DEXARBOXYLASE MEDIUM

INTENDED USE

Decarboxylase media are used in the biochemical differentiation of gram-negative enteric bacilli based on the production of arginine dihydrolase and lysine and ornithine decarboxylase.

SUMMARY AND EXPLANATION

Moeller introduced the decarboxylase media for detecting the production of lysine and ornithine decarboxylase and arginine dihydrolase.[1-3] These media are a useful adjunct to other biochemical tests for the speciation and identification of the *Enterobacteriaceae* and other gram-negative bacilli.[4-6] 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.[6]

PRINCIPLE

Decarboxylase basal media consist of peptones and beef or yeast extract to supply the nitrogenous and other 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, ornithine or arginine are added to the basal medium at a concentration of 10.0 g/L 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. Arginine is first hydrolyzed to form ornithine, which is then decarboxylated to form 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.[7]

Each isolate to be tested must also be inoculated into a tube of the basal medium that 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.

REAGENTS (FORMULA)

Peptone	5.0	g
Beef Extract	5.0	g
Dextrose	0.5	g
Bromcresol Purple	0.01	g
Cresol Red	5.0	mg
Pyridoxal	5.0	mg
Deionized Water	1000.0	ml

PROCEDURE

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 hours before reporting as negative. The medium will become yellow initially, if the dextrose is fermented, and then will gradually turn purple if the decarboxylase or dihydrolase reaction occurs and elevates the pH.

EXPECTED 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.

QUALITY CONTROL

All lot numbers have been tested and have been found to be acceptable. Customers can test products using the following quality control organisms. Testing of control organisms should be performed in accordance with established laboratory quality control procedures. If aberrant quality control results are noted, sample results should not be reported.

Organisms	Incubation	Results
Escherichia coli ATCC 25922	35 ± 2°C for 18-48 hours	Lysin Decarboxylase Positive
Shigella flexneri ATCC 12022	35 ± 2°C for 18-48 hours	Lysin Decarboxylase Negative

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Viikinkaari 6, 00790 Helsinki Room 306A5

Phone: +358 (45) 8016507 Email: info@abbadis-life.com Website: https://abbadis-life.com