2) for the same. Cytochrome oxidase is a iron-containing porphyrin enzyme that participates in the electron transfer mechanisms and in the nitrate metabolic pathways of some bacteria. Although the test can be performed by flooding the agar surface of an inoculated plate with the reagent after incubation or with the help of oxidase reagent impregnated filter paper.

Peptic digest of animal tissue and meat extract provide nitrogenous compounds, carbon, sulphur and trace ingredients. Sodium chloride maintains osmotic equilibrium.

Nutrient Agar plates are streak inoculated to obtain isolated colonies. The isolated colony is used for oxidase testing on an impregnated filter paper. A dark purple colour that develops within 10 seconds is a positive oxidase test. Coliform bacteria are oxidase negative.

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Gelling
Firm, comparable with 1.5% Agar gel

Colour and Clarity of Prepared medium
Yellow coloured clear to slightly opalescent gel forms in Petri plates

Reaction
Reaction of 2.2% w/v aqueous solution at 25°C. pH : 7.3±0.2

pH
7.10-7.50

Cultural Response
M1274: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

Organism	Growth	Oxidase
Cultural Response		
Aeromonas hydrophila ATCC 7966	luxuriant	positive reaction, deep purple blue colour develops

		within 10 seconds
Escherichia coli ATCC 25922	luxuriant	negative reaction
Enterobacter aerogenes ATCC 13048	luxuriant	negative reaction
Pseudomonas aeruginosa ATCC 27853	luxuriant	positive reaction, deep purple blue colour develops within 10 seconds
Vibrio cholerae ATCC 15748	luxuriant	positive reaction, deep purple blue colour develops within 10 seconds

Storage and Shelf Life

Store below 30°C in tightly closed container and the prepared medium at 2 - 8°C. Use before expiry date on the label.

Reference

1. Greenberg A. E., Clesceri L. S. and Eaton A. D., (Eds.), 1992, Standard Methods for the Examination of Water and Wastewater, 18th Ed., APHA, Maryland.
2. International Organization for Standardization (ISO), 1990, Draft, ISO/DIS 9308-1.

Revision : 2 / 2015