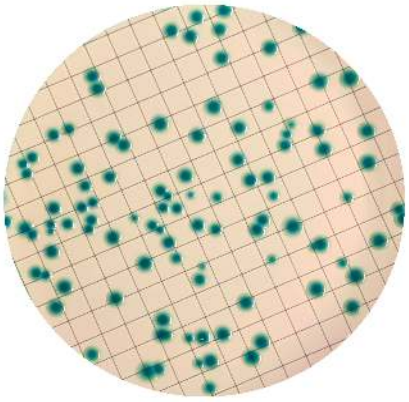
# MEMBRANE LACTOSE GLUCURONIDE AGAR (MLGA)

**Dehydrated culture medium and ready to use medium in plates**



MLG Agar: *E.coli* ATCC 25922 on membrane filter

## 1 - INTENDED USE

For the detection and enumeration of *Escherichia coli* and other coliforms with the membrane filter technique.

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

| | |
|---|---|
| Peptone | 40.00 g |
| Yeast Extract | 6.00 g |
| Lactose | 30.00 g |
| Phenol Red | 0.20 g |
| Sodium Lauryl Sulphate | 1.00 g |
| Sodium Pyruvate | 0.50 g |
| Agar | 10.0 g |
| X-Glucuronide | 0.20 g |

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

## 3 - PRINCIPLE OF THE METHOD AND EXPLANATION OF THE PROCEDURE

The count of *Escherichia coli* and coliforms is a sensitive parameter for evaluating the faecal contamination of samples and judging the quality of the water before and after sanitization treatments.[1]
The ISTISAN 14/182 report and "The Environment Agency"[1] recommend the use of Membrane Lactose Glucuronide Agar (MLGA) with the single membrane filtration technique.
MLGA medium differs from Membrane Lauryl Sulphate Broth (mLSB) for the addition of X-glucuronide (BCIG), sodium pyruvate and agar; MLGA simplifies the membrane filtration technique for *E. coli* and coliforms by reducing the required filtrations from two to one and reduces the need for additional confirmation steps.
Peptone and yeast extract are sources of nitrogen and carbon and are factors for microbial growth. Sodium pyruvate protects damaged cells, promotes the recovery of coliforms and improves their growth; sodium lauryl sulphate inhibits Gram positive bacteria. The differentiation of *E. coli* and coliforms is allowed by two biochemical reactions within the medium: the fermentation of lactose and the determination of beta-glucuronidases.
Lactose is fermented by coliforms with the production of acids which cause the phenol red to turn yellow. The chromogenic substrate 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide (BCIG) is cleaved by the beta-glucuronidase enzyme of *E.coli* and produces a blue chromophore that accumulates in colonies.
Coliforms other than *E. coli*, which are lactose positive, will produce yellow colonies; *E. coli* will grow with green colonies, a combination of the yellow of lactose fermentation and the blue of the beta-glucuronidase reaction.

## 4- DIRECTIONS FOR MEDIUM PREPARATION (DEHYDRATED MEDIUM)

Suspend 88 g in 1000 mL of cold purified water, heat to boiling with frequent agitation and sterilise by autoclaving at 121°C for 15 minutes. Cool to 47-50°C and distribute into sterile Petri dishes.

## 5 - PHYSICAL CHARACTERISTICS

| | |
|---|---|
| Dehydrated medium appearance | from pink to straw yellow, fine, homogeneous, free-flowing powder |
| Prepared plates appearance | Bright red, clear |
| Final pH (at 20-25°C) | 7.4 ± 0.2 |

## 6 - MATERIALS PROVIDED – PACKAGING

| Product | Type | REF | Pack |
|---|---|---|---|
| Membrane Lactose Glucuronide Agar | Dehydrated medium | 4017502 | 500 g (5.68 L) |
| Membrane Lactose Glucuronide Agar | Ready-to-use plates | 491315 | 3 x 10 plates ø 55 mm |

## 7 - MATERIALS REQUIRED BUT NOT PROVIDED

Autoclave, water-bath, sterile loops, swabs and pipettes, incubator and laboratory equipment as required, Petri dishes, Erlenmeyer flasks, filter membranes, ancillary culture media and reagents.

## 8 - SPECIMENS

Water samples and food chain samples. For specimen collection, storage, transport and preparation, follow good laboratory practices and refer to applicable international standards and regulations.[1,2]

## 9 - TEST PROCEDURE

Filter the water sample to be analyzed through a membrane filter (diameter 47 mm, pore size 0.45 µm). The volume and dilution of the filtered water should be chosen to obtain 20 to 80 colonies on the membrane surface. For waters expected to contain a low number of coliforms, filter a volume of 100 mL. For polluted waters use a smaller volume or a diluted sample.
Place the membrane filter on an MLGA plate ensuring that no air bubbles are trapped under the membrane. Incubate plates at 30 ± 1°C for 4 ± 0.5 hours, then at 44 ± 1°C for 16 ± 2 hours[2].

## 10 - READING AND INTERPRETATION

After incubation, observe the bacterial growth and record the specific morphological and chromatic characteristics of the colonies on plating out media and count all the yellow and green colonies.
Consider the yellow colonies as coliforms other than *E. coli*
Consider green colonies as *E. coli* and no further confirmation is required. However, if deemed necessary, confirmatory tests can be performed to demonstrate the production of acid from lactose, the formation of indole from tryptophan at 44°C and the cytochrome oxidase reaction[2].
The sum of the two represents the number of total coliform bacteria. Express the result in colony forming units per sample volume (CFU/mL).
Read results within 15 minutes of removal from thermostat as yellow colour may change over time.

## 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 25922 | 30°C/4H + 37°C/14H-A | good growth, green colonies |
| *E. aerogenes* ATCC 13048 | 30°C/4H + 37°C/14H-A | good growth, yellow colonies |
| *P.aeruginosa* ATCC 27853 | 30°C/4H + 37°C/14H-A | good growth, pink colonies |
| *B. subtilis* ATCC 6633 | 30°C/4H + 37°C/14H-A | inhibited |

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

## 12- LIMITATION OF THE METHOD

- This method is not suitable for sludge samples that have been treated with lime or subjected to drying or pasteurization, treatments that significantly reduce bacterial concentrations to values below 10 viable *E. coli* cells per gram of wet weight, or for those samples where an intense microbial reduction is expected[2].
- Sludges with a high solids content (>20% w/v) tend to block the filter membrane at lower dilutions or may mask or inhibit the growth of target organisms lowering the limit of detection[2].

## 13 - PRECAUTIONS AND WARNINGS

- These products 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.
- Dehydrated medium must be handled with adequate protection. 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.
- Apply good manufacturing practices in the plates preparation process.
- 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 supplements and the inoculated plates with samples or microbial strains in accordance with current local legislation.
- Do not use the culture medium as active ingredients for pharmaceutical preparations or as production materials intended for human and animal consumption.
- The Certificates of Analysis and the Safety Data Sheet 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.

## 14 - STORAGE CONDITIONS AND SHELF LIFE

**Dehydrated medium**
Upon receipt, store at +2°C /+8°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).
**Ready to use plates**
Upon receipt, store plates in their original pack at +2°C /+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°C /+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).

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/flasks) and the applied storage conditions (temperature and packaging).

## 15 - REFERENCES

1. The Environment Agency - Methods for Examination of Waters and Associated Material - The Microbiology of Drinking Water 2002.

2. Bonadonna L, Musmeci L (Ed.). *Metodi analitici di riferimento per la valutazione microbiologica dei fanghi di depurazione e di matrici ad essi assimilabili.* Roma: Istituto Superiore di Sanità; 2014. (Rapporti ISTISAN 14/18).

**TABLE OF APPLICABLE SYMBOLS**

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

**REVISION HISTORY**

| Version | Description of changes | Date |
|---|---|---|
| Revision 0 | First version | 2024/05 |

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

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