

Also known as

MMN Medium

Specification

Semi-solid culture medium for the differentiation of bacteria based on their mobility and their ability to ferment mannitol and reduce nitrates

Formula * in g/L

Peptone.....	20.00
Potassium nitrate.....	1.00
D-Mannitol.....	2.00
Phenol red.....	0.04
Agar.....	4.00

Final pH 7.4 ±0.2 at 25 °C

* Adjusted and /or supplemented as required to meet performance criteria

Directions

Suspend 27.04 g of powder in 1 L of distilled water and bring to a boil until completely dissolved. Distribute in suitable containers (tubes) and sterilize in the autoclave for 15 minutes at 121 °C.

Description

The use of semisolid media to verify the motility of bacteria was proposed and demonstrated by Tittler and Sandholzer in 1936 and in 1949 Roland and Bourbon suggested the addition of mannitol to a semisolid medium for the identification of Enterobacteriaceae but gas bubbles from fermentation of mannitol sometimes hampered mobility verification. This problem was solved by Le Minor in 1967 with the addition of a small amount of potassium nitrate to the medium, which inhibits the production of the fermentation gas while at the same time allowing to verify the reduction of nitrates.

This fluid motility, mannitol and nitrates medium, when used together with the Iron Three Sugar Agar, allow a rapid differentiation between lactose-negative enterobacteria and non-fermenting gram-negative bacilli from clinical samples.

Technique

The medium is inoculated by driving the seeding needle to the bottom of the tube and incubated à 36±1 ° C for 20-24 hours.

After incubation, the nitrate test is carried out by depositing 4-6 drops of sulfanilic acid on the surface of the culture medium, followed by an equal amount of α-naphthylamine. The appearance of a bright red ring indicates a positive test for reduction of nitrate to nitrite. If colour does not occur, a little zinc powder should be added. If the red colour then appears, it indicates that there are nitrates without reducing and, on the contrary, if the red colour continues without occurring, there is a total reduction of nitrate to nitrogen.

The colour change of the medium from red to yellow indicates the fermentation of mannitol.

Interpretation of changes after incubation of the medium:

- Motility is observed by the cloudiness of the medium from the inoculums line.
- Clear, transparent and red: Medium not inoculated.
- Turbidité limited to the sting line: Immobile microorganism.
- Turbidité limited to the surface layer: Immobile, aerobic microorganism unable to use nitrate as an electron acceptor.
- Turbidité with slight intensification of the original red colour of the medium: Non-fermentation of mannitol.
- Turbidité that diffuses the sting line laterally in greater or lesser profusion: Mobile organism (degree of spread, indicative, slow to very active motility).
- Turbidité with general yellowing of the medium except the surface that remains intense red: Mannitol fermentation.
- Turbidité with slight intensification of the original red colour of the medium, with the appearance of trapped gas bubbles along the pit line and, sometimes, rupture of the culture medium: Non-fermentation of mannitol with progressive reduction of nitrates to nitrites and final ly to nitrogen gas.

Quality control
Incubation temperature: 36 °C ±1

Incubation time: 18 h ±2

Inoculum: Pure cultures using and inoculating needle
Microorganism
Growth
Remarks
Escherichia coli ATCC® 25922

Good

Mot. (+) Man. (+) Nit. (+)

Proteus mirabilis ATCC® 25933

Good

Mot. (+) Man. (-) Nit. (+)

Klebsiella pneumoniae ATCC® 13883

Good

Mot. (-) Man. (+) Nit. (+)

References

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Storage

For laboratory use only. Keep tightly closed, away from bright light, in a cool dry place (+4 °C to 30 °C).