DECARBOXYLASE MEDIA

PRODUCTS:

Tubed Media:[a]

Moeller Decarboxylase Broth (Control)	T6945
Moeller Arginine Dihydrolase Broth	T6940
Moeller Lysine Decarboxylase Broth	T6950
Moeller Ornithine Decarboxylase Broth	T6955
Moeller Decarboxylase Agar (Control)	T3310
Moeller Ornithine with Agar	T3313,T6956
Moeller Arginine Agar	T3311
Moeller Lysine Agar	T3312

[a]see catalog for ordering options

PURPOSE:

The decarboxylase tests are used primarily to aid in the identification of organisms within the family *Enterobacteriaceae,* but may be used to differentiate other gram-negative bacilli as well.

PRINCIPLE:

Moeller[6] discovered the first practical application of amino acid decarboxylation as a means of identifying different members of the family *Enterobacteriaceae.* The decarboxylase test detects the enzymatic ability of an organism to decarboxylate an amino acid to form an amine, which results in alkaline by-products in the media and a pH change.[7] Bacteria that possess specific decarboxylase enzymes are capable of attacking amino acids, yielding an amine, or diamine, and carbon dioxide. Decarboxylases are induced enzymes and are formed only in an acid environment and in the presence of a specific substrate containing the amino acid. In the test, the organism is grown in a media containing a specific amino acid and dextrose, and is overlaid with mineral oil to create an anaerobic environment. All unbound oxygen is utilized by the organism in the growth phase, and an acid environment is created due to the fermentation of dextrose. The reduced pH induces the decarboxylase enzyme if present in the organism. Decarboxylation takes place, and the pH shifts to the alkaline range as amines and NH_3 are produced. This causes the pH indicator to change to a purple or gray-purple color. Organisms, which do not possess the enzyme, will utilize the dextrose, dropping the pH to acid, changing the indicator to yellow.

The breakdown of arginine is a two-step process involving two enzyme systems. Arginine is first broken down by a dihydrolase enzyme. An NH_3 group is removed from arginine to form citrulline. Citrulline is broken down further to ornithine, which is then decarboxylated by the other enzyme system to the final end products that result in an alkaline pH.

FORMULAS:

Approximate, per liter deionized filtered water.

(1) Moeller Decarboxylase Broth (Control):

Peptic Digest of Animal Tissue	5.00 g
Beef Extract	5.00
Bromcresol Purple	0.01
Dextrose	0.50
Cresol Red	5.00 mg
Pyridoxal	5.00

Final pH 6.0 ± 0.2 at 25°C

(2) Moeller Arginine Dihydrolase Broth:
Same as **(1)** with the addition of 10.0 g of L-Arginine HCl.

(3) Moeller Lysine Decarboxylase Broth:
Same as **(1)** with the addition of 10.0 g of L-Lysine HCl.

(4) Moeller Ornithine Decarboxylase Broth:
Same as **(1)** with the addition of 10.0 g of L-Ornithine HCl.

(5) Moeller Decarboxylase Agar (Control):
Same as **(1)** with the addition of 3.0 g of Agar
Final pH 6.0 ± 0.2 at 25°C

(6) Moeller Ornithine with Agar:
Same as **(4)** with the addition of 3.0 g of Agar.

(7) Moeller Arginine Agar:
Same as **(5)** with the addition of 10.0 g of L-Arginine HCl.

(8) Moeller Lysine Agar:
Same as **(5)** with the addition of 10.0 g of L-Lysine HCl.

(9) Moeller Ornithine Agar:
Same as **(5)** with the addition of 10.0 g of L-Ornithine HCl.

PRECAUTIONS:*

For *in vitro* diagnostic use. Observe approved biohazard precautions.

Storage: Upon receipt store at 2-8°C away from direct light. Media should not be used if there are signs of contamination, deterioration (shrinking, cracking, evaporation, or discoloration), or if the expiration date has passed.

Limitations: Results of the Moeller decarboxylation tests should not be interpreted until the tubes/plates have been incubated at least 18-24 hours. Earlier interpretations may give a false-negative result. Within the first 10-12 hours of incubation, glucose is fermented, which results in a yellow color due to acid by-products; only after glucose is fermented will the decarboxylase activity be initiated.

A decarboxylase test may show two layers of different colors in the broth, yellow and purple, after standing. Shake the tube gently before attempting to interpret the results.

A positive decarboxylase tube test may be difficult to interpret due to the presence of an indistinct yellow-purple color. If this occurs, always compare with an uninoculated tube. Any trace of purple color denotes a positive test if the tube has been incubated at least 24 hours.

Some organisms may be slow in decarboxylation and may require an increased incubation of up to 10 days.

The incorporation of agar into the media eliminates the need for the use of mineral oil.

A pH rise can occur independently of decarboxylation in this media, probably due to oxidation, which may produce a false-positive result. Mineral oil must be added to prevent this phenomenon.[6] In plated media, which must be incubated anaerobically, the reading of test results must be within 5 minutes of removing the plates from the anaerobic jar; false-positive test results are produced from excessive exposure to air.

These tests rely on the ability of the organism to ferment glucose to initiate decarboxylation. Any organism which does not have the ability to utilize glucose in the absence of oxygen may not give reliable test results. The control tube containing no amino acid should remain yellow if the organism being tested ferments dextrose after 18-24 hours of incubation. An uninoculated purple-colored control tube invalidates all the amino acid decarboxylase tests, and no interpretation should be made in this case.

Other biochemical tests must be performed to secure a complete identification for the organisms being tested.

PROCEDURE:*

Specimen Collection: Not applicable since these media are not for primary isolation. These media are used in characterizing pure cultures. Isolated organisms, established isolation techniques, and tests for purity are necessary before inoculating these media. Direct inoculation of specimens will produce erroneous results. Information on specimen collection may be found in standard reference texts.

Method of Use, Broth: Prior to inoculation, the media should be brought to room temperature. Lightly inoculate each culture media with organisms taken from a pure 18 to 24 hour culture (TSI or broth culture such as Tryptone or Trypticase Soy Broth).[6] A control tube without an amino acid should always be inoculated with each test run.[1,2] **Overlay all broth tubes**, including the control, with 2-3 ml of sterile mineral oil.[6] Incubate at 35°C for 18 hours to 4 days. Examine daily. Prolonged incubation from 6-10 days or longer may be required to demonstrate weak reactions due to an organism's delayed decarboxylation activity.

Method of Use, Agar: Touch a well-isolated colony or broth culture with a straight wire and stab the media to the bottom of the tube. **Do not overlay with mineral oil.** Incubate aerobically at 35°C for 18-24 hours.

Interpretation: With Moeller broth or agar plates, all amino acids give the same colors in their reactions:

Positive Test:	Purple to a faded out yellow-purple color.
Negative Test:	Bright yellow color for dextrose fermenters, little or no color change in comparison to uninoculated tube for nonfermenters.

Materials Required but Not Provided: Standard microbiological supplies and equipment such as those commonly found in a microbiological laboratory are not provided.

QUALITY CONTROL:*

Microorganisms Used (ATCC #):

	Expected Results:			
	Control	Arginine	Lysine	Ornithine
Klebsiella pneumoniae (13883)	(-)	(-)	(+)	(-)
Enterobacter cloacae (13047)	(-)	(+)	(-)	(+)

Key: See “Interpretation”

User Quality Control: Check for signs of contamination and deterioration. Moeller Decarboxylase broth media should appear as a clear liquid and olive-tan in color. Prepared plated media should appear moderately firm, translucent, and olive-tan in color.

BIBLIOGRAPHY:

1. Edwards, P. R., and W. H. Ewing, *Identification of Enterobacteriaceae*, 3rd ed., Burgess Publishing, Minneapolis, 1972.
2. Kauffmann, F., and V. Moeller, *Acta Pathol. Microbiol. Scand.*, *36*:173, 1955.
3. Koneman, E. W., et al., *Color Atlas and Textbook of Diagnostic Microbiology,* 2nd ed., J. B. Lippincott, Philadelphia, 1983.
4. Lennette, E. H., et al., *Manual of Clinical Microbiology*, 4th ed., American Society for Microbiology, Washington, D. C., 1985.
5. MacFaddin, J. F., *Biochemical Tests for Identification of Medical Bacteria,* 2nd ed., Williams and Wilkins, Baltimore, 1980.
6. Moeller, V., *Acta Pathol. Microbiol. Scand.*, *36*:158, 1955.
8. Smith, D. T., N. F. Conant, and H. P. Willett, *Zinsser Microbiology,* 14th ed., Appleton-Crofts, New York, 1968.

*For more detailed information, consult appropriate references.

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