DOUBLE MODIFIED LYSINE IRON AGAR (DMLIA)
DMLIA NOVOBIOCIN SUPPLEMENT

Dehydrated culture medium, selective supplement and ready-to-use plates

Salmonella Derby on DMLIA

1 - INTENDED USE
For selective and differential isolation of *Salmonella* from foods.

2 – COMPOSITION*

DOUBLE MODIFIED LYSINE IRON AGAR (DMLIA) – DEHYDRATED MEDIUM
TYPICAL FORMULA AFTER RECONSTITUTION WITH 1 L OF WATER

Peptone	5.00 g
Yeast extract	3.00 g
Glucose	1.00 g
L-Lysine HCl	10.00 g
Ferric ammonium citrate	0.80 g
Sodium thiosulphate	6.80 g
Bile salts n° 3	1.50 g
Lactose	10.00 g
Sucrose	10.00 g
Bromocresol purple	0.02 g
Agar	15.00 g

DMLIA NOVOBIOCIN SUPPLEMENT
VIAL CONTENTS FOR 500 mL OF MEDIUM

Novobiocin	7.5 mg

DOUBLE MODIFIED LYSINE IRON AGAR DMLIA – READY TO USE PLATES

Double Modified Lysine Iron Agar	63.12 g
Novobiocin	15.00 mg
Purified water	1000 mL

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

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE
Double Modified Lysine Iron Agar (DMLIA), prepared by adding 15 mg/L novobiocin to the base medium, corresponds to the formula of the selective and differential medium described by Rappold and Bolderdijk[1] in 1979 and recommended by the USDA[2-4] for the isolation of H_2S-positive and -negative strains of *Salmonella* in a wide variety of foodstuffs.
The dehydrated medium consists mainly of Lysine Iron Agar supplemented with bile salts, lactose, sucrose and additional amounts of sodium thiosulphate and ferric ammonium citrate.
Peptone provides nitrogen, carbon, minerals for bacterial growth; yeast extract is a source of vitamins, particularly of the B-group; glucose, lactose and sucrose are fermentable carbohydrates; bromocresol purple is a pH indicator, yellow at pH below 5.2 and purple at pH above pH 6.8; ferric ammonium citrate and sodium thiosulphate are the indicator system for the formation and detection of hydrogen sulphide. Lysine is included as a substrate for detection of lysine decarboxylase: when lysine is decarboxylated it is converted in cadaverine causing an alkaline reaction (the medium remains purple). Bile salts and novobiocin (included in the selective supplement) are the inhibitory agents, active mostly against Gram-positive bacteria but also against a few Gram-negative bacteria.
Salmonella spp. decarboxylates lysine and, being lactose/sucrose positive, induce an alkaline reaction in the medium with the development of colonies with different shades of purple (mauve colonies); the formation of hydrogen sulphide is indicated by the formation of colonies with a black centre.

4- DIRECTIONS FOR MEDIUM PREPARATION
Suspend 31.56 g in 500 mL of cold purified water. Heat to boiling with frequent stirring to dissolve completely. Do not autoclave. Cool to approximately 45-50°C and add the contents of one vial of DMLIA Novobiocin Supplement (REF 4240029) reconstituted with 5 mL of sterile purified water. Mix well and distribute 15-20 mL into sterile Petri dishes.

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	green, fine, homogeneous, free-flowing powder
Solution and prepared plates appearance	purple, slightly opalescent
Freeze-dried selective supplement	short, dense, white pellet; colourless and clear solution after reconstitution
Final pH of complete medium (at 20-25°C)	6.7 ± 0.2

6 - MATERIALS PROVIDED – PACKAGING

Product	Type	REF	Pack
Double Modified Lysine Iron Agar (DMLIA)	Dehydrated medium	4013252	500 g (7.9 L)
DMLIA Novobiocin Supplement	Freeze-dried supplement	4240029	10 vials, each for 500 mL of medium base
Double Modified Lysine Iron Agar (DMLIA)	Ready-to-use plates	541325	2 x 10 plates ø 90 mm

7 - MATERIALS REQUIRED BUT NOT PROVIDED
Water-bath, sterile inoculation needles, swabs and pipettes, incubator and laboratory equipment as required, sterile Petri dishes, Erlenmeyer flasks, ancillary culture media and reagents.

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8 - SPECIMENS

Meat, poultry, pasteurized eggs and siluriformes (fish) products and environmental sponges. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to the applicable International Standard.[2]

9 - TEST PROCEDURE

Transfer a loopful of growth from the selective enrichment broths by streaking onto four quadrants of a Double Modified Lysine Iron Agar (DMLIA) plate to obtain isolated colonies.
Incubate at 35 ± 2 °C for 18-24 hours. If there is no growth or no typical colonies, re-incubate for a further 18-24 hours.
For analytical details refer to the cited USDA document.[2]

10 - READING AND INTERPRETATION

After incubation, observe bacterial growth and record each specific morphological and colour characteristic of the colonies.
Consider mauve-coloured colonies with or without a black centre as typical for *Salmonella*.

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.[5]

CONTROL STRAINS	INCUBATION T°/ T - ATM	EXPECTED RESULTS
Salmonella sp. H_2S positive	35°C / 24h / A	good growth, mauve-coloured colonies with a black centre
Salmonella sp. H_2S negative	35°C / 24h / A	good growth, mauve-coloured colonies without a black centre

A: aerobic incubation

12- PERFORMANCES CHARACTERISTICS

Prior to release for sale, representative samples of all lots of dehydrated and ready to use Double Modified Lysine Iron Agar (DMLIA) and DMLIA Novobiocin Supplement, are tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.
The productivity characteristics are assessed by semi-quantitative ecometric technique with the following non-target strains: S. Typhimurium ATCC 14028, *S. arizonae* ATCC 13314, S. Derby CBAES1.5, S. Gallinarum CB506, S. Dublin CB9.2, S. Choleraesuis CBX4. After incubation, the amount of growth and the colony characteristics are evaluated: all target strains exhibit good growth with mauve colonies, black centred.
Specificity is tested with the non-target strain *S. sonnei* ATCC 9290 by semi-quantitative ecometric technique. After incubation, *S. sonnei* grows with colourless colonies.
The selectivity is assessed with modified Miles-Misra surface drop method by inoculating the plates with suitable decimal dilutions in saline of a 0.5 McFarland suspension of *E. coli* ATCC 8739, *C. freundii* ATCC 8090, and *E. faecalis* ATCC 19433. *C. freundii* exhibits yellow colonies, the growth of *E. coli* is partially inhibited while the growth of *E. faecalis* is totally inhibited.
CB: Biolife Microbial Collection

13 - LIMITATIONS OF THE METHOD

- Lactose-positive *Salmonella* variants have been described with an incidence rate of less than 1%.[5,6] These salmonellae may grow with yellowish colonies on DMLIA plate.
- Complete identification of the colonies must be carried out by biochemical, immunological, molecular or mass spectrometric techniques, after purification of the colonies by subculture on appropriate medium. For analytical details refer to the cited USDA document.[2]

14 - PRECAUTIONS AND WARNINGS

- The medium base, the supplement and the ready-to-use plates are for microbiological control and for professional use only; they are to be used by adequately trained and qualified laboratory personnel, observing approved biohazard precautions and aseptic techniques.
- The medium base and the supplement shall be used in association according to the described directions. Apply Good Manufacturing Practice in the production process of prepared media.
- Dehydrated media and antibiotics containing supplements must be handled with suitable protection. DMLIA Novobiocin Supplement is classified as dangerous. Before use, consult the Material Safety Data Sheets.
- 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 the 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.
- Be careful when opening the metal ring of the supplement vials to avoid injury.
- The supplement is sterilized by membrane filtration.
- Each plate of this culture medium is for single use only.
- Ready-to-use plates are not to be considered a "sterile product" as they are not subject to terminal sterilization, but a product with controlled bio contamination, within the limits of defined specifications reported on the Quality Control Certificate.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder and supplement or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and supplement and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium and the supplement as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheets are available on the website www.biolifeitaliana.it.
- 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

Ready to use plates

Upon receipt, store plates in their original pack at 2-8°C away from direct light. If properly stored, the plates may be used up to the expiration date. Do not use the plates beyond this date. Plates from opened plastic sachet can be used for 7 days when stored in a clean area at 2-8°C. Do not use the plates if the plastic sachet is damaged or if the dish is broken. Do not use the plates with signs of deterioration (e.g., microbial contamination, dehydration, shrinking or cracking of the medium, atypical colour, excess of moisture).

Dehydrated medium

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).

Freeze-dried supplement

Upon receipt, store the product in the original package at 2-8°C away from direct light. If properly stored, the product may be used up to the expiry date printed on the label; do not use beyond this date. Once the vial has been opened and the lyophilised product has been reconstituted, the resulting solution should be used immediately. Before use, examine the lyophilized and reconstituted product and discard if there are obvious signs of deterioration (e.g., contamination, atypical colour or other abnormal characteristics).

The user is responsible for the manufacturing and quality control processes of prepared media and the validation of their shelf life, according to the type (plates/tubes/bottles) and the applied storage conditions (temperature and packaging). According to USDA document the self-prepared plates can be stored at +2°C +8°C for up to 3 weeks.[3]

16 - REFERENCES

1. Rappold H, Bolderdijk R. Modified lysine iron agar for isolation of Salmonella from food. Appl Environ Microbiol 1979; 38(1):162-3.
2. United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science. Laboratory Guidebook, Notice of Change: Isolation and Identification of Salmonella from Meat, Poultry, Pasteurized Eggs and Siluriformes (Fish) Products and Environmental Sponges. MLG 1.11, Effective Date: 08/16/21.
3. United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science. Laboratory Guidebook, Notice of Change: Media and Reagents. MLG Appendix 1.10, Effective Date: 03/07/22.
4. United States Department of Agriculture Food Safety and Inspection Service, Office of Public Health Science Laboratory Guidebook, Notice of Change: Flow Chart Specific for FSIS Laboratory Isolation and Identification of Salmonella. Chapter MLG 4.0, , Effective Date 02/24/20.
5. Ewing W H. Edwards and Ewing's identification of the Enterobacteriaceae. 4th ed. New York, N.Y: Elsevier Science Publishing Co., Inc.; 1986. Differentiation of Enterobacteriaceae by biochemical reactions; pp. 47–72.
6. Patrick L. McDonough, Sang J. Shin, Donald H. Lein. Diagnostic and Public Health Dilemma of Lactose-Fermenting Salmonella enterica Serotype Typhimurium in Cattle in the Northeastern United States. J Clin Microbiol 2000; 38(3): 1221–1226.

TABLE OF APPLICABLE SYMBOLS

REF or REF Catalogue number	LOT Batch code	Manufacturer	This side up	Store in a dry place	Fragile
Temperature limitation	Content sufficient for <n> tests	Consult Instructions for Use	Use by	Keep away from direct light	For single use only

REVISION HISTORY

Version	Description of changes	Date
Revision 0	First issue	2022/07

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