MIO Medium (Motility Indole Ornithine Medium)

M378

Motility Indole Ornithine Medium (MIO Medium) is used for the identification of *Enterobacteriaceae* on the basis of motility, indole production and ornithine decarboxylase activity.

Composition**

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Peptic digest of animal tissue	10.000
Yeast extract	3.000
L-Ornithine hydrochloride	5.000
Dextrose	1.000
Bromocresol purple	0.020
Agar	2.000
Final pH (at 25°C)	6.5±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 31.02 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense in test tubes in 5 ml amounts. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool the tubes in an upright position.

Principle And Interpretation

Motility, indole production and ornithine decarboxylation are routine biochemical tests employed during identification of *Enterobacteriaceae* . Motility can be demonstrated microscopically (hanging drop) or macroscopically (tube method), where motility is observed as a diffused zone of growth flaring out from the line of inoculation. Indole test is carried out to determine the ability of an organism to split indole from tryptophan by the tryptophanase enzyme. On reaction with Kovacs reagent, indole combines with the colour in the alcohol layer, which is visualized as a red ring (in the alcohol layer) (1). If the test organisms possess the specific decarboxylase enzyme, then ornithine is decarboxylated to putrescine, an amine, resulting in a subsequent rise in the pH of the medium towards alkalinity. This causes the pH indicator bromocresol purple to change from purple to yellow colour. MIO (Motility Indole Ornithine Medium) is used for identification of *Enterobacteriaceae* on the basis of motility, indole production and ornithine decarboxylation in a single tube. This medium was formulated by Ederer and Clark (2) and evaluated by Oberhofer and Hajkowski (3).

Casein enzymic hydrolysate and peptic digest of animal tissue provide amino acids and other nitrogenous substances. Yeast extract is the source of vitamin B complex. Dextrose is the fermentable carbohydrate. Test cultures are stab-inoculated into the medium butts.

Motility and ornithine decarboxylation reactions are read before testing indole production. On addition of the Kovacs reagent, colour of the medium changes to yellow. Therefore positive ornithine decarboxylase test (purple) could be misinterpreted as negative (yellow).

Organisms ferment dextrose to form acid, which causes the pH indicator bromocresol purple to change from purple to yellow. Organisms possessing ornithine decarboxylase enzyme, decarboxylate ornithine to putrescine which increases the pH making it alkaline, indicated by a colour change from yellow to purple throughout the medium. Decarboxylase negative reaction is indicated by yellow colour or yellow with a purple band near the top of the medium. Indole is produced from tryptophan present in casein enzymic hydrolysate (4, 5). The indole produced combines with the aldehyde present in the Kovacs reagent to form a red complex.

Quality Control

Appearance

Light yellow to pale green homogeneous free flowing powder

Gelling
Semisolid, comparable with 0.2% Agar gel.

Colour and Clarity of prepared medium
Purple coloured clear to slightly opalescent gel forms in tubes as butts

Reaction
Reaction of 3.1% w/v aqueous solution at 25°C. pH : 6.5±0.2

pH
6.30-6.70

Cultural Response
M378: Cultural characteristics observed after an incubation at 35-37°C for 40-48 hours.

Organism	Inoculum (CFU)	Growth	Motility	Indole production	Ornithine Decarboxylation
Escherichia coli ATCC 25922	50-100	luxuriant	positive, growth away from stabline causing turbidity	positive reaction, red ring at the interface of the medium	positive reaction, purple colour
Enterobacter aerogenes ATCC 13048	50-100	luxuriant	positive, growth away from stabline causing turbidity	negative reaction	positive reaction, purple colour
Klebsiella pneumoniae ATCC 13883	50-100	luxuriant	negative, growth along the stabline, surrounding medium remains clear	negative reaction	negative reaction
Proteus mirabilis ATCC 25933	50-100	luxuriant	motility is temperature dependent,it is more pronounced at 20°C and almost absent at 35°C	negative reaction	positive reaction, purple colour

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. MacFaddin J. F., 2000, Biochemical tests for Identification of Medical Bacteria, 3rd Ed., Lippincott, Williams and Wilkins, Baltimore.
2. Ederer G. M. and Clark M., 1970, Appl. Microbiol., 20:849.
3. Oberhofer J. R. and Hajkowski R., 1970, Am. J. Clin. Pathol., 54:726.
4. MacFaddin J. F., 1985, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria, Vol. 1, Williams and Wilkins, Baltimore.
5. Ewing W. H., 1986, Edwards and Ewings Identification of Enterobacteriaceae, 4th Ed., Elsevier Science Publishing Co., Inc., New York.

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