MIO MEDIUM and MI CONTROL MEDIUM

INTENDED USE

Remel MIO (Motility Indole Ornithine) Medium and MI (Motility Indole) Control are semisolid media recommended for use in qualitative procedures for differentiation of enteric gram-negative bacilli on the basis of motility, production of indole, and ornithine decarboxylase.

SUMMARY AND EXPLANATION

Ederer and Clark developed MIO Medium in 1970 for identification of enteric gram-negative bacilli.[1] MIO Medium combines three tests into a single tube to facilitate inoculation and identification of *Enterobacteriaceae*. Oberhofer and Hajkowski further investigated the use of MIO Medium for nonlactose-fermenting members of the *Klebsiella-Enterobacter-Serratia* group of organisms.[2]

PRINCIPLE

Peptone and tryptone supply nitrogenous compounds and amino acids necessary for the growth of enteric gram-negative bacilli. Yeast extract is a source of B-complex vitamins and dextrose is an energy source. Agar is added to enable detection of motility. Brom cresol purple is a pH indicator that is purple at the initial pH of the medium. Enteric gram-negative bacilli ferment dextrose producing acid which changes the pH indicator color to yellow (tube butt) and stimulates enzyme activity. Organisms possessing the enzyme ornithine decarboxylase convert ornithine to putrescine which is alkaline. This causes the pH indicator to revert to purple. Organisms which do not possess the enzyme produce a yellow color in the butt of the tube (acidic). Tryptophanase, an enzyme produced by some enteric gram-negative bacilli, degrades tryptophan present in the medium to form indole. This reaction is detected by adding Kovacs' Reagent to the surface of the medium. Indole combines with ρ-dimethylaminobenzaldehyde (Kovacs' Reagent) to produce a red complex. MI Control Medium contains dextrose and brom cresol purple. At the initial pH of the medium, the color is purple. Enteric gram-negative bacilli ferment dextrose which produces acid and turns the pH indicator to yellow.

REAGENTS (CLASSICAL FORMULA)*

MI Control:

Peptone	10.0	g	Dextrose	1.0	g
Tryptone	10.0	g	Brom Cresol Purple	20.0	mg
Yeast Extract	3.0	g	Agar	2.0	g
			Demineralized Water	1000.0	ml

pH 6.6 ± 0.2 @ 25°C

MIO Medium:

Peptone	10.0	g	Dextrose	1.0	g
Tryptone	10.0	g	Brom Cresol Purple	20.0	mg
L-Ornithine HCl	5.0	g	Agar	2.0	g
Yeast Extract	3.0	g	Demineralized Water	1000.0	ml

pH 6.5 ± 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PROCEDURE

Note: To improve the consistency of this medium for detection of motility, gently heat tubes in a boiling water bath with caps loosened. Allow media to cool and re-solidify in an upright position, prior to inoculation.

1. Inoculate MIO Medium and MI Control from a pure, 18-24 hour culture of the test isolate. Using an inoculating needle, stab down the center of the medium to within ¼ inch of the bottom of the tube.
2. Incubate the tubes aerobically with caps loosened at 33-37°C for 18-24 hours.
3. Examine the MI Control Medium for development of a yellow color indicating dextrose has been fermented by the test isolate.
4. Examine the MIO Medium for motility and ornithine decarboxylation as described under Interpretation.
5. To detect indole production, add 3-4 drops of Kovacs' Reagent (REF R21227) to the MIO Medium tube and observe for a pink to red color development.

INTERPRETATION OF THE TEST

MI Control, Dextrose Fermentation:
Positive Test - Yellow color development
Negative Test - No color development, test invalid

MIO Medium, Motility:
Positive Test - Growth diffusing outward from the stab line of inoculation
Negative Test - Growth along the stab line, only

MIO Medium, Indole:
Positive Test - Pink to red color development in the upper layer of the medium after the addition of Kovacs' Reagent
Negative Test - Yellow color in the upper layer of the medium after the addition of Kovacs' Reagent

MIO Medium, Ornithine:
Positive Test - Deep purple color development (alkaline) throughout the medium
Negative Test - Yellow color development throughout the medium or no change in the color

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QUALITY CONTROL

All lot numbers of MI Control and MIO Medium have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, patient results should not be reported.

CONTROL	INCUBATION	RESULTS
MI Control:		
Escherichia coli ATCC® 25922	Aerobic, 18-24 h @ 33-37°C	Dextrose (+)
Klebsiella pneumoniae ATCC® 27736	Aerobic, 18-24 h @ 33-37°C	Dextrose (+)
MIO Medium:		
Escherichia coli ATCC® 25922	Aerobic, 18-24 h @ 33-37°C	Motility (+), Indole (+), Ornithine (+)
Klebsiella pneumoniae ATCC® 27736	Aerobic, 18-24 h @ 33-37°C	Motility (-), Indole (-), Ornithine (-)

LIMITATIONS

1. Interpret the motility and ornithine results before adding Kovacs' Reagent to interpret the indole reaction.[3]

BIBLIOGRAPHY

1. Ederer, G.M. and M. Clark. 1970. Appl. Microbiol. 20:849-850.
2. Oberhofer, T.R. and R. Hajkowski. 1970. Am. J. Clin. Pathol. 54:720-725.
3. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.

Refer to the front of Remel *Technical Manual of Microbiological Media* for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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