

Amino acid metabolism tests

Of the various amino acids, lysine, ornithine, arginine and phenyl alanine are used to test for the bacterial ability to metabolize them. These amino acids are decarboxylated, dihydrolysed or deaminated depending on the organism and the amino acid in question. Amino acids are metabolized variably by gram negative aerobic and facultatively anaerobic bacteria as well as gram positive cocci.

Principle:

Strains to be tested are grown in a liquid or semi-solid medium containing glucose, 1% amino acid and a pH indicator. Peptone or other sources (beef extract, yeast extract etc) provide minimal protein barely enough for the bacterial growth. Similarly, the concentration of glucose is kept low at 0.1%. The pH of the medium is set to 6.2, at which the colour of the medium is purple. The test organism is inoculated into the tubes, sealed with mineral oil and incubated.

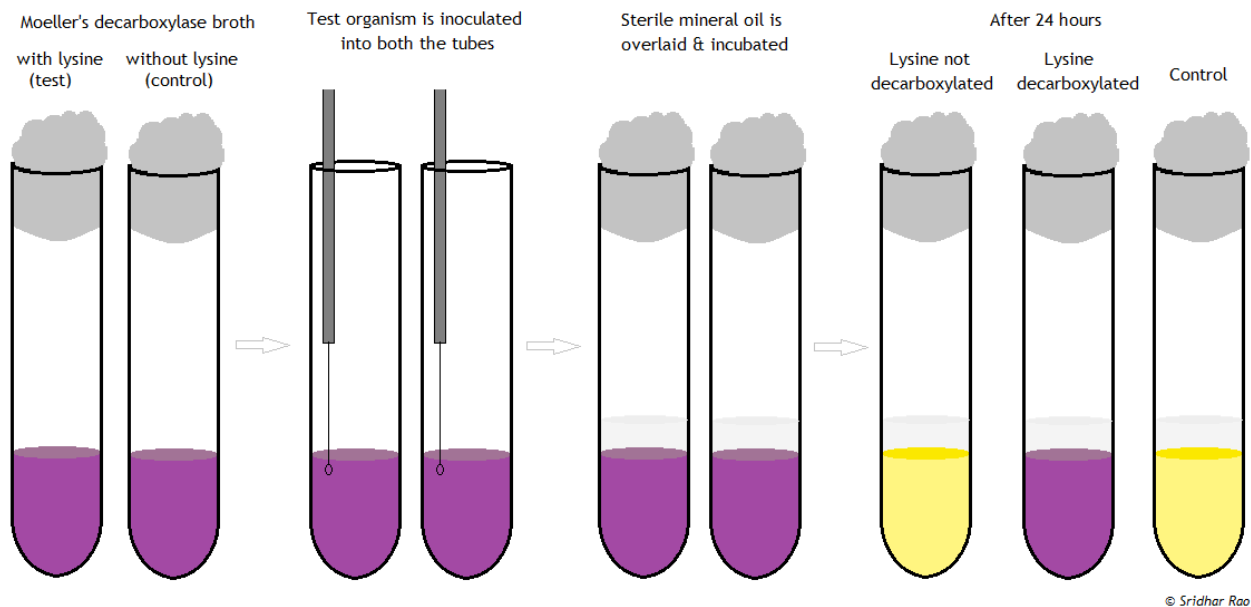
Facultatively fermenting bacteria ferment glucose resulting in the production of acid, which lower the pH of the medium. At this pH, bromocresol purple turns yellow from its original purple colour. Under anaerobic conditions and low pH, the enzyme decarboxylase is activated, which acts upon L-amino acid in the medium. Pyridoxal acts as a coenzyme for this reaction.

Lysine decarboxylase removes COOH group from the lysine to produce CO₂ and cadaverine, an alkaline polyamine. Similarly, ornithine decarboxylase acts on ornithine to produce CO₂ and putrescine, also an alkaline amine. Degradation of arginine is a two-step process, occurring simultaneously or separately. Arginine is first degraded to citrulline and then to ornithine by arginine dihydrolase. The ornithine produced is subsequently decarboxylated to form putrescine. The resulting alkalinity leads to the reversion of the pH indicator's colour from yellow to purple.

Non-fermenting bacteria don't ferment glucose in anaerobic conditions of the test. However, they decarboxylate the amino-acid when the medium is overlaid with mineral oil. Since the glucose is not fermented, they don't turn the medium yellow at any time. Due to decarboxylation, the alkalinity makes the tube deep purple. This can be checked by comparing with uninoculated tube. A variant of this method excludes glucose in the medium and lowers the pH to 5.5 so that the medium is orange and acidic at the time of inoculation. When non-fermenters decarboxylate amino acids, the colour of the medium changes from orange to purple. When testing non-fermenters, higher inoculum is recommended.

Procedure:

Moeller’s decarboxylase broth with 1% amino acid is inoculated with fresh culture from agar plate. A tube with Moeller’s decarboxylase broth but without amino acid is included as a control and inoculated with the test organism. The tubes are overlaid with 1 cm of sterile mineral oil and incubated at 37°C for 18-24 hours. If the test organism is a fermenter and produces decarboxylase, the medium with amino acid would be purple whereas the control tube without amino acid would be yellow. If the organism doesn’t produce decarboxylase, the medium would remain yellow. In case of non-fermenters, there would be no change in the control tube whereas the test would have developed a deep purple colour, indicating decarboxylation. It is important to seal the tube with mineral oil, otherwise oxidative deamination of peptone would render the top part of the medium alkaline.

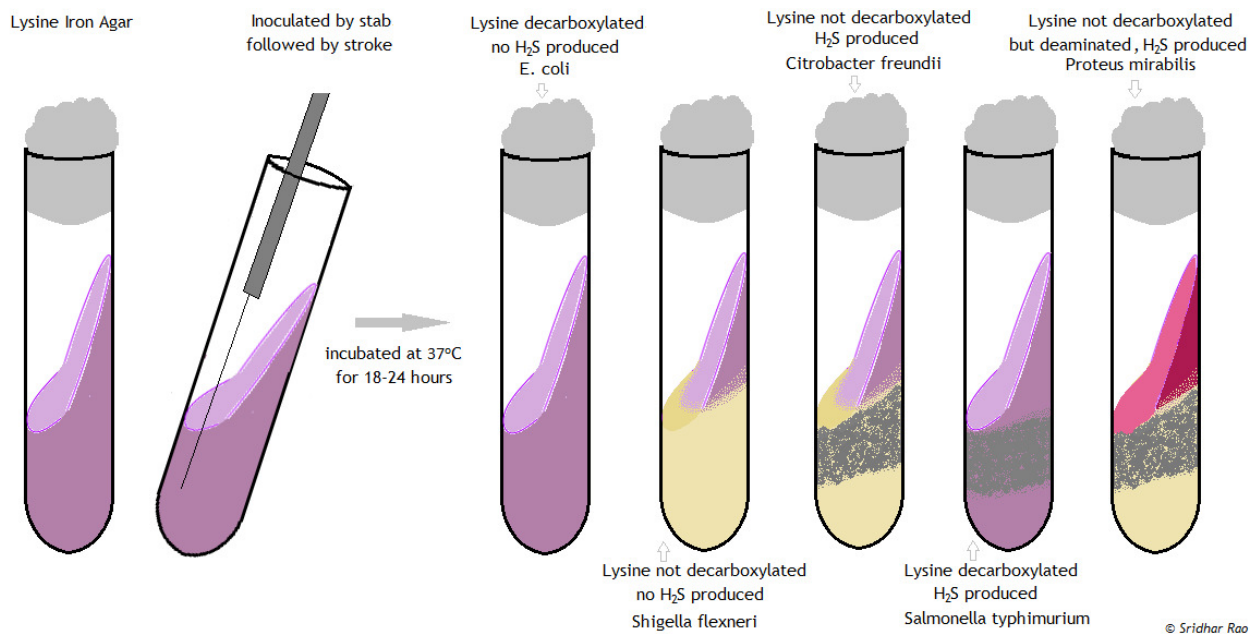


Controls:

Amino acid	Positive control	Negative control
Lysine	<i>Escherichia coli</i> ATCC 25922	<i>Shigella flexneri</i> ATCC 12022
Ornithine	<i>Proteus mirabilis</i> ATCC 25933	<i>Klebsiella pneumoniae</i> ATCC 13883
Arginine	<i>Enterobacter cloacae</i> ATCC 13047	<i>Klebsiella pneumoniae</i> ATCC 33495

Organisms that decarboxylate lysine include *K. pneumoniae*, *K. oxytoca*, *E. aerogenes*, *S. marcescens*, *S. typhi*, *E. tarda*, *M. morgani*, *V. cholerae*, *V. parahemolyticus*, *P. shigelloides* etc. Organisms that decarboxylate ornithine include *K. ornitholytica*, *S. cholerae*, *E. tarda*, *C. diversus*, *S. marcescens*, *P. mirabilis*, *M. morgani*, *E. aerogenes*, *E. cloacae*, *Shigella sonnei*, *S. paratyphi* A, *H. alvei*, *V. cholerae*, *V. parahemolyticus*, *P. shigelloides* etc. Organisms that decompose arginine include *E. cloacae*, *E. sakazakii*, *V. fluvialis*, *P. shigelloides*, *S. aureus*, *E. faecalis*, *E. faecium* etc.

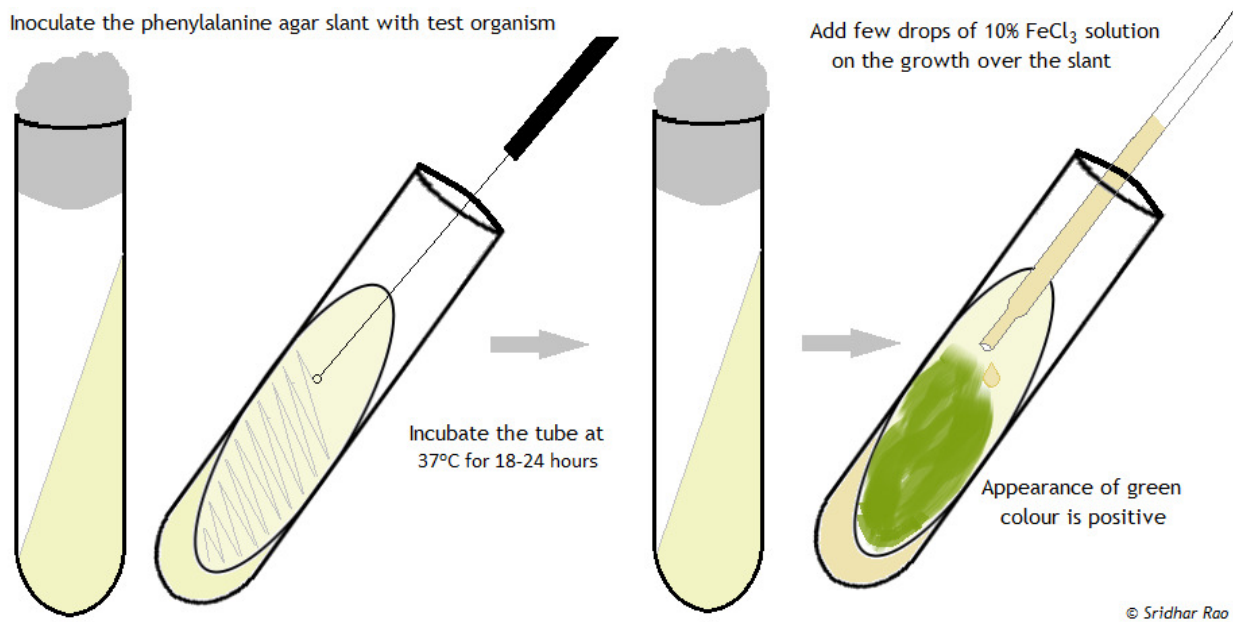
Lysine Iron Agar (LIA): This medium in test tubes has a butt and a slope (slant); more of the former and less of the latter. The test organism is inoculated with a straight wire by stabbing into the butt and stroking on the slope. Deeper butt and presence of agar prevents oxygen from dissolving into the medium. Fermenters would initially turn the medium yellow due to glucose fermentation and later reverse it to purple by decarboxylation event. *Proteus* spp can deaminate lysine aerobically in the exposed slope, producing alpha - ketocarboxylic acid. This reacts with the ferric salts to produce a reddish-brown compound in the slope. This medium can also be used for simultaneous detection of H₂S production by including sodium thiosulphate and ferric ammonium citrate. Presence of black precipitate in the butt indicates H₂S production.



Motility-Indole-Lysine Medium: It is a medium for the detection of motility, lysine decarboxylase, lysine deaminase and indole production. Apart from peptic digest of animal tissue, casein enzymic hydrolysate, yeast extract, lysine, glucose and bromocresol purple, this medium also contains ferric ammonium citrate and 0.2% agar. The test organism is stab inoculated and incubated at 37°C for 18-24 hours without mineral oil overlay. Since the upper surface of the medium is exposed to air, decarboxylation occurs only in the bottom of the tube. If lysine is decarboxylated, the bottom part of the medium turns purple whereas the upper part remains yellow due to glucose fermentation. The medium remains yellow with non-decarboxylating organisms. If the organism can oxidatively deaminate other amino acids in the peptone, the top part may turn red-brown in colour. If the organism produces H₂S, a black precipitate may be formed in the medium. Motile organism form fuzzy growth around the stab line whereas non-motile organism grow along the stab line. Upon addition of Kovac's reagent, appearance of red ring indicates a positive indole test. Addition of Kovac's reagent is the last event, after lysine decarboxylation, H₂S production and motility are observed.

Phenylalanine deamination test: This test relies on the ability of certain organisms to oxidatively deaminate phenylalanine by the enzyme phenylalanase. Deamination results in the production of phenylpyruvic acid (PPA), which is then detected by the addition of ferric chloride solution. Phenylpyruvic acid reacts with ferric chloride to give rise to a green coloured product. This property is a characteristic of members of the Proteae tribe (*Proteus*, *Morganella* & *Providencia*).

Procedure: The long slant of the phenylalanine agar is inoculated with the test organism by streaking on the surface. The tube is incubated at 37°C for 18-24 hours. Four-five drops of 10% ferric chloride solution is allowed to run down over the slope. Immediate appearance of green colour is a positive test.



Rapid filter-paper method: A suspension of the test organism is made in saline in a test tube. Filter paper strips containing 0.5% phenylalanine in phosphate buffer (pH 7.4) is prepared and dried in advance. This strip is placed inside the tube such that the tip of the paper touches the suspension. The tube is placed in a water bath and incubated at 37°C for one hour. The strip is removed and one drop of 8% ferric chloride solution is added to it. The paper strip would turn green in positive reactions.

Rapid tube method: The test organism is incubated in a solution of phenylalanine in phosphate buffer and incubated in a water bath at 37°C for two hours. To this, 0.04 ml 2% ferric chloride solution is added. Appearance of green colour is considered as a positive test.

Proteus vulgaris ATCC 13315 serves as a positive control whereas *Enterobacter aerogenes* ATCC 13048 serves as a negative control.