INSTRUCTIONS FOR USE

DECARBOXYLASE MOELLER BASE BROTH

Dehydrated culture medium

Enterobacter aerogenes - from left: uninoculated tube, Moeller arginine (-), Moeller lysine (+), Moeller ornithine (+)

1 - INTENDED USE

In vitro diagnostic. Decarboxylase Moeller Base Broth, when supplemented with amino acids, aids in the differentiation of *Enterobacteriaceae* isolated from clinical and other specimens.

2 - COMPOSITION TYPICAL FORMULA (AFTER RECONSTITUTION WITH 1 L OF WATER) *

Peptone	5.000 g
Beef Extract	5.000 g
Pyridoxal	0.005 g
Glucose	0.500 g
Bromocresol Purple	0.010 g
Cresol Red	0.005 g

*the formula may be adjusted and/or supplemented to meet the required performances criteria.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

Decarboxylase Moeller Base Broth, devised by Moeller[1] in 1955, is a liquid medium to which amino acids can be added, for the evaluation of Gram negative enteric bacteria ability to decarboxylate them with the formation of amines with change to alkalinity of the culture medium.[2] The most commonly used amino acids are L-lysine, L-ornithine, L-arginine. Cadaverine is produced from the decarboxylation of lysine, putrescine is produced from ornithine; arginine is first converted into citrulline by the enzyme dihydrolase with removal of an NH_2 group; citrulline is then converted into ornithine and the latter is decarboxylated into putrescine.[3] The amines that are formed in these enzymatic reactions increase the pH to alkalinity, with a colour change of the indicator system bromocresol purple and cresol red. Glucose, included in the medium is fermented by *Enterobacteriaceae* with the production, during the first hours of incubation, of an acidic environment, necessary for the full performance of the decarboxylase enzyme. When the medium containing amino acids is inoculated with a glucose fermenting and decarboxylase positive strain, it turns first to yellow for the production of acids from the fermentation of glucose, then to purple for the production of amines. The positive test for the decarboxylase is therefore indicated by the formation of a purple colour, the negative test by the presence of a yellow colour. Pyridoxal is a coenzyme that activates the decarboxylase enzyme; the peptone and the meat extract provide nitrogen, carbon and trace elements necessary for bacterial growth. Since the peptones can be oxidized and deaminated with production of ammonia, to avoid false positives, it is necessary to create anaerobic conditions for performing the test, covering the medium with mineral oil. Together with the test tubes containing amino acids, inoculate a test tube of basal medium without amino acids: if the latter turns to alkalinity with purple colour formation, the test is invalidated.[2]
The medium is intended for the differentiation of *Enterobacteriaceae* colonies isolated from clinical or other specimens. The production of ornithine decarboxylase is particularly useful for differentiating *Klebsiella* from *Enterobacter*: *Klebsiella* spp. are non-motile and do not produce ornithine decarboxylase, while most *Enterobacter* spp are motile and usually produce the enzyme.[4]
Positive lysine decarboxylase species include *K.pneumoniae, K.oxytoca, E.aerogenes, S.marcescens*, S.Typhi, *E.tarda, M.morganii, V.cholerae, V.parahemolyticus, P.shigelloides*; the species that decompose arginine include *E.cloacae, C.sakazakii, V.fluvialis, P.shigelloides, S.aureus, E.faecalis, E.faecium*.[3]

4- DIRECTIONS FOR MEDIUM PREPARATION

Suspend 10.5 g in 1000 mL of cold purified water. Add 10 g of the chosen amino acid in L form (final concentration 1% w/v) or 20 g/L of the DL form (final concentration 2% w/v). Mix well and, if necessary, heat to dissolve. If required, especially when L-ornithine is added, readjust the pH with 1N NaOH. Distribute 4-5 mL into screw-cap tubes and sterilise at 121°C for 15 minutes. Also prepare tubes omitting the addition of amino acids (control tubes).

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	violet, limpid or slightly opalescent
Final pH at 20-25 °C	6.0 ± 0.2 (after amino acid addition)

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Decarboxylase Moeller Base Broth	Dehydrated medium	4013662	500 g (47.6 L)

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile microbiological loops or needles, incubator and laboratory equipment as required, Erlenmeyer flasks, ancillary culture media and reagents (L-lysine, L-ornithine, L-arginine, mineral oil or liquid paraffin).

8 - SPECIMENS

The specimens consist of bacteria strains isolated from clinical specimens or other samples, purified on appropriate medium (e.g. Tryptic Soy Agar or Blood Agar).

9 - TEST PROCEDURE

With an inoculating needle or loop, transfer one colony into the tube with the amino acid and mix well; a tube with Decarboxylase Moeller Base Broth without amino acid is included as a control and inoculated with the test organism.
To all the inoculated tubes, with and without amino acids, add 2 mL of sterile mineral oil (about 1 cm) to the surface.
Incubate the tubes, with the caps tightened, at 35-37°C for up to 4 days with daily observation.

10 - READING AND INTERPRETATION

After 18-24 h, 48 h, 72 h and 96 h of incubation, observe the presence of growth (turbidity) and the colour change of the medium.
Positive reaction: the medium initially turns yellow due to the fermentation of glucose and then turns purple due to the formation of amines.
Negative reaction: the medium is turbid with a light yellow colour (glucose fermentation).
Test tube without amino acids: the medium is light yellow in colour (glucose fermentation); if this test tube shows a purple colour, the test is invalidated.
The following table summarizes the reactive models on Moeller Medium with added amino acids.

Microorganism	**Lysine**	**Ornithine**	**Arginine**
Escherichia coli	+	*var*	*var*
Enterobacter cloacae	-	+	+
Enterobacter aerogenes	+	+	-
Edwardsiella tarda	+	+	-
Salmonella typhi	+	-	*var*
Citrobacter freundii	-	*var*	*var*
Proteus vulgaris	-	-	-
Proteus mirabilis	-	+	-
Shigella dysenteriae	-	-	-
Shigella sonnei	-	+	-
Serratia liquefaciens	*var*	-	-
Serratia marcescens	+	+	-
Klebsiella pneumoniae	-	+	-

+: positive (alkaline reaction, purple colour); -: negative reaction (yellow colour); var: variable reaction

11 - USER QUALITY CONTROL

All manufactured lots of the product are released for sale after the Quality Control has been performed to check the compliance with the specifications. However, the end user can perform its own Quality Control in accordance with the local applicable regulations, in compliance with accreditation requirements and the experience of the Laboratory. Here below are listed some test strains useful for the quality control.

CONTROL STRAINS	INCUBATION T°/ T / ATM	EXPECTED RESULTS		
		ARGININE	LYSINE	ORNITHINE
P.vulgaris ATCC 9484	35-37°C ° / 44-48H /A	- (yellow)	- (yellow)	- (yellow)
S.Enteritidis ATCC 13076	35-37°C ° / 44-48H /A	+ (purple)	+ (purple)	+ (purple)

A: aerobic incubation; ATCC is a trademark of American Type Culture Collection

12 - PERFORMANCES CHARACTERISTICS

Prior to release for sale a representative sample of all lots of dehydrated Decarboxylase Moeller Base Broth, supplemented with L-lysine, L-ornithine and L-arginine is tested for specific performance characteristics by comparing the results with a previously approved Reference Batch. Pure cultures, grown for 18–24 h on Tryptic Soy Agar, of the following strains are inoculated directly into the tubes covered with liquid paraffin: *P.vulgaris* ATCC 9484, S.Typhimurium ATCC 14028, S.Enteritidis ATCC 13076, *E.aerogenes* ATCC 13048, *S.flexneri* ATCC 12022, *K.pneumoniae* ATCC 27736, *S.marcescens* ATCC 8100. Tubes are incubated with closed caps at 35–37 °C for 44-48 hours. The colour changes of medium are observed and recorded: for all strains and all amino acids, the decarboxylase reactions conform to the specifications.

13 - LIMITATIONS OF THE METHOD

- 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 to yellow at any time. Due to decarboxylation, the alkalinity makes the tube deep purple;[3] this can be checked by comparing with uninoculated tube. Some non-fermenting strains can give a delayed reaction and an absent or very slight colour change of the medium and, for this reason, Moeller's medium is not always considered satisfactory for glucose non-fermenting strains.[2,5]
- The lysine decarboxylation test does not measure the amount of intracellular enzyme, rather they indicated whether the amount of amines produced is sufficient to raise the pH of the culture medium. The change in growth conditions (concentrations of glucose, lysine and amino acids other than lysine) can significantly influence the activity of the enzyme lysine decarboxylase in coliforms.[6]
- With the tubes held vertically during incubation, the decarboxylase test may show two layers of different colours; shake the tube gently before attempting to make the interpretation.[2]
- Sometimes there may be problems of interpretation of the positive result due to the formation of an indistinct yellowish-purple colour. If this occurs, always compare with an uninoculated control tube. Any trace of purple colour, after at least 24 hours of incubation, must be interpreted as a positive test.[2]
- In some cases, after a particularly prolonged incubation, an indistinct colour or even a discolouration of the medium may appear due to the destruction of bromocresol purple.[2] This discolouration or indistinct colour occurs frequently with *P.mirabilis* and *P.vulgaris*.[1]
- *Salmonella* Gallinarum gives a delayed positive ornithine decarboxylation reaction requiring 5-6 days of incubation due to a poor bacterial permeability to the amino acid.[2]
- Many *E.coli* strains, including adonitol fermenting strains, exhibit a delayed ornithine decarboxylation reaction.[2]
- The amino acid decarboxylation is one of the tests necessary for the identification of *Enterobacteriaceae*. The results of the decarboxylation tests must be interpreted together with other tests for a correct identification of the strains. Therefore, it is recommended that biochemical, immunological, molecular, or mass spectrometry testing be performed on isolates, from pure culture, for complete identification. If relevant, perform antimicrobial susceptibility testing.

- This culture medium is intended as an aid in the diagnosis of infectious diseases; the interpretation of the results must be made considering the patient's clinical history, the origin of the sample and the results of other diagnostic tests.

14 - PRECAUTIONS AND WARNINGS

- This product is a qualitative *in vitro* diagnostic, for professional use only; it is to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- Dehydrated media must be handled with suitable protection. Before use, consult the Safety Data Sheet.
- This culture medium contains raw materials of animal origin. The *ante* and *post mortem* controls of the animals and those during the production and distribution cycle of the raw materials, cannot completely guarantee that this product doesn't contain any transmissible pathogen. Therefore, it is recommended that the culture medium be treated as potentially infectious and handled observing the usual specific precautions: do not ingest, inhale, or allow to come into contact with skin, eyes, mucous membranes. Download the TSE Statement from the website www.biolifeitaliana.it, describing the measures implemented by Biolife Italiana for the risk reduction linked to infectious animal diseases.
- Apply Good Manufacturing Practice in the production process of prepared media.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as culture medium or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized plates inoculated with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredient for pharmaceutical preparations or as production material intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet of the product are available on the website www.biolifeitaliana.it.
- Notify Biolife Italiana Srl (complaint@biolifeitaliana.it) and the relevant Authorities of any serious incident occurring in connection with the use of the *in vitro* diagnostic.
- The information provided in this document has been defined to the best of our knowledge and ability and represents a guideline for the proper use of the product but without obligation or liability. In all cases existing local laws, regulations and standard procedures must be observed for the examination of samples collected from human and animal organic districts, for environmental samples and for products intended for human or animal consumption. Our information does not relieve our customers from their responsibility for checking the suitability of our product for the intended purpose.

15 - STORAGE CONDITIONS AND SHELF LIFE

Upon receipt, store at +10°C /+30°C away from direct light in a dry place. If properly stored, it may be used up to the expiration date. Do not use beyond this date. Avoid opening the bottle in humid places. After use, the container must be tightly closed. Discard the product if the container and/or the cap are damaged, or if the container is not well closed, or in case of evident deterioration of the powder (colour changes, hardening, large lumps).
The user is responsible for the manufacturing and quality control processes of prepared media and for the validation of the shelf life of the finished products, according to the type (tubes/bottles) and the storage method (temperature and packaging).

16 - REFERENCES

1. Moeller V. Simplified tests for some amino acid decarboxylases and for the arginine dihydrolase system. Acta Pathol Microbiol Scand 1955; 36(2):158-72.
2. MacFaddin JF. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Baltimore: Williams & Wilkins; 1985.
3. Rao S. Amino acid metabolism tests. www.microrao.com
4. Farmer *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology,7th ed. American Society for Microbiology, Washington,DC. 1999
5. Barrow, G.I., Feltham R.K.A. Bacterial characters and characterization. Cowan and Steel's Manual for the Identification of Medical Bacteria. 3rd ed. Cambridge: Cambridge University Press; 1999.
6. Piluscki RW, Claytn NW, Cabelli VJ, Cohen PS. Limitations of the Moeller Lysine and Ornithine Decarboxylase Tests. App Environ Microbiol. 1979; 37(2): 254-260.

401366 DECARBOXYLASE MOELLER BASE BROTH
SDS
Regulation (EU) 2020/878
Contains: CITRIC ACID
Classification: none
Labelling none
Hazard statements: **EUH210** Safety data sheet available on request.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	IVD *In vitro* Diagnostic Medical Device	Manufacturer	Use by
Temperature limitation	Contents sufficient for <n> tests	Consult Instructions for Use	Keep away from direct light	Store in a dry place

REVISION HISTORY

Version	Description of changes	Date
Revision 2	Updated layout and content	2020/06
Revision 3	Update of "precautions and warnings" and "storage conditions and shelf life"	2022/03
Revision 4	Removal of obsolete classification	2023/04
Revision 5	Addition of SDS section	2025/05

Note: minor typographical, grammatical, and formatting changes are not included in the revision history.