HAL009
Harlequin™ membrane Lactose Glucuronide Agar (mLGA)

Description
Harlequin™ membrane Lactose Glucuronide Agar (mLGA) is a medium for the detection and identification of *E. coli* and coliforms from water samples.

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

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g/litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>40.0</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>6.0</td>
</tr>
<tr>
<td>Lactose</td>
<td>30.0</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.2</td>
</tr>
<tr>
<td>Sodium lauryl sulphate</td>
<td>1.0</td>
</tr>
<tr>
<td>Sodium pyruvate</td>
<td>0.5</td>
</tr>
<tr>
<td>X-Glucuronide (BCIG)</td>
<td>0.2</td>
</tr>
<tr>
<td>Agar</td>
<td>10.0</td>
</tr>
</tbody>
</table>

**Grams per litre** 88

Appearance
Powder: fine, free-flowing, homogeneous, buff
Finished medium: clear, red gel

pH: 7.4 ± 0.2

Hazard classification
NR – Not regulated

Method for reconstitution
Disperse 88 grams of powder in 1 litre of deionised water. Allow to soak for 10 minutes, swirl to mix, then sterilise by autoclaving at 115°C for 10 minutes. Cool to 47°C and mix well before dispensing into sterile Petri dishes. Dry the agar surface prior to use.

Storage
Dehydrated culture media: 10-25°C
Final medium: up to 7 days at 2-8°C in the dark
Media stored at 2-8°C may show formation of surface crystals. This is normal and does not affect the performance of the product. Crystals will disappear when plates are warmed to >20°C.
Inoculation – according to The Microbiology of Drinking Water (2009) Part 4B

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 care not to trap air bubbles between the filter and agar. ‘Rolling’ the filter onto the surface minimises the risk of air bubbles becoming trapped.

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

Incubation
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.

Interpretation

<table>
<thead>
<tr>
<th>Organism</th>
<th>Colony size (mm)</th>
<th>Shape &amp; surface</th>
<th>Colour</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escherichia coli</td>
<td>0.5 - 1.5</td>
<td>CV.E.G.</td>
<td>Green*</td>
<td>Yellow if Glucuronidase -ve</td>
</tr>
<tr>
<td>Lactose fermenters e.g. Enterobacter aerogenes</td>
<td>0.5 – 1.5</td>
<td>CV.E.G.</td>
<td>Yellow</td>
<td></td>
</tr>
<tr>
<td>Non-lactose fermenters e.g. Salmonella spp.</td>
<td>0.5 – 1.5</td>
<td>CV.E.G.</td>
<td>Red</td>
<td></td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td></td>
<td></td>
<td></td>
<td>Inhibited</td>
</tr>
</tbody>
</table>

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

Minimum Q.C. organisms
Escherichia coli ATCC 25922 (green)
Enterobacter aerogenes coli ATCC 13048 (yellow)
Salmonella Typhimurium ATCC 14028 (red)
Enterococcus faecalis ATCC 29212 (inhibited)

References
