# BBL™ Moeller Decarboxylase Broth with Lysine
# BBL™ Moeller Decarboxylase Broth with Ornithine

**L007470 • Rev. 10 • January 2015**

## QUALITY CONTROL PROCEDURES

### I INTRODUCTION

**BD BBL™** Moeller Decarboxylase Broth Base, when supplemented with lysine or ornithine, aids in the differentiation of enteric bacilli by means of decarboxylase reactions.

### 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 **BD 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 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™ | Lysine | Ornithine |
|---|---|---|---|
| **Klebsiella pneumoniae* subsp. *pneumoniae* | 33495 | + | – |
| **Enterobacter cloacae* | 13047 | – | + |

**Note:** + = positive reaction (alkaline, purple or red)
– = negative reaction (acid, yellow)
The basal medium is negative with both organisms.
*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

**BD BBL** Moeller Decarboxylase media are used in the biochemical differentiation of gram-negative enteric bacilli based on the production of lysine and ornithine decarboxylase.

### V SUMMARY AND EXPLANATION

In 1955, Moeller introduced the decarboxylase media for detecting the production of lysine and ornithine decarboxylase.[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] The production of ornithine decarboxylase is particularly useful for differentiating *Klebsiella* and *Enterobacter* species. *Klebsiella* species are non-motile and, except for *K. ornithinolytica*, do not produce ornithine decarboxylase, while most *Enterobacter* species are motile and, except for *E. agglomerans*, usually produce this enzyme.[4]

### VI PRINCIPLES OF THE PROCEDURE

**BD BBL** 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 acids lysine and ornithine are added to the basal medium to detect the production of the enzyme specific for these substrates.

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. Decarboxylation of lysine yields cadaverine, while decarboxylation of ornithine yields putrescine. The production of these amines 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 alkalinization at the surface of the medium, which could cause a decarboxylase-negative organism to appear positive.

## VII REAGENTS

**BD BBL 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.

**BD BBL** Moeller Decarboxylase Broth with Lysine or Ornithine consists of **BD BBL** Moeller Decarboxylase Broth Base with 10.0 g/L of the amino acid specified.

**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: BD BBL** Moeller Decarboxylase Broth with Lysine or **BD BBL** Moeller Decarboxylase Broth with Ornithine

**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. Overlay the medium in each tube with 1 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 decarboxylase 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 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 media containing the specific amino acids with the color of control tubes of basal media (without amino acid) 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 a specific amino acid.

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.[3,4,6,7]

## XII PERFORMANCE CHARACTERISTICS

In a study by Yabuuchi, Yamanaka and Ohyama, to develop a new profile system for identification of nonfermenters in conjunction with the Minitek System, **BD BBL** Moeller Decarboxylase media were used as controls. Six hundred twenty-five (625) strains of nonfermenters were tested. Agreement between **BD BBL** Moeller Decarboxylase with Lysine and the Minitek System was 95.4%. Agreement between **BD BBL** Moeller Decarboxylase with Ornithine and the Minitek System was 90.9%.[8]

In another study, Westbrook et al. used **BD BBL** Moeller Decarboxylase with Ornithine as part of an identification scheme to determine the incidence of *Klebsiella planticola* and *K. oxytoca* in 352 stock isolates and 84 fresh clinical specimens of *Klebsiella* sp.[9]

## XIII AVAILABILITY

| Cat. No. | Description |
|---|---|
| 221661 | **BD BBL™** Moeller Decarboxylase Broth with Lysine, Pkg. of 10 size K tubes |
| 221662 | **BD BBL™** Moeller Decarboxylase Broth with Lysine, Ctn. of 100 size K tubes |
| 221663 | **BD BBL™** Moeller Decarboxylase Broth with Ornithine, Pkg. of 10 size K tubes |
| 221664 | **BD BBL™** Moeller Decarboxylase Broth with Ornithine, Ctn. of 100 size K tubes |

## XIV REFERENCES


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