Moeller Decarboxylase Broth with Ornithine, Lysine or Arginine

I. PRINCIPLE

Decarboxylases are a group of substrate-specific enzymes that are capable of reacting with the carboxyl (COOH) portion of amino acids, forming alkaline-reacting amines. Each decarboxylase enzyme is specific for an amino acid. Lysine, ornithine, and arginine are the three amino acids used routinely in the identification of *Enterobacteriaceae*, *Aeromonas, Plesiomonas* and *Vibrio* species. The decarboxylation of lysine and ornithine yield cadaverine and putrescine, respectively. Arginine is converted to citrulline by a dihydrolase reaction. All decarboxylase tests must be accompanied by a control tube containing the base without an added amino acid to verify that the organism utilizes glucose. Since decarboxylation is an anaerobic reaction, the contents of each tube must be overlaid with mineral oil prior to incubation. If the organism is viable, both the control and the test tube with amino acid should turn yellow because of fermentation of the small amount of glucose in the medium. If the amino acid is decarboxylated, the alkaline amines cause the indicator (bromcresol purple) in the acid medium to revert to its original purple color.

II. REAGENTS AND MATERIALS

A. Moeller decarboxylase base
B. Moeller decarboxylase broth with ornithine, lysine or arginine
C. Sterile inoculating loop or needle
D. Mineral oil

III. QUALITY CONTROL

A. Quality control is performed on each new lot or shipment received and includes the following organisms and their expected reactions:
    1. Ornithine decarboxylase
        E. cloacae ATCC 13047 = positive (purple)
        K. pneumoniae ATCC 13882 = negative (yellow)
    2. Lysine decarboxylase
        Klebsiella pneumoniae ATCC 13882 = positive (purple)
        E. cloacae ATCC 13047 = negative (yellow)
    3. Arginine dihydrolase
        Enterobacter cloacae ATCC 13047 = positive (purple)
        K. pneumoniae ATCC 13882 = negative (yellow)
B. If controls do not display expected results, quality control must be repeated. Notify the supervisor.

IV. PROCEDURE

A. Organisms with the ability to ferment glucose (fermenters)
    1. Using a sterile inoculating loop, use one colony to inoculate a Moeller decarboxylase base as a control.
    2. Inoculate a Moeller decarboxylase broth containing ornithine, lysine and/or arginine in the same manner, using a sterile loop or needle for each broth.
    3. Overlay the contents of all tubes with 1 ml of sterile mineral oil.
    4. Incubate in a non-CO_2 incubator at 35°C for 18-24 hr.

5. Examine for a color change. Negative reactions are examined daily for no more than 4 days.

B. Organisms that do not ferment glucose (non-fermenters)
   1. Using a sterile inoculating loop, prepare a very heavy suspension, using fresh growth (18-24h) from a blood agar plate to inoculate a Moeller decarboxylase base as a control.
   2. Inoculate one each of Moeller decarboxylase broth containing ornithine, arginine and/or lysine in the same manner, using a sterile loop or needle for each broth.
   3. Overlay the contents of all tubes with 1 mL of sterile mineral oil.
   4. Incubate in a non-CO_2 incubator at 35°C for 18-24 h.
   5. Examine for a color change. Negative reactions are examined daily for no more than 6 days.

V. INTERPRETATION

A. Control tube = remains its original color or turns yellow if the organism is a glucose fermenter (turbidity must be apparent in the tube)

B. Fermenters:

	Control*	**Ornithine, Lysine, Arginine**
Positive	yellow (acid)	turbid purple to faded yellow purple
Negative	yellow (acid)	yellow

C. Non fermenters:

	Control*	**Ornithine, Lysine, Arginine**
Positive	No color change	turbid purple to faded yellow purple
Negative	No color change	No color change

* Turbidity must be apparent

VI. LIMITATIONS AND PRECAUTIONS

A. A control tube containing only the basal medium must be inoculated each time the test is performed.

B. If the reaction is difficult to interpret, compare the tube with an uninoculated tube. After 24 h incubation, any trace of purple denotes a positive test.

C. If oil is not added, the reactions cannot be interpreted.

VII. REFERENCES

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