



HALO10

Harlequin™ *Listeria* Chromogenic Agar (ISO)

Description

Listeria Chromogenic Agar (according to the formulation of Ottaviani and Agosti) is a selective medium for the isolation and presumptive identification of *Listeria monocytogenes* from foodstuffs and related materials as described in ISO 11290-1:1997.

Lithium chloride in the base medium and supplementary antimicrobial compounds Ceftazidime, Polymyxin, Nalidixic acid and Cycloheximide provide the medium's selectivity. Chromogenic activity is as a result of a chromogenic substrate for the detection of the β -glucosidase enzyme, common to all *Listeria spp.* and to a few strains of Enterococci and Bacilli.

The specific differential activity of this agar is obtained with a proprietary lecithin substrate for the detection of the phospholipase enzyme that will only be present in the *L. monocytogenes* colonies growing on this media. This enzyme activity will result in a halo of precipitation surrounding the target colonies.

With the combination of both the chromogenic and phospholipase enzyme reactions, it is possible to differentiate *Listeria monocytogenes* (blue colonies surrounded by an opaque halo) from other *Listeria spp* (blue colonies without an opaque halo).

Formulation

	g/litre
Meat peptone	18.0
Tryptone	6.0
Yeast extract	10.0
Lithium chloride	10.0
Sodium chloride	5.0
Disodium hydrogen orthophosphate anhydrous	2.5
Sodium pyruvate	2.0
Glucose	2.0
Glycerophosphate	1.0
Magnesium sulphate	0.5
5-bromo-4-chloro-3-indolyl- β -D-glucoopyranoside	0.05
Agar	13.5
Grams per litre	70.5

Appearance

Powder: fine, free-flowing, homogeneous, buff
Finished medium: opaque, cream-yellow gel

pH: 7.2 \pm 0.2

Hazard classification

NR, Not regulated

Method for reconstitution

Weigh 70.5 grams of powder and disperse in 950mL of deionised water. Allow to soak for 10 minutes, swirl to mix and sterilise by autoclaving for 15 minutes at 121°C. Cool to 48-50°C, and add 2 vials of reconstituted X072 supplement. Swirl to mix. Add 2 vials of X010 supplement (**pre-heated to 48-50°C**). Mix well with gentle end-over-end mixing and dispense into Petri dishes. Dry the agar surface prior to use.



Storage

Dehydrated culture media: 10-25°C

Final medium: 7 days at 2-8°C in the dark

Inoculation

Surface inoculation - streak out to single colonies. This medium is highly selective and a heavy inoculum can be used.

Incubation

37°C aerobically for 48 hours.

Minimum Q.C. organisms

Listeria monocytogenes NCTC 11994

Listeria monocytogenes NCTC 10527

Escherichia coli ATCC 25922 (Inhibited)

Enterococcus faecalis ATCC 29212 (Inhibited)

Interpretation

Organism	Colony size (mm)	Colony shape	Colony Colour
<i>Listeria monocytogenes</i>	1-2	Round, regular	Blue to blue-green, surrounded by opaque halo
<i>Listeria</i> spp.	1-2	Round, regular	Blue to blue-green, without opaque halo

Isolates presumptively identified as *Listeria* spp. and *Listeria monocytogenes* must be subjected to further biochemical tests to confirm their identity. Some strains of *Listeria ivanovii* may demonstrate lecithinase activity.

References

ISO 11290-1:1997 Microbiology of food and animal feeding stuffs - Horizontal method for the detection of *Listeria monocytogenes* - Part 1: Detection method. Incorporating Amendment 1.

ISO/TS 11133-2:2003. Microbiology of food and animal feed stuffs- Guidelines on preparation and production of culture media – Part 2: Practical guidelines on performance testing of culture media.