IFU

Instructions for Use

MIO (MOTILITY, INDOLE, ORNITHINE) MEDIUM

Cat. no. Q20	MIO Medium, 16x100mm Tube, 6.5ml	20 or 100 tubes/box
Cat. no. R24	MIO Medium, 13x100mm Tube, 4ml	20 or 100 tubes/box

INTENDED USE

Hardy Diagnostics MIO (Motility, Indole, Ornithine) Medium is recommended for use in testing motility, indole production, and ornithine-decarboxylase activity of enteric bacilli.

SUMMARY

Ederer and Clark, et al., developed MIO (Motility Indole Ornithine) Medium to be used as an aid in the identification of members of the Enterobacteriaceae family.[7] Motility, indole production, and ornithine-decarboxylation are the three differentiating tests that are provided in the one culture tube.

The addition of agar to the medium allows for the detection of motility along the stab line of inoculation. Motile organisms extend from the stab line and produce turbidity or cloudiness throughout the medium. Non-motile organisms grow only along the stab line and leave the surrounding medium clear.

Tryptophan, present in the basal medium, is degraded by organisms that possess the enzyme tryptophanase. Degradation of tryptophan produces indole which is detected upon addition of Kovacs Reagent (Cat. no. Z67) to the surface of the medium. Indole combines with p-dimethylaminobenzaldehyde and produces a red band at the top of the medium. A negative indole test results in no color change upon addition of Kovacs Reagent.

Bromcresol purple serves as the pH indicator which allows for detection of decarboxylase activity. Organisms that ferment dextrose will produce acids, thereby lowering the pH. A decreased pH results in the indicator changing from purple to yellow. The presence of acid also results in stimulation of enzyme activity. Once the enzyme has decarboxylated ornithine, the by-product diamine putrescine is produced. Production of putrescine causes an alkaline shift which turns the medium a dark purple. Organisms which do not produce decarboxylase remain acidic due to dextrose-fermentation, and the medium retains a yellow (acidic) color throughout or yellow with a purple band near the top of the tube.

FORMULA

Ingredients per liter of deionized water:*

Pancreatic Digest of Gelatin	10.0gm
Pancreatic Digest of Casein	10.0gm
L-Ornithine	5.0gm
Yeast Extract	3.0gm

Dextrose	1.0gm
Bromcresol Purple	0.02gm
Agar	2.0gm

Final pH 6.5 +/- 0.2 at 25ºC.

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

STORAGE AND SHELF LIFE

Upon receipt store at 2-30ºC away from direct light. Media should not be used if there are any signs of deterioration (shrinking, cracking, or discoloration), contamination, or if the expiration date has passed. Product is light and temperature sensitive; protect from light, excessive heat, moisture, and freezing.

The expiration date on the product label applies to the product in its intact packaging when stored as directed. The product may be used and tested up to the expiration date on the product label and incubated for the recommended incubation times as stated below.

Refer to the document "Storage" for more information.

PRECAUTIONS

This product may contain components of animal origin. Certified knowledge of the origin and/or sanitary state of the animals does not guarantee the absence of transmissible pathogenic agents. Therefore, it is recommended that these products be treated as potentially infectious, and handle observing the usual Universal Precautions for blood. Do not ingest, inhale, or allow to come into contact with skin.

This product is for *in vitro* diagnostic use only. It is to be used only by adequately trained and qualified laboratory personnel. Observe approved biohazard precautions and aseptic techniques. All laboratory specimens should be considered infectious and handled according to "standard precautions." Refer to the document "Guidelines for Isolation Precautions" from the Centers for Disease Control and Prevention.

For additional information regarding specific precautions for the prevention of the transmission of all infectious agents from laboratory instruments and materials, and for recommendations for the management of exposure to infectious disease, refer to CLSI document M29: *Protection of Laboratory Workers from Occupationally Acquired Infections.*

Sterilize all biohazard waste before disposal.

Refer to the document "Precautions When Using Media" for more information.

PROCEDURE

Specimen Collection: This medium is not intended for use as a primary isolation medium. It is used in characterizing pure cultures. Consult listed references for information regarding the processing and inoculation of specimens.[2-5]

Method of Use: Allow medium to warm to room temperature prior to inoculation. Using a straight needle, select isolated colonies from a pure 18-24 hour culture and stab the center of the medium to about one-half its length. Incubate the tubes aerobically at 35 degrees C. for 18-24 hours. Caps should be loose during incubation. Examine for motility and ornithine production. Record the above reactions then add a few drops of Kovacs Reagent (Cat. no. Z67) and observe for indole production.

INTERPRETATION OF RESULTS

Positive motility is denoted when turbidity or cloudy growth extends from the line of inoculation. Growth only along the stab line is indicative of a negative motility test.

A positive test for indole is denoted when a pink to red color band is formed at the top of the medium after addition of Kovacs Reagent. A yellow color denotes a negative indole test after addition of Kovacs Reagent.

A positive test for ornithine is denoted by a dark, turbid purple color in the medium. A yellow color throughout the medium denotes a negative ornithine result.

LIMITATIONS

It is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on colonies from pure culture for complete identification of bacteria and/or fungi.

Weakly motile organisms or organisms that possess damaged flagella (due to heating, shaking, or other trauma) often result in false-negative motility tests. Motility results may be confirmed by performing a hanging drop motility test. Consult listed references for procedure.[2-4,6]

Motility and Ornithine results must be interpreted prior to addition of Kovacs Reagent.

A purple color near the top of the tube may result due to oxidation.

Erroneous results may occur if caps are not loose during incubation.

Refer to the document "Limitations of Procedures and Warranty" for more information.

MATERIALS REQUIRED BUT NOT PROVIDED

Standard microbiological supplies and equipment such as loops, slides, coverslips, primary isolation media, incinerators, incubators, and microscopes, etc., as well as Kovacs Reagent (Cat. no. Z67) and other serological and biochemical reagents, are not provided.

QUALITY CONTROL

Hardy Diagnostics tests each lot of commercially manufactured media using appropriate quality control microorganisms and quality specifications as outlined on the Certificate of Analysis (CofA) and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media*. The following microorganisms are routinely used for testing at Hardy Diagnostics:

Test Organisms	Inoculation Method*	Incubation			Results
		Time	Temperature	Atmosphere	
Escherichia coli ATCC® 25922	D	18-48hr	35°C	Aerobic	Growth; Motility: positive, Indole: positive (turns pink after adding Kovacs Reagent), Ornithine: positive (purple throughout tube)
Klebsiella pneumoniae ATCC® 13883	D	18-48hr	35°C	Aerobic	Growth; Motility: negative, Indole: negative, Ornithine: negative (purple top layer, rest of tube yellow)

* Refer to the document "Inoculation Procedures for Media QC" for more information.

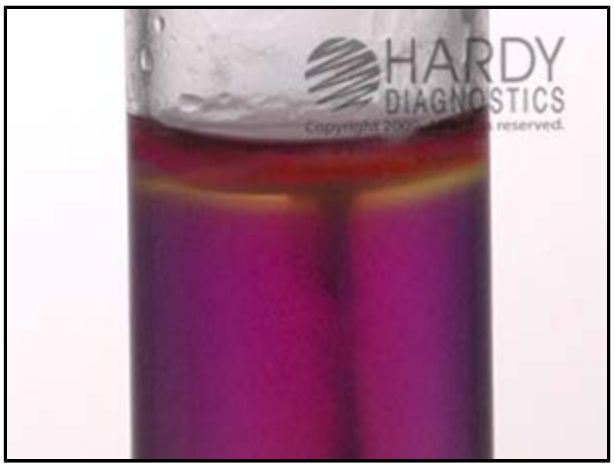
USER QUALITY CONTROL

End users of commercially prepared culture media should perform QC testing in accordance with applicable government regulatory agencies, and in compliance with accreditation requirements. Hardy Diagnostics recommends end users check for signs of contamination and deterioration and, if dictated by laboratory quality control procedures or regulation, perform quality control testing to demonstrate growth or a positive reaction and to demonstrate inhibition or a negative reaction, if applicable. Hardy Diagnostics quality control testing is documented on the certificate of analysis (CofA) available from Hardy Diagnostics Certificate of Analysis website. Also refer to the document "Finished Product Quality Control Procedures," and the CLSI document M22-A3 *Quality Assurance for Commercially Prepared Microbiological Culture Media* for more information on the appropriate QC procedures. See the references below.

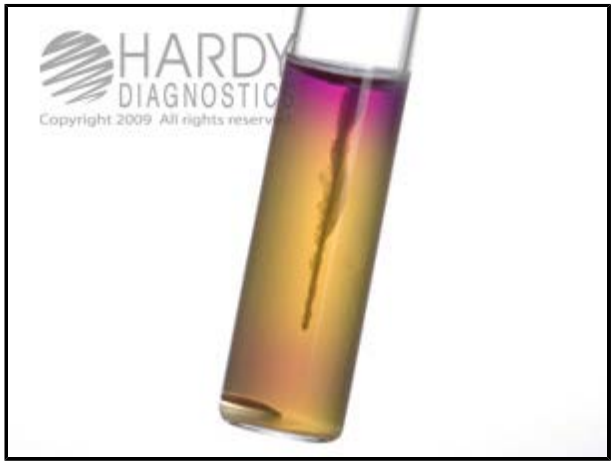
PHYSICAL APPEARANCE

MIO Medium should appear clear, semi-solid, and purple in color.

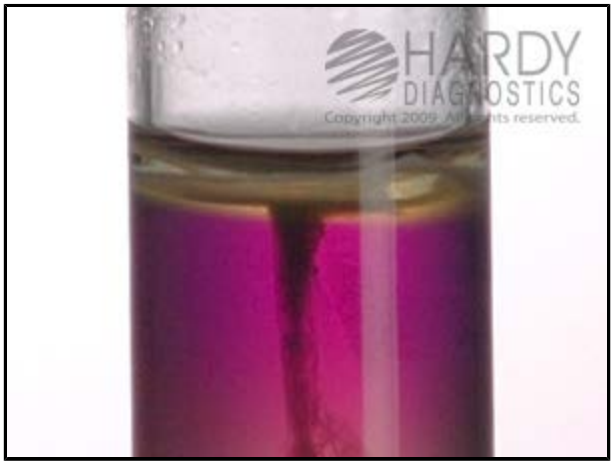
Escherichia coli (ATCC® 25922) growing in MIO Medium (Cat. no. Q20). Incubated aerobically for 24 hours at 35ºC. Showing positive motility and positive ornithine decarboxylation.



Escherichia coli (ATCC® 25922) growing in MIO Medium (Cat. no. Q20). Incubated aerobically for 24 hours at 35ºC. Five drops of Indole Kovacs Reagent (Cat. no. Z67) were added subsequent to incubation. The red color development is indicative of a positive indole reaction.



Klebsilla pneumoniae (ATCC® 13883) growing in MIO Medium (Cat. no. Q20). Incubated aerobically for 24 hours at 35ºC. Showing negative motility and negative ornithine decarboxylation.



Klebsilla pneumoniae (ATCC® 13883) growing in MIO Medium (Cat. no. Q20). Incubated aerobically for 24 hours at 35ºC. Five drops of Indole Kovacs Reagent (Cat. no. Z67) were added subsequent to incubation. No red color development is indicative of a negative indole reaction.

Uninoculated tube of MIO Medium (Cat. no. Q20).

REFERENCES

1. Anderson, N.L., et al. *Cumitech 3B; Quality Systems in the Clinical Microbiology Laboratory*, Coordinating ed., A.S. Weissfeld. American Society for Microbiology, Washington, D.C.

2. Jorgensen., et al. *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, D.C.

3. Tille, P., et al. *Bailey and Scott's Diagnostic Microbiology*, C.V. Mosby Company, St. Louis, MO.

4. Koneman, E.W., et al. *Color Atlas and Textbook of Diagnostic Microbiology*, J.B. Lippincott Company, Philadelphia, PA.

5. Isenberg, H.D. *Clinical Microbiology Procedures Handbook*, Vol. I, II & III. American Society for Microbiology, Washington, D.C.

6. MacFaddin, J.F. *Biochemical Tests for Identification of Medical Bacteria*,, Lipincott Williams & Wilkins, Philadelphia, PA.

7. Ederer and Clark. 1970. *Appl. Microbiol.*; 2:849.

IFU-10537[B]

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The Hardy Diagnostics manufacturing facility and quality management system is certified to ISO 13485.

HDQA 2207F [D]