MINERALS MODIFIED GLUTAMATE MEDIUM (MMGM) BASE
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SODIUM GLUTAMATE

Dehydrated culture medium and ready-to-use tubes

1 - INTENDED USE

Chemically defined enrichment broth for detection of *Escherichia coli* according to ISO 16649 and other coliform organisms in food, water and wastewater samples.

2 - COMPOSITION - TYPICAL FORMULA *

DEHYDRATED MEDIUM AND READY-TO-USE TUBES

401737 MINERALS MODIFIED GLUTAMATE MEDIUM BASE (MMGM)

	DOUBLE STRENGTH	SINGLE STRENGTH
Lactose	20.000 g	10.000 g
Sodium formate	0.500 g	0.250 g
L-cystine	0.04 g	0.020 g
L (-) aspartic acid	0.048 g	0.024 g
L (+) arginine	0.040 g	0.020 g
Thiamine	0.002 g	0.001 g
Nicotinic acid	0.002 g	0.001 g
Pantothenic acid	0.002 g	0.001 g
Magnesium sulphate heptahydrate	0.200 g	0.100 g
Ammonium iron (III) citrate	0.02 g	0.010 g
Calcium chloride dihydrate	0.020 g	0.010 g
Di-potassium hydrogen phosphate	1.800 g	0.900 g
Bromocresol purple	0.020 g	0.010 g
4123642 Sodium Glutamate		
Sodium glutamate**	12.70 g	6.35 g
Ammonium chloride**	5.00 g	2.50 g
Water	1000 mL	1000 mL

*The formula may be adjusted and/or supplemented to meet the required performances criteria.
** Not included in the dehydrated medium; it must be added to the basal medium.

3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

A chemically defined medium based on glutamic acid was first advocated by Folpmers[1] in 1948 for the enumeration of the coliform group of bacteria in water. This basic formulation was modified by Burman and Oliver in 1952[2] and by the UK Public Health Laboratory Service[3] substituting glucose with lactose. The lactose medium was then further improved by Gray in 1959[4], who increased the pH and added sodium formate to increase gas production. Further modifications were made by Windle Taylor[5]: the concentration of lactose was increased and the phosphate decreased, and the modified medium was adopted in UK in place of MacConkey broth for all routine samples examined by the multiple tube method. Simultaneously and independently Gray[6] also made further modifications in 1964 resulting in the publication of an improved formate lactose glutamate medium.
Comparative trials with MacConkey Broth[7] demonstrated that Gray's modification of the medium gave significantly higher numbers of positive results for coliform organisms and *E. coli*, after only 24 hours incubation. In a comparative evaluation with Lauryl Tryptose Broth (LTB)[8], at 48 h of incubation, Minerals Modified Glutamate Medium (MMGM) gave better results for total coliform organisms including *E. coli* in chlorinate waters, especially if the numbers were small. MMGM was evaluated by Abbis et al[9] with reference to Lauryl Sulfate Tryptose Broth, MacConkey Broth and Brilliant Green Bile Broth for the enumeration of coliforms in different food samples and provided the best sensitivity results.
MMGM is recommended by ISO 16649-3[10] and, supplemented with agar, by ISO 16649-1[11]. Both the techniques include a resuscitation step and are used for the enumeration of β-glucuronidase positive *E.coli* from foodstuffs likely to contain sub-lethally injured cells.
Essential growth factors are provided by sodium glutamate and sodium formate; lactose is a fermentable carbohydrate. The presence of B-complex vitamins, amino acids and magnesium ions allows an increased rate of growth and fermentation. The addition of ammonium chloride (not included in the dehydrated medium) increases gas production by target strains, while di-potassium hydrogen phosphate acts as buffer system during lactose fermentation. Bromocresol purple serves as an acid-base indicator giving a yellow colour to the broth with lactose fermenting bacteria while non-lactose-fermenting bacteria develop a blue colour. To improve the stability of the dehydrated medium on storage, the sodium glutamate is supplied separately (REF 4123642) and must be added to the basal medium REF 401737.

4 - DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Single strength medium
Dissolve 2.5 g of ammonium chloride in 1000 mL of cold purified water. Add 11.4 g of Minerals Modified Glutamate Medium Base, and 6.35 g of Sodium Glutamate (REF 4123642).
Double strength broth
Dissolve 5 g of ammonium chloride in 1000 mL of cold purified water. Add 22.7 g of Minerals Modified Glutamate Medium Base, and 12.7 g of Sodium Glutamate (REF 4123642).

Mix and heat if necessary to dissolve the medium completely.
Dispense the single strength medium in 10 mL volumes into tubes or bottle of dimensions at least 16 mm x 160 mm.
Dispense the double strength medium in 10 mL volumes into tubes or bottles of dimensions at least 18 mm x180 mm or 20 mm x 200 mm.
Place an inverted fermentation tube in each container if required. Sterilise by autoclaving for 10 minutes at 116°C.

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Minerals Modified Glutamate Medium Base may be supplemented with 13 g/L of Agar Bios LL (REF 401030) before sterilisation for the preparation of Minerals Modified Glutamate Agar.[11]

5 - PHYSICAL CHARACTERISTICS

Dehydrated medium appearance	whitish to mauve, fine, homogeneous, free-flowing powder
Prepared tubes appearance	purple, limpid
Final pH at 20-25 °C	6.7 ± 0.1

6 - MATERIALS PROVIDED - PACKAGING

Product	Type	REF	Pack
Minerals Modified Glutamate Medium (MMGM) Base	Dehydrated medium	4017372	500 g (44 or 22 L)
Sodium Glutamate	Raw material/supplement	4123642	300 g (46.9 or 23.4 L)
Minerals Modified Glutamate Medium (MMGM)	Ready-to-use tubes	551737	20 x 10 mL
Minerals Modified Glutamate Medium (MMGM) 2x	Ready-to-use tubes	551737D	20 x 10 mL

7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops and pipettes, incubator and laboratory equipment as required, Erlenmeyer flasks, test-tubes, ammonium chloride, Sodium Glutamate (REF 4123642), Agar Bios LL (REF 411030) ancillary culture media and reagents.

8 – SPECIMENS

Products intended for human consumption and the feeding of animals, and environmental samples in the area of food production and food handling; water and wastewater. When collecting, storing, transporting and preparing samples, follow the rules of good laboratory practice and refer to applicable International Standards and regulations.

9 - TEST PROCEDURE

Enumeration of *E. coli* by MPN technique in foodstuffs (ISO 16649-3)[10]

1. Inoculate 3 or 5 double-strength MMGM tubes with 10 mL aliquots of the test sample, if liquid, or with 10 mL aliquots of the initial suspension in the case of other products.
2. Inoculate 3 or 5 tubes of single-strength MMGM with 1 mL aliquots of the test sample, if liquid or with 1 mL aliquots of the initial suspension in the case of other products.
3. Repeat the inoculation of the single strength liquid medium for each of the further decimal dilutions, using a fresh pipette for each dilution.
4. Incubate the tubes at 37°C for 24 ± 2 hours
5. From each of the incubated tubes showing yellow colour subculture with a loop on a plate of TBX Agar (402156) by streaking to obtain isolated colonies and incubate at 44°C for 24 ± 2 hours
6. Examine the TBX Agar plates for the presence of green-blue colonies (*E. coli* beta-glucuronidase positive).
7. Express the results as the Most Probable Number of *E. coli* on the basis of the presence of green-blue colonies on TBX plates.

Enumeration of coliforms/*E.coli* in water

Inoculate the water sample into the medium in the following volumes:

- 50 mL of sample into 50 mL of double-strength medium or 5 x 10 mL of sample into 5 x 10 mL of double-strength medium (for suspected low number of target organisms).
- 5 x 1 mL of sample into 5 x 5 mL of single-strength medium or 5 x 1 mL of a 1:10 dilution of the sample into 5 x 5 mL of single-strength medium (for suspected high number of target organisms).
- Incubate the tubes at 37°C. Examine after 18-24 hours incubation and again at 48 hours.

Enumeration of *E. coli* in foodstuffs with MMG Agar (ISO 16649-1)[11]

This technique requires the use of Minerals Modified Glutamate Medium (MMGM) Base supplemented as described above and with 13 g/L of Agar Bios LL (REF 411030): MMG Agar.

- Transfer in the centre of the membrane 1 mL of the sample or 1 mL of the initial suspension and spread the inoculum on the surface of the membrane. Repeat the procedure with further decimal dilutions if necessary.
- Using a sterile spreader, spread the inoculum evenly over the whole membrane surface, avoiding any spillage from the membrane.
- Leave the plates at room temperature for 15 minutes in order the medium adsorbs the liquid sample.
- Incubate the plates for 4 h ± 0.25 h at 37 °C, with the membrane/agar surface uppermost.
- After this resuscitation step transfer the membranes onto TBX Agar plates and incubate at 44°C for 18-24 hours.

10 - READING AND INTERPRETATION

MMGM becomes turbid when bacteria are growing; lactose fermentation can be detected by gas formation and by yellow colour development. Each presumptive positive tube should be confirmed with appropriate sub-cultures and with additional biochemical tests.
Examine the TBX Agar plates for the presence of typical, blue or blue-green colonies indicating the presence of β-glucuronidase-positive *E. coli*.

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
E. coli ATCC 8739	37°C/24 H/A	growth, with gas production; the medium turns yellow
E. faecalis ATCC 29212	37°C/24 H/A	inhibited

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 and ready to use MMGM is tested for productivity, specificity and selectivity by comparing the results with a previously approved Reference Batch.
Productivity is tested by dilution to extinction method, by inoculating 1 mL of appropriate decimal dilutions of target organisms in test tubes, incubating at 37°C for 24 hours and recording the highest dilution showing growth, gas production and yellow colour in Reference Batch

(Gr_{RB}) and in Test Batch (Gr_{TB}). Productivity is tested with the following target strains: *E. coli* ATCC 8739, *E. coli* NCTC 13216, *E. coli* ATCC 25922, *C. freundii* ATCC 43864. The productivity index Gr_{RB}-Gr_{TB} for each test strain is ≤ 1 and the tubes exhibit gas and yellow colour.
Specificity is tested with appropriate dilutions of non-target strains S. Typhimurium ATCC 14028 and *P. aeruginosa* ATCC 27853. After incubation, the strains exhibit good growth without turning yellow the medium and without gas production.
Selectivity is tested with appropriate dilutions of non-target strains *E. faecalis* ATCC 29212 and *S. aureus* ATCC 25923. After incubation, the growth of non-target strains is inhibited.

13 – Limitations of the method

- The pH of the medium significantly influences its performance. Avoid overheating and check the pH of each preparation batch.
- Some strains of *E. coli* may grow poorly or not at all in media incubated at 44 °C. Consequently, some strains of *E. coli*, including pathogenic ones, will not be detected by the methods reported above taken from ISO Standards. β-glucuronidase activity may also be exhibited at 44 °C by certain other members of the *Enterobacteriaceae*, notably *Shigella* and *Salmonella*.[10]
- Some organisms other than coliform bacteria can grow in the medium with acid and gas production. Each presumptive positive tube should be confirmed with appropriate sub-cultures and with additional biochemical tests.

14 - Precautions and warnings

- This culture medium is for microbiological control and 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.
- Apply Good Manufacturing Practice in the production process of prepared media.
- Be careful when opening screw cap tubes to prevent injury due to breakage of glass.
- Ready-to-use tubes are subject to terminal sterilization by autoclaving
- Each ready-to-use tube of this culture medium is for single use only.
- All laboratory specimens should be considered infectious.
- The laboratory area must be controlled to avoid contaminants such as medium powder or microbial agents.
- Sterilize all biohazard waste before disposal. Dispose the unused medium and the sterilized medium 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 Sheets of the products 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

Dehydrated medium and Sodium Glutamate

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 the validation of their shelf life, according to the type and the applied storage conditions (temperature and packaging).

Ready-to-use medium in tubes

Upon receipt, store tubes in their original pack at +2°C /+8°C away from direct light. If properly stored, the tubes may be used up to the expiration date. Do not use the tubes beyond this date. Tubes from opened secondary packages can be used up to the expiration date. Opened tubes must be used immediately. Before use, check the closing and the integrity of the screw cap. Do not use tubes with signs of deterioration (e.g., microbial contamination, abnormal turbidity, precipitate, atypical colour).

16 - References

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3. Public Health Laboratory Service, Water Sub-Committee. A comparison between MacConkey broth and glutamic acid media for the detection of coliform organisms in water. J Hyg Camb 1958; 56:377-88.
4. Gray RD. Formate lactose glutamate: a chemically defined medium as a possible substitute for MacConkey broth in the presumptive coliform examination of water. J Hyg Camb 1959;57: 249-65.
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8. Joint Committee of the P. H. L. S. and the Standing Committee of Analysts. A comparison between minerals-modified glutamate medium and lauryl tryptose lactose broth for the enumeration of Escherichia coli and coliform organisms in water by the multiple tube method. J. Hyg., Camb.1980;85:35-49.
9. Abbiss JS, Wilson JM, Blood RM, Jarvis B. A comparison of minerals modified glutamate medium with other media for the enumeration of coliforms in delicatessen foods. J Appl Bacteriol 1981; 51:121-127.
10. ISO 16649-3:2016. Microbiology of the food chain — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide
11. ISO 16649-1:2018. Microbiology of the food chain — Horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli. Part 1: Colony-count technique at 44 °C using membranes and 5-bromo-4-chloro-3-indolyl beta-D-glucuronide.

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 1	Updated layout and content	2022/09

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

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