Harlequin® membrane Lactose Glucuronide Agar (mLGA) (NCM1009)

Intended Use

Membrane Lactose Glucuronide Agar (mLGA) is a medium for the detection and identification of *E. coli* and coliforms from water samples and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Traditionally, membrane Lauryl Sulphate Broth (mLSB) has been used as the standard media for isolating coliforms (including *E. coli*) from water potentially contaminated with sewage. Harlequin membrane Lactose Glucuronide Agar (mLGA) is a modification of mLSB aimed at reducing costs by reducing the number of filters used per test sample and aiding in the recovery and identification of coliforms and *E. coli*. The medium has been modified from the mLSB formulation by the incorporation of X-glucuronide (BCIG), sodium pyruvate and agar.

X-glucuronide is a chromogenic substrate which detects the β-glucuronidase enzyme - highly specific for *E. coli** - and allows for the presumptive isolation of *E. coli*. Sodium pyruvate aids the recovery of chlorine stressed organisms and agar is incorporated to remove the need for absorbent pads.

This medium is recommended for the enumeration of coliform bacteria and *E. coli* by a single membrane filtration technique in The Microbiology of Drinking Water 2009 (previously Report 71).

*96-97% of *E. coli* strains positive. A notable exception is *E. coli* O157:H7.

Typical Formulation

Peptone	40.0 g/L
Yeast Extract	6.0 g/L
Lactose	30.0 g/L
Phenol Red	0.2 g/L
Sodium Lauryl Sulphate	1.0 g/L
Sodium Pyruvate	0.5 g/L
X-Glucuronide (BCIG)	0.2 g/L
Agar	10.0 g/L

Final pH: 7.4 ± 0.2 at 25°C
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

1. Refer to SDS

Preparation

1. Suspend 88 grams of the medium in one liter of purified water.
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 115ºC for 10 minutes.
4. Cool to 45-50°C.

Test Procedure

E. coli and coliform counts can be performed on the same sample of water.

- The volume and dilution of test sample should be chosen so as the number of colonies on the membrane lies between 20 and 80. With waters expected to contain low numbers of coliforms, a sample of 100ml should be filtered.
- After filtration of the sample, carefully transfer the membrane filter to the mLGA surface, taking

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care not to trap air bubbles between the filter and agar. 'Rolling' the filter onto the surface minimizes the risk of air bubbles becoming trapped.

- Incubate for 4 hours (± 15 minutes) at 30°C followed by incubation at 37°C for a minimum of 14 hours. Alternatively, plates may be incubated at 37°C for 18-24 hours.

For full methodology refer to The Microbiology of Drinking Water 2009 section 4B – The enumeration of coliform bacteria and *E. coli* by a single membrane filtration technique.

Quality Control Specifications
Dehydrated Appearance: Powder is homogeneous, free flowing and beige.

Prepared Appearance: Prepared medium is clear, red gel.

Minimum QC:

Escherichia coli ATCC 25922	
Enterobacter aerogenes coli ATCC 13048	yellow
Salmonella Typhimurium ATCC 14028	red
Enterococcus faecalis ATCC 29212	inhibited

Results

Organism	Colony Size (mm)	Shape & Surface	Color	Other
Escherichia coli	0.5 - 1.5	CV.E.G.	Green	Yellow if Glucuronidase -ve
Lactose fermenters e.g. *Enterobacter aerogenes*	0.5 - 1.5	CV.E.G.	Yellow	
Non-lactose fermenters e.g. *Salmonella* spp.	0.5 - 1.5	CV.E.G.	Red	
Enterococcus faecalis				Inhibited

Expiration
Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing or appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedures
Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium.

Storage
Store dehydrated culture media at 2-30°C away from direct sunlight. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.

References
1. Calabrese, J.P. & Bisssonette, G.K. (1990). Improved membrane filtration method incorporating catalase and sodium pyruvate for detection of chlorine stressed coliform bacteria. *Applied and Environmental Microbiology*. 56. 3558-3564.

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2. The Microbiology of Drinking Water (2009) – Part 4 – Methods for the isolation and enumeration of coliform bacteria and *Escherichia coli* (including *E. coli* O157:H7). Section B – The enumeration of coliform bacteria and *Escherichia coli* by a single membrane filtration technique using membrane lactose glucuronide agar incubated at 37°C. The Environment Agency.

3. Sartory, D.P. & Howard, L. (1992). A medium detecting B-glucuronidase for the simultaneous membrane filtration enumeration of *Escherichia coli* and coliforms from drinking water. *Letters in Applied Microbiology*. 15. 273-276.

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