

Lysine Decarboxylase Broth without Peptone**M376I**

Lysine Decarboxylase Broth w/o Peptone are used for differentiating *Salmonella* Arizonae from the Bethesda Ballerup group of *Enterobacteriaceae*.

Composition***

| Ingredients | Gms / Litre |
|------------------------|-------------|
| L-Lysine hydrochloride | 5.000 |
| Yeast extract | 3.000 |
| Dextrose | 1.000 |
| Bromocresol purple | 0.015 |
| Final pH (at 25°C) | 6.8±0.2 |

***Formula adjusted, standardized to suit performance parameters

Directions

Suspend 9.01 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense 5 ml amount into screw-capped test tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes.

Principle And Interpretation

Decarboxylase tests are based on the ability of some bacteria to decarboxylate an amino acid to the corresponding amine with the liberation of carbon dioxide (1). Decarboxylase media were first described by Moeller (2-4) for detecting lysine and ornithine decarboxylase and arginine dihydrolase. Falkow developed a lysine decarboxylase medium for the identification and differentiation of *Salmonella* and *Shigella* (5). Falkows Medium was further modified by Taylor (6) by deleting peptone from the formulation (M376I), thus eliminating false positives caused by *Citrobacter freundii* and its paracolons. Taylors modification of decarboxylase medium has been recommended by the ISO committee (7). Lysine Decarboxylase Broth is especially suited to study the decarboxylase reactions for members of *Enterobacteriaceae*.

During the initial stages of incubation, following inoculation, fermentation of dextrose by the organisms leads to acid production, which causes a subsequent colour change of the bromocresol purple indicator to yellow. The acidic condition thus generated stimulates decarboxylase activity, which leads to decarboxylation of lysine to cadavarine. The alkaline conditions generated due to cadaverine production cause the bromocresol purple indicator (changed to yellow) to revert to purple colour. If the organisms do not produce decarboxylase enzyme, the colour of the medium remains yellow. Dextrose non-utilizers will not show any change in the medium colour. Use light inocula and do not read the tests after 24 hours incubation, as some organisms require longer incubation time upto 4 days.

Inoculate 25 grams of the test sample into Buffered Peptone Water (M614S). After incubation at 35-37°C for 16-20 hours, inoculate into RVS Broth (M1491) and Fluid Selenite Cystine Broth (M1533I) and incubate at 35-37°C for 24-48 hours. From the second enrichment, streak a loopful on Brilliant Green Agar Base w/ phosphates (M971S). Presumptive *Salmonella* so isolated on M971S are further confirmed by performing biochemical testing using the following media i.e. Nutrient Agar, pH 7.0 (M561A), Triple Sugar Iron Agar (M021S), Urea Agar Base, Christensen (M112I), Lysine Decarboxylase Broth w/o peptone (M376I), VP test, Indole test (7).

Quality Control**Appearance**

Light yellow to greenish yellow homogeneous free flowing powder

Colour and Clarity of prepared medium

Purple coloured clear solution without any precipitate

Reaction

Reaction of 0.9% w/v aqueous solution at 25°C. pH : 6.8±0.2

Cultural Response

M376I: Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours.

(Inoculated tubes are overlaid with sterile paraffin oil).

| Organism | Inoculum (CFU) | Lysine decarboxylation | | | | |
|---------------------------------------|----------------|----------------------------------|--|--|--|--|
| <i>Citrobacter freundii</i> ATCC 8090 | 50-100 | negative reaction, yellow colour | | | | |

| | | | | | | |
|--|--------|----------------------------------|--|--|--|--|
| <i>Escherichia coli</i> ATCC 25922 | 50-100 | positive reaction, purple colour | | | | |
| <i>Enterobacter aerogenes</i> ATCC 13048 | 50-100 | positive reaction, purple colour | | | | |
| <i>Klebsiella pneumoniae</i> ATCC 13883 | 50-100 | positive reaction, purple colour | | | | |
| <i>Proteus mirabilis</i> ATCC 25933 | 50-100 | negative reaction, yellow colour | | | | |
| <i>Proteus vulgaris</i> ATCC 13315 | 50-100 | negative reaction, yellow colour | | | | |
| <i>Salmonella Arizonae</i> ATCC13314 | 50-100 | Positive reaction, purple colour | | | | |
| <i>Salmonella Paratyphi A</i> ATCC 9150 | 50-100 | negative reaction, yellow colour | | | | |
| <i>Salmonella Typhi</i> ATCC 6539 | 50-100 | positive reaction, purple colour | | | | |
| <i>Serratia marcescens</i> ATCC 8100 | 50-100 | positive reaction, purple colour | | | | |
| <i>Shigella dysenteriae</i> ATCC 13313 | 50-100 | negative reaction, yellow colour | | | | |

Reference

- 1.Collee J. G., Duguid J. P., Fraser A. G., Marmion B. P., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1989, 13th Edition, Churchill Livingstone
- 2.Moeller V., 1954, Acta. Pathol. Microbiol. Scand., 34:102.
- 3.Moeller V., 1954, Acta. Pathol. Microbiol. Scand., 34:259.
- 4.Moeller V., 1955, Acta. Pathol. Microbiol. Scand., 36:158.
- 5.Falkow, 1958, Am. J. Clin. Pathol., 29:598.
- 6.Taylor W. I., 1961, Appl. Microbiol., 9:487.
- 7.International Organization For Standardization (ISO), 1993, Draft ISO/DIS 6579.

Storage and Shelf Life

Store below 30°C and prepared medium at 2-8°C. Use before expiry period on the label.