

## Decarboxylase Broth Protocol

Created: Wednesday, 02 September 2015

Author

- Archana Lal
- Naowarat Cheeptham

### Information HISTORY

The decarboxylase media, used to identify bacteria's ability to decarboxylate amino acids, were first introduced by Moeller (12) for detecting lysine and ornithine decarboxylase and arginine dihydrolase among bacteria belonging to Enterobacteriaceae. The Moeller's basal media contained peptone, beef extract, bromocresol purple, cresol red, pyridoxal, and glucose. To test decarboxylase activity, 1% of the appropriate amino acid was added. The medium was poured in narrow tubes as a column of about 2 cm in height and autoclaved, after which a layer of about 5 mm sterile paraffin oil was poured in each tube. The decarboxylase activity was measured on the basis of a pH rise of the amino acid reagent that was made visible by an indicator. After incubation the color changes in the tubes were followed for up to 10 days (13).

Utilizing the lysine decarboxylase reaction, Calquist developed a medium to differentiate *Salmonella arizonae* from *Citrobacter* (4). Falkow developed a lysine decarboxylase medium for the identification and differentiation of *Salmonella* and *Shigella* (7). Falkow's medium was further extended to examine ornithine and arginine decarboxylase reactions (5).

Gilardi suggested that these tests may also be used in identification of various non-fermentative Gram-negative bacilli (10 & 11). All of the above methods for detecting ornithine decarboxylase activity required up

*vulgaris* and *P. rettgeri* are ornithine decarboxylase negative (6)). With slight modification of Fay & Barry's medium, Brooker et al. detected lysine decarboxylase in various members of Enterobacteriaceae (2). Similar to Fay & Barry's ornithine decarboxylase media, Brooks & Sodeman (1974) published a technique to rapidly assay for lysine and arginine decarboxylase and arginine dihydrolase (3). They compared the rapid test of Fay & Barry with the Moeller method to test several species of the Enterobacteriaceae and Gram -negative, non-fermenting rod-shaped bacteria and found close agreement between the two methods. Oberhofer et al. evaluated the rapid decarboxylase and dihydrolase test and reported them to be accurate, extremely sensitive, and specific for the differentiation of a wide range of non-fermentative bacteria (15).

## THEORY

The decarboxylase test has long been used to determine decarboxylase production and amino acid decarboxylation in bacteria particularly in the family of Enterobacteriaceae and *Aeromonas* species (1 & 3). This simple test is able to measure the ability of bacteria to produce decarboxylase or dihydrolase that are used to remove carboxyl group from amino acids resulting in the formation of amines. Decarboxylases remove the carboxyl group from amino acids like lysine and ornithine while arginine decarboxylation occurs with the help of dihydrolase in addition to decarboxylase (Figs 1, 2, and 3).

Decarboxylase medium contains beef extract and peptic digests of animal tissues that provide nitrogenous nutrients for the growth of the organisms. Glucose in the medium is a fermentable carbohydrate. Bromocresol purple and cresol red are the pH indicators that change colors under acidic and alkaline conditions and help in the detection of glucose fermentation and amino acid decarboxylation. Pyridoxal is the co-factor for the decarboxylase (9). Decarboxylation and dihydrolation are anaerobic reactions therefore



## RECIPE

### Moeller's Decarboxylase Broth Composition (g/liter) (14)

#### Base broth

Peptone	5 g
Beef Extract	5 g
Glucose	0.5 g
Bromocresol Purple	0.01 g

Pyridoxal 255.24 Tm [(Py)1(r)4(i)-16(d)-4(o)4(x)1(a)-2(l)]TJ 0 Tc -0.002 Tw 4.614 0 Td [( )1( )1

boil. When L - ornithine is added, re -adjust the pH. Final pH of the medium should be  $6.0 \pm 0.2$  at  $25^{\circ}\text{C}$ .

Dispense in 5 ml quantities in screw -cap tubes (12.5 cm x 1.5 cm tubes for a column height of about 3.5 cm).

Autoclave the medium at  $121^{\circ}\text{C}$  for 15 min at 15 psi. Prepared broth is light brown in color. It should be stored below  $8^{\circ}\text{C}$  away from the direct light.

Decarboxylase broth is also commercially available as pre -mixed dehydrated powder from biological supply companies. The manufacturer's instructions should be followed to prepare the broth. It can also be purchased as pre -made broth from biological supply companies. Sterilized mineral oil can also be purchased from biological supply companies. (Please see comments and tips)

#### PROTOCOL

##### Inoculation:

Use a fresh (18 - to 24 -hour) pure culture of test bacteria as an inoculation source. Use a single isolated colony as inoculum and aseptically transfer it to the decarboxylase broth tube with one of the amino acid. Inoculate a control tube of decarboxylase broth base (without amino acid) in parallel with the amino acid containing decarboxylase broth (as a control).

After inoculation, overlay 1 ml of sterile mineral oil to each tube to separate the medium from external oxygen and promote fermentation of glucose and decarboxylation reactions and tighten the caps on the test tubes.

##### Incubation:

Incubate tubes at  $37^{\circ}\text{C}$  in an incubator. Check the tubes every day for color changes for up to 4 days. Final readings should be made after 4 days of inoculation. For some microorganisms, increased incubation for up to 10 days may be required.

##### Decarboxylase Test Interpretations:

Check the color of the medium (every day for up to 10 days) for signs of fermentation and decarboxylation.

Uninoculated Moeller's base media and media with an amino acid are \_\_\_\_\_

If there is no change in the c.434 0 9.96 167.16 283.08 Tm [(d)-7(3(039d)-7(3r))-8(i)- the dwhel

If the color of the media changes to purple \_\_\_\_\_ it indicates decarboxylation of amino acid and formation of amine (alkaline by \_\_\_\_\_ products) by the test organism meaning that the organism is decarboxylase positive (+) for that amino acid. The lack of a purple color indicates that amino acid has not been decarboxylated and the organism did not produce the decarboxylase enzyme.

Table 1: Summary of result interpretations

Media color	Bacterial reaction
No Change (Media remains light brown)	Decarboxylase negative (-)
Changes to yellow but does not change to purple	Decarboxylase negative (-)
Changes to purple	Decarboxylase positive (+)

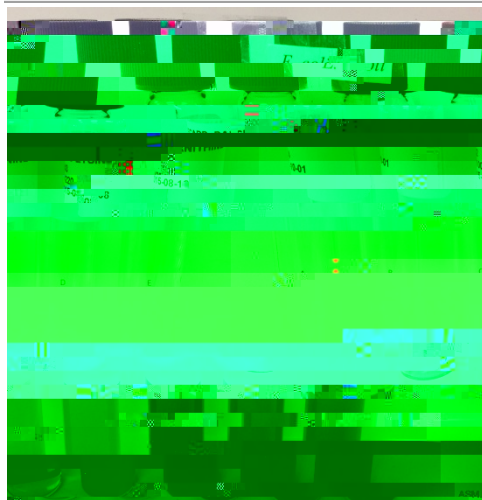


FIG 4 Moeller's Decarboxylase media. A: Uninoculated base, B: Base inoculated with *E. coli*, C: Arginine broth inoculated with *E. coli*, D: Lysine broth inoculated with *E. coli*, E: Ornithine broth inoculated with *E. coli*. Purple color in tubes C, D, and E indicate that this strain of *E. coli* is arginine, lysine, and ornithine decarboxylase positive.

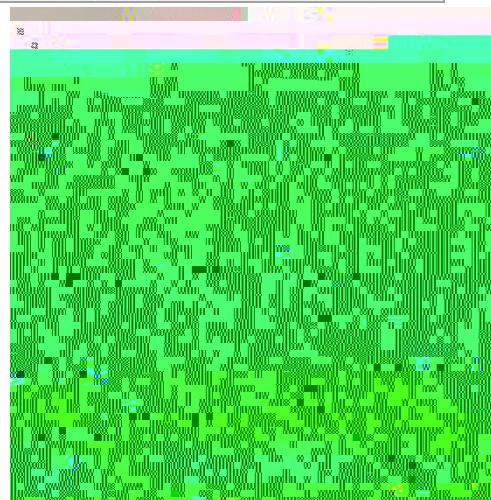


FIG 5 Moeller's Decarboxylase media. A: Uninoculated base, B: Base inoculated with *Enterobacter cloacae*, C: Arginine broth inoculated with *E. cloacae*, D: Lysine broth inoculated with *E. cloacae*, E: Ornithine broth inoculated with *E. cloacae*. Purple color in tubes C and E indicate that *E. cloacae* is arginine and ornithine decarboxylase positive.

while yellow color in tube D  
(absence of purple color) indicates  
that it is lysine decarboxylase  
negative.

FIG 6 Moeller's Decarboxylase  
media. A: Uninoculated base, B:  
Base inoculated with *Klebsiella*  
*oxytoca*, C: Arginine broth  
inoculated with *K. oxytoca*, D:  
Lysine broth inoculated with

3.	Klebsiella pneumoniae	1 day	-	+	-
4.	Klebsiella oxytoca	1 day	-	+	-

#### ACKNOWLEDGEMENTS

Thanks to Dr. Norman Reed, Associate Professor of Organic Chemistry at Thompson Rivers University, for his time, knowledge, and assistance on making diagrams of the chemical reactions (Figures 1, 2, and 3) used in this article.

#### SAFETY

The ASM advocates that students must successfully demonstrate the ability to explain and practice safe laboratory techniques. For more information, read the laboratory safety section of the [ASM Curriculum Recommendations: Introductory Course in Microbiology](#) and the [Guidelines for Biosafety in Teaching Laboratories](#).

#### COMMENTS AND TIPS

1. Each isolate to be tested should be inoculated into a basal medium tube without the amino acid as a growth control and negative reference.
2. Inoculated broth should be protected from air by adding a layer of sterile mineral oil before incubation. Exposure to air may cause alkalization of the surface of the medium giving false positive.
3. The broths containing different amino acids look the same. Label them carefully as to which amino acid is present in which tube.
4. Test interpretation should only be made after at least 24 hours of incubation.
5. For some microorganisms, increased incubation of up to 10 days may be required.
5. Autoclaving mineral oil is not recommended as it is problematic due to incompleteness of sterility. Sometimes water/condensation is introduced



3.

---

Min-Ken Liao  
Furman University, Greenville, SC

Patricia Shields