BBL™ Motility Indole Ornithine (MIO) Medium

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Motility Indole Ornithine (MIO) Medium is a semisolid medium useful in the identification of members of the *Enterobacteriaceae*.

II PERFORMANCE TEST PROCEDURE

1. Loosen caps, boil* and cool before use.
   *__NOTE:__ Use of a microwave oven is not recommended.
2. Inoculate representative samples with the cultures listed below.
   a. Inoculate tubes by stabbing with an inoculating needle to within 1/4 inch of the bottom of the medium using 10-1 dilutions of 18- to 24-h **Trypticase**™ Soy Broth cultures.
   b. Incubate tubes with loosened caps at 35 ± 2 °C in an aerobic atmosphere.
3. Examine tubes after 18–24 h for growth, presence of motility and ornithine decarboxylase and indole reactions. If the indole reaction is negative, incubate an additional 24 h.
4. Expected Results

Organisms	ATCC™	Motility	Indole	Ornithine
**Escherichia coli*	25922	+	+	+
**Enterobacter aerogenes*	13048	+	–	+
**Klebsiella pneumoniae* subsp. *pneumoniae*	33495	–	–	–
Morganella morganii	8019	+	+	+
Salmonella enterica subsp. *enterica* serotype Typhi	19430	+	–	–

*Recommended organism strain for User Quality Control.

III ADDITIONAL QUALITY CONTROL

1. Examine tubes as described under “Product Deterioration.”
2. Visually examine representative tubes to assure that any existing physical defects will not interfere with use.
3. Incubate uninoculated representative tubes at 20–25 °C and 30–35 °C and examine after 7 days for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Motility Indole Ornithine (MIO) Medium is used to demonstrate motility, indole production and ornithine decarboxylase activity for the differentiation of *Enterobacteriaceae*.

V SUMMARY AND EXPLANATION

Motility Indole Ornithine Medium was formulated by Ederer and Clark[1] and Oberhofer and Hajkowski[2] for detection of motility, indole and ornithine decarboxylase production in one tube as an aid in the identification of members of the *Enterobacteriaceae* family.

VI PRINCIPLES OF THE PROCEDURE

The casein and gelatin peptones, yeast extract and dextrose provide nitrogenous and carbonaceous substances, vitamins and minerals essential for bacterial metabolism. When ornithine decarboxylase is present, the ornithine is decarboxylated to putrescine which causes a rise in the pH and corresponding color change of the bromcresol purple from yellow to purple.

VII REAGENTS

Motility Indole Ornithine Medium

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	9.5 g	L-Ornithine Monohydrochloride	5.0 g
Pancreatic Digest of Gelatin	10.0 g	Bromcresol Purple	0.02 g
Yeast Extract	3.0 g	Agar	2.0 g
Dextrose	1.5 g		

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

Warnings and Precautions: For *in vitro* Diagnostic Use.

Tubes with tight caps should be opened carefully to avoid injury due to breakage of glass.

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared tubes, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store tubes in the dark at 2–25 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Tubed media stored as labeled until just prior to use may be inoculated up to the expiration date and incubated for the recommended incubation times.

Product Deterioration: Do not use tubes if they show evidence of microbial contamination, discoloration, drying or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Specimens suitable for culture may be handled using various techniques. For detailed information, consult appropriate texts.[3,4] Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Motility Indole Ornithine (MIO) Medium

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

Loosen caps, heat the medium to boiling* and cool to room temperature prior to inoculation. Inoculate tubes of medium by a single stab to 1/4 inch from the bottom of the tube using growth from a primary isolation plate or other pure culture. Incubate all tubes for 18–24 h at 35 ± 2 °C in an aerobic atmosphere.

***NOTE:** Use of a microwave oven is not recommended.

User Quality Control: See "Quality Control Procedures."

Quality Control requirements must be performed in accordance with applicable local, state and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. It is recommended that the user refer to pertinent CLSI guidance and CLIA regulations for appropriate Quality Control practices.

X RESULTS

Read motility and decarboxylase activity prior to the addition of the reagent for the detection of indole production.

1. Motility is indicated by growth extending from the line of inoculation. Nonmotile organisms grow only along the line of inoculation.
2. Decarboxylation of ornithine is indicated by the development of a turbid purple to a faded yellow-purple color. A negative reaction is indicated by a yellow color.
3. Indole production is indicated by the formation of a pink to red color after the addition of three or four drops of Kovacs' reagent to the surface of the medium and gentle shaking. A negative reaction is indicated by the development of a yellow color. Refer to appropriate texts for typical reactions produced by various members of the *Enterobacteriaceae*.[3-5]

XI LIMITATIONS OF THE PROCEDURE

For identification, organisms must be in pure culture. Morphological, biochemical, and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[3-7]

XII PERFORMANCE CHARACTERISTICS

Prior to release, all lots of Motility Indole Ornithine (MIO) Medium are tested for performance characteristics. Prior to use, representative samples of the lot are placed in a boiling water bath (after the caps have been loosened) and then cooled to reestablish the semi-solid nature of the medium. The tubes are stab inoculated with an inoculating needle to one-fourth inch from the bottom of the tube with **Trypticase** Soy Broth cultures diluted 10^{-1} of *Escherichia coli* (ATCC 25922), *Morganella morganii* (ATCC 8019), *Enterobacter aerogenes* (ATCC 13048), *Klebsiella pneumoniae* (ATCC 33495), and *Salmonella* Typhi (ATCC 19430). The tubes are incubated with loosened caps at 35 ± 2 °C. After 18–24 h incubation, the tubes are observed for the amount of growth and ornithine decarboxylase. All cultures exhibit moderate to heavy growth. All cultures exhibit motility, indicated by a spread of growth throughout the medium from the line of inoculation, except for *K. pneumoniae* which is nonmotile and growth is evident only along the line of inoculation. *E. aerogenes*, *E. coli* and *M. morganii* are positive for ornithine decarboxylase demonstrated by a turbid purple to a faded yellow-purple color while *K. pneumoniae* and *Salmonella* Typhi are negative indicated by a yellow color. Subsequently, 3–4 drops of Kovacs' Reagent is added to the surface of each tube to test for indole production. *E. coli* and *M. morganii* are positive for indole production which is indicated by the formation of a pink to red color in the medium. *E. aerogenes*, *K. pneumoniae* and *Salmonella* Typhi are negative for indole production and no reaction (no color change) occurs in the medium.

XIII AVAILABILITY

Cat. No.	Description
221517	**BD BBL™** Motility Indole Ornithine (MIO) Medium, 5 mL, Pkg. of 10 size K tubes
221518	**BD BBL™** Motility Indole Ornithine (MIO) Medium, 5 mL, Ctn. of 100 size K tubes

XIV REFERENCES

1. Ederer, G.M., and M. Clark. 1970. Motility-indole-ornithine medium. Appl. Microbiol. *2*:849-850.
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3. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R.H. Yolken (ed.) 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
4. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey & Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
5. Ewing, W.H. 1986. Edwards and Ewing's identification of *Enterobacteriaceae*, 4th ed. Elsevier Science Publishing Co., New York.
6. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
7. Farmer, J.J., III. 1999. *Enterobacteriaceae*: introduction and identification, p. 442-458. *In* P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

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