Technical Information

Nitrate MiVeg Agar

Product Code : VM1072

Application:- Nitrate MiVeg Agar is recommended for detection of nitrate reduction by bacteria.

Composition

Ingredients	Gms / Litre
MiVeg peptone No. 2	5.00
MiVeg extract	3.00
Potassium nitrate	1.00
Agar	12.00
Final pH (at 25°C)	6.8±0.2

** Formula adjusted, standardized to suit performance parameters.

Principle & Interpretation

Nitrate MiVeg Agar is prepared by using vegetable peptones in place of animal based peptones, thereby making the medium free from BSE/TSE risks. Nutritionally rich in nitrogen source, MiVeg peptone No. 2 and MiVeg extract is used in this medium instead of Gelatine peptone and Beef extract respectively. It is a modification of conventional Nitrate Medium 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 a valuable property for differentiating and identifying various types of bacteria especially those belonging to the *Enterobacteriaceae* family (2).Nonfermenters and other miscellaneous gram-negative bacilli vary in their ability to reduce nitrates. Some members of this group are capable of denitrification which is reduction of nitrate to nitrogen gas. Production of nitrogen gas from nitrate is an important differential test for detecting gram-negative glucose fermenters (3).

Potassium nitrate present in the medium serve as a substrate for determining nitrate reduction by bacteria. Certain bacteria convert nitrate to nitrite, ammonia or nitrogen gas. The presence of nitrite is determined by addition of 0.5 ml each of Sulphanilic Acid and alpha - Naphthylamine solution to the incubated tubes. The development of red violet colour 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 which results in red colour. The red colour indicates that nitrate is still present and was not reduced previously. After the addition of zinc dust, an absence of red colour 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.

The colour of the medium becomes red due to reduction of nitrate to nitrite which reacts with sulfanilic acid and N,N-dimethyl-1-naphthylamine by the members of *Enterobacteriaceae*. 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 : Put few drops of each reagent into the tube containing culture to be tested. A distinct red or pink colour indicates nitrate reduction. A control (uninoculated) tube should also be tested. If there is no pink colour formation, add pinch of zinc dust to confirm the absence of nitrate in the medium (3).

Nitrate reduction should not be considered as a confirmatory test. For complete identification of bacteria should include the morphology, gram reaction, biochemical and serological tests. Addition of excess zinc may result in false-negative reaction.

Methodology

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

Quality Control

Physical Appearance

Yellow coloured may have slightly greenish tinge, 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 pH: 6.8 ±0.2 at 25°C

pH range

6.6-7.0

Cultural Response/Characteristics

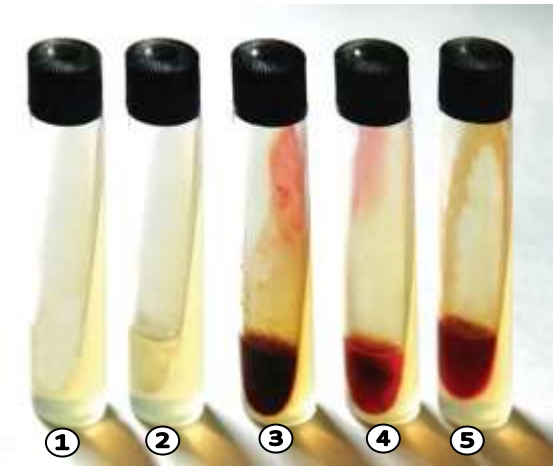
Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours

Organisms (ATCC)	Inoculum (CFU)	Growth	Nitrate reduction*
Acinetobacter calcoaceticus (19606)	10^2-10^3	luxuriant	-
Enterobacter aerogenes (13048)	10^2-10^3	luxuriant	+
Escherichia coli (25922)	10^2-10^3	luxuriant	+
Salmonella serotype Typhimurium (14028)	10^2-10^3	luxuriant	+

Key : + = Development of distinct red or pink colour
- = No change in colour or no development of red or pink colour.
* = On addition of 0.5 ml of Sulphanilic acid and 0.5 ml of alpha –Naphthylamine solution.

Storage and Shelf Life

Dried Media: Store below 30°C in tightly closed container and use before expiry date as mentioned on the label.
Prepared Media: 2-8^0 in sealable plastic bags for 2-5 days.



VM1072 Nitrate MiVeg Agar

1. Control
2. *Acinetobacter calcoaceticus*
4. *Escherichia coli*
5. *Salmonella* serotype Typhimurium

3. *Enterobacter aerogenes*

Further Reading

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(ed), Biochemical Tests for Identification of Medical Bacteria, 3rd edition, Lippinicott Williams and Wilkins, New York

Disclaimer :

- User must ensure suitability of the product(s) in their application prior to use.
- The product conform solely to the technical information provided in this booklet and to the best of knowledge research and development work carried at **CDH** is true and accurate
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