



BBL™ Moeller Decarboxylase Broth Base
BBL™ Moeller Decarboxylase Broth with Arginine

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QUALITY CONTROL PROCEDURES

I INTRODUCTION

Moeller Decarboxylase Broth Base, when supplemented with arginine, aids in the differentiation of enteric bacilli by means of a dihydrolase reaction.

II PERFORMANCE TEST PROCEDURE

1. Inoculate representative samples with the cultures listed below.
 - a. Inoculate lightly the broth tubes using 0.01 mL calibrated loops with growth from 18- to 24-h **Trypticase™** Soy Agar Slant cultures. Include tubes of Moeller Decarboxylase Broth Base without an amino acid as growth controls for all organisms.
 - b. Overlay all tubes with 1.0 - 2.0 mL of sterile mineral oil.
 - c. Incubate tubes with tightened caps at 35 ± 2°C.
2. Examine tubes after 24, 48, 72 and 96 h for reactions.
3. Expected Results

Organisms	ATCC™	Base	Arginine
* <i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	33495	-	-
* <i>Enterobacter cloacae</i>	13047	-	+

Note: + = positive reaction (alkaline, purple or red)
 - = negative reaction (acid, yellow)

*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. Slight color variations may be observed within a lot. This color variation does not affect performance.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 6.0 ± 0.2.
4. 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

Moeller Decarboxylase Broth with Arginine is used in the biochemical differentiation of gram-negative enteric bacilli based on the production of arginine dihydrolase.

V SUMMARY AND EXPLANATION

In 1955, Moeller introduced the decarboxylase media for detecting the production of lysine and ornithine decarboxylase and arginine dihydrolase.¹ These media are a useful adjunct to other biochemical tests for the speciation and identification of the *Enterobacteriaceae* and other gram-negative bacilli.²⁻⁴

VI PRINCIPLES OF THE PROCEDURE

Moeller Decarboxylase basal medium consists of peptone and beef extract to supply the nitrogenous nutrients necessary to support bacterial growth. Pyridoxal is an enzyme co-factor for the amino acid decarboxylase. Dextrose is a fermentable carbohydrate. Bromcresol purple and cresol red are pH indicators. The amino acid arginine is added to the basal medium to detect the production of the enzyme specific for this substrate.

When the medium is inoculated with a bacterium that is able to ferment dextrose, acids are produced that lower the pH of the medium and change the color of the indicator from purple to yellow. The acidic condition also stimulates decarboxylase activity. If the organism produces the appropriate enzyme, the amino acid in the medium is degraded, yielding a corresponding amine. Arginine is first hydrolyzed to form ornithine, which is then decarboxylated to form putrescine. The production of this amine elevates the pH of the medium, changing the color of the indicator from yellow to purple or violet. If the organism does not produce the appropriate enzyme, the medium remains acidic (yellow). Consult the reference for more information.⁵

Each isolate to be tested must also be inoculated into a tube of the basal medium, which does not contain the amino acid. If this tube becomes alkaline, the test is invalid.

To obtain the appropriate reactions, the inoculated tubes must be protected from air with a layer of sterile mineral oil. Exposure to air may cause alkalization at the surface of the medium, which could cause a decarboxylase-negative organism to appear positive.

VII REAGENTS

Moeller Decarboxylase Broth Base

Approximate Formula* Per Liter Purified Water

Peptic Digest of Animal Tissue	5.0 g	Cresol Red	0.005 g
Beef Extract	5.0 g	Dextrose	0.5 g
Bromcresol Purple	0.01 g	Pyridoxal	0.005 g

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

Moeller Decarboxylase Broth with Arginine consists of Moeller Decarboxylase Broth Base with 10.0 g/L of arginine.

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 2–8°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. Allow the medium to warm to room temperature before inoculation.

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.^{3,6} Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Moeller Decarboxylase Broth Base or Moeller Decarboxylase Broth with Arginine

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

Test Procedure: Observe aseptic techniques.

Subculture the isolate to be tested onto a suitable medium, streaking to obtain isolated colonies, and incubate at 35 ± 2°C for 18–24 h.

Inoculate the broth media by transferring one or two colonies from the surface of a fresh culture with an inoculating loop or needle and mix to distribute the culture throughout the medium. Include tubes of Moeller Decarboxylase Broth Base without an amino acid as growth controls for all organisms. Overlay the medium in each tube with 1- 2 mL sterile mineral oil.

Incubate the tubes with caps tightened at 35 ± 2°C. Examine for growth and decarboxylase reactions after 18–24, 48, 72 and 96 h before reporting as negative. The medium will become yellow initially, if the dextrose is fermented, and then will gradually turn purple if the dihydrolase reaction occurs and elevates the pH.

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 (formerly NCCLS) guidance and CLIA regulations for appropriate Quality Control practices.

A single electrode of sufficiently small size to fit into the tubes should be used to determine the pH potentiometrically of tubed media. The tip of the electrode should be placed below the surface of broth media.

X RESULTS

Compare the color of tubes of medium containing arginine with the color of control tubes of the basal medium (without arginine) that have been inoculated with the same isolate. If inoculated control tubes show an alkaline reaction, the test is invalid, i.e., either improperly performed or the test organisms can degrade the peptone sufficiently to produce an alkaline reaction in the absence of arginine.

The medium becomes purple to violet if the reaction is positive (alkaline). A yellow color indicates a negative test, i.e., the organism does not produce the appropriate enzyme.

Consult appropriate texts for information needed to interpret the results.³⁻⁶

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.^{3,6,7}

XII AVAILABILITY

Cat. No.	Description
221731	BBL™ Moeller Decarboxylase Broth Base, 5 mL, Pkg. of 10 size K tubes
221659	BBL™ Moeller Decarboxylase Broth with Arginine, 5 mL, Pkg. of 10 size K tubes
221660	BBL™ Moeller Decarboxylase Broth with Arginine, 5 mL, Ctn. of 100 size K tubes

XIII REFERENCES

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