



Nitrate Agar

M072

Nitrate Agar is recommended for detection of nitrate reduction by bacteria.

Composition**

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.000
Beef extract	3.000
Potassium nitrate	1.000
Agar	12.000
Final pH (at 25°C)	6.8±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 21 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow to cool the tubes in slanted position.

Principle And Interpretation

Nitrate Agar is prepared in accordance with the formula published in Pure Culture Study of Bacteria of the Society of American Bacteriologist (1). The ability to reduce nitrate is valuable for differentiating and identifying various types of bacteria especially *Enterobacteriaceae* family (2). Non-fermenters and other miscellaneous gram-negative bacilli vary in their ability to reduce nitrates. Some members of this group are capable of denitrification which is the reduction of nitrate to nitrogen gas. For the glucose fermenting gram-negative bacilli, the production of nitrogen gas from nitrate is an important differential test (3).

Potassium nitrate in the medium acts as a substrate for determining nitrate reduction by bacteria. Certain bacteria convert nitrate to nitrite, ammonia or nitrogen gas. The presence of nitrites can be detected by the addition of 0.5 ml each of sulphanilic acid (R015) and alpha-naphthylamine solution (R009). The development of red violet colour, due to the formation of a red diazonium dye i.e. p-sulfobenzene-azo-a-naphthylamine, indicates nitrate reduction to nitrite. If no colour develops, it means that either nitrate is not reduced or further reduction to ammonia or nitrogen gas has taken place. This can be verified by adding a pinch of zinc dust to the tube. Zinc reduces nitrate to nitrite resulting in a red colour. The red colour indicates that nitrate is still present and was not reduced previously. An absence of red colour after the addition of zinc dust indicates that no nitrate is present and thus the nitrate was reduced further than nitrite. Therefore the nitrate reduction test is evidenced by either the presence of a catabolic end product or the absence of nitrate in the medium.

Members of *Enterobacteriaceae* characteristically reduce nitrate to nitrite which reacts with sulfanilic acid and N, N-dimethyl-1-naphthylamine to produce the red colour. This reaction is known as Griess reaction. If an organism grows rapidly and reduces nitrate actively, the test should be performed after an early incubation period since the nitrite may be further reduced to nitrogen.

For the test: Add few drops of each reagent i.e. sulphanilic acid (R015) and a-naphthylamine solution (R009) into the tube containing culture to be tested. A distinct red or pink colour indicates nitrate reduction. A control (un-inoculated) tube should also be tested. If there is no pink colour formation, add a pinch of zinc dust to confirm the absence of nitrate in the medium (3).

Note: Nitrate reduction is not a confirmatory test. Complete identification of bacteria should include the morphology, gram reaction, biochemical and serological tests. Addition of excess zinc may result in false negative reaction. Also during performance of nitrate reduction test with a-naphthylamine, the colour produced in a positive reaction may fade quickly (4).

Quality Control

Appearance

Cream to yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.2% Agar gel.

Colour and Clarity of Prepared medium

Light amber coloured clear to slightly opalescent gel forms in tubes as slants

Reaction

Reaction of 2.1% w/v aqueous solution at 25°C. pH : 6.8±0.2

pH

6.60-7.00

Cultural Response

M072: Cultural characteristics observed after incubation at 35 - 37°C for 18 - 24 hours. Nitrate reduction observed on addition of 0.5ml of sulphanilic acid (R015) and 0.5ml of α-naphthylamine Solution(R009).

Organism	Inoculum (CFU)	Growth	Nitrate reduction
Cultural Response			
<i>Acinetobacter calcoaceticus</i> ATCC 23055	50-100	luxuriant	negative reaction
<i>Enterobacter aerogenes</i> ATCC 13048	50-100	luxuriant	Positive reaction, distinct red-pink colour developed within 1-2 minutes
<i>Escherichia coli</i> ATCC 25922	50-100	luxuriant	Positive reaction, distinct red-pink colour developed within 1-2 minutes
<i>Salmonella Typhimurium</i> ATCC 14028	50-100	luxuriant	Positive reaction, distinct red-pink colour developed within 1-2 minutes

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. Society of American Bacteriologist, Pure Culture Study of Bacteria, 1944, 12 : Leaflet 11: 8.
2. Ewing, 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th ed., Elsevier Science Pub. Co., Inc., N.Y.
3. MacFaddin J. F., 2000, Biochemical Tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore

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