CHROMOGENIC LISTERIA AGAR (ISO)

Code: CM1084

a medium for isolation, enumeration and presumptive identification of Listeria species and Listeria monocytogenes from food samples

Typical Formula*	**gm/litre**
Enzymatic digest of animal tissues	18.0
Enzymatic digest of casein	6.0
Sodium pyruvate	2.0
Glucose	2.0
Magnesium glycerophosphate	1.0
Magnesium sulphate (anhydrous)	0.5
Sodium chloride	5.0
Yeast extract	10.0
Lithium chloride	10.0
Disodium hydrogen phosphate (anhydrous)	2.5
X-glucoside chromogenic mix	0.05
Agar	12.0
Final pH 7.2 ± 0.2 @ 25°C	

*** Adjusted as required to meet performance standards**

OCLA (ISO) SELECTIVE SUPPLEMENT

Code: SR0226

Typical Formula	**SR0226E** (1 vial per 500ml medium)	**per litre**
Nalidixic acid	10.0mg	20.0mg
Polymyxin B	38,350IU	76,700IU
Ceftazidime	10.0mg	20.0mg
Amphotericin	5.0mg	10.0mg

OCLA (ISO) DIFFERENTIAL SUPPLEMENT†

Code: SR0244

Typical Formula	SR0244E (1 vial per 500ml medium)	per litre
L-α-phospotidylinositol solution	20.0ml	40.0ml

BRILLIANCE LISTERIA DIFFERENTIAL SUPPLEMENT†

Code: SR0228

Typical Formula	SR0228E (1 vial per 500ml medium)	per litre
Lecithin solution	20.0ml	40.0ml

† Please use OCLA (ISO) Differential Supplement (SR0244) or *Brilliance* Listeria Differential Supplement (SR0228), in accordance with method being followed

Directions

Modified ISO Formulation

Suspend 34.5g of Chromogenic Listeria Agar (ISO) in 480ml of distilled water. Mix well and sterilize by autoclaving at 121°C for 15 minutes. Cool the medium to 46°C and add one vial of Chromogenic Listeria Selective Supplement (ISO), reconstituted as directed and one vial of *Brilliance* Listeria Differential Supplement. Mix well and pour into sterile Petri dishes.

ISO Formulation

Suspend 34.5g of Chromogenic Listeria Agar (ISO) Base in 480ml of distilled water. Mix well and sterilise by autoclaving at 121ºC for 15 minutes. Cool the medium to around 50ºC, and add one vial of Chromogenic Listeria Selective Supplement (ISO), re-suspended as directed, and one vial of OCLA (ISO) Differential Supplement. Mix well and pour into sterile Petri dishes.

Description

Listeria monocytogenes is the most common pathogenic *Listeria* spp. and has been shown to be pathogenic to both man and animals. Some *Listeria ivanovii* strains also possess these enzymes and, although *Listeria ivanovii* are primarily pathogenic to animals, there are strains which have been shown to cause infection in humans[2]. Studies have shown this medium to be superior to PALCAM and Oxford medium for the isolation of *Listeria monocytogenes*[3].

OCLA (ISO) uses the chromogen X-glucoside for presumptive identification of *Listeria* spp. This chromogen is cleaved by ß-glucosidase, which is common to all *Listeria* species. Other organisms that possess this enzyme, such as enterococci, are inhibited by the selective agents within the medium: lithium chloride, polymyxin B and nalidixic acid, whilst amphotericin inhibits the growth of yeasts and moulds that may be present in the sample. *Listeria monocytogenes* and pathogenic *Listeria ivanovii* are then further differentiated by their ability to produce the phospholipase enzymes

PIPLC and PCPLC which hydrolyse phosphotidyliositol or lethicin in the medium, producing an opaque white halo around the colony.

This medium is designed to identify *Listeria* spp. based on their utilisation of a chromogenic substrate. OCLA Base (ISO), when used with OCLA (ISO) Selective Supplement (SR0226) and OCLA (ISO) Differential Supplement (SR0244) following the manufacturer's instructions, conform to the formulation described by Ottaviani and Agosti (ALOA) in ISO 11290-1:1997 (Incorporating Amendment No.1)[1]. This ALOA formulation incorporates phosphatidylinositol so that phosphatidylinositol phospholipase C (PIPLC), produced by *Listeria monocytogenes*, is detected. Alternatively, adding lecithin (*Brilliance* Listeria Differential Supplement (SR0228)) instead of phosphatidylinositol means that phosphatidylcholine phospholipase C (PCPLC) activity can be detected. Both PIPLC and PCPLC are associated with the virulence of *Listeria* and, therefore, detection of either enzyme is a useful indicator of pathogenicity.

Technique
Chromogenic Listeria Agar (ISO) can be used following a variety of enrichment procedures i.e. ISO, NMKL, FDA, AFNOR (UNI 03/04 - 04/05[4]), etc.

The following method is a summary of ISO 11290-1:1997 (Incorporating Amendment No. 1):

1. Add 25g of food sample to 225ml of Half Fraser Broth CM0895 & SR0166 and stomach for a minimum of 30 seconds to mix the sample.
2. Incubate the broth without agitation at 30ºC for 24 ± 3 hours.
3. Gently agitate the bag then, using a microbiological loop inoculate onto Chromogenic Listeria Agar (according to Ottaviani and Agosti) and a second selective medium (e.g. PALCAM Agar - CM0877 & SR0150). Incubate at 37ºC for 24h ± 3h, and if necessary for an additional 24h ± 3h (PALCAM Agar should be incubated under micro-aerobic conditions for best results).
4. Examine the PALCAM plate for black colonies and the OCLA plate for blue colonies with and without halos.
5. From the same incubated Half Fraser Broth remove 0.1ml and inoculate into 10ml of Fraser Broth CM0895 & SR0156. Incubate at 37ºC for 48h ± 3h and then repeat Steps 3 & 4 followed by step 6.
6. Confirm presumptive colonies on the agar plates as *Listeria monocytogenes* or *Listeria* spp. by appropriate methods - refer to ISO 11290-1:1997 (Incorporating Amendment No.1)[1].

Storage conditions and Shelf life
The dehydrated medium should be stored at 10-30°C and used before the expiry date on the label. Prepared medium may be stored for up to 2 weeks at 2-8°C.

Appearance:
Dehydrated medium: Straw coloured, free-flowing powder
Prepared medium: Translucent cream gel

Quality control

Positive controls:	**Expected results**
Listeria monocytogenes ATCC®7644 *	Good growth: blue/green plus halo

Listeria innocua ATCC®33090 *	Good growth: blue/green no halo
Negative contol:	
Enterococcus feacalis ATCC®29212 *	Inhibited

*** This organism is available as a Culti-Loop®**

Reference

1. ISO 11290-1:1997 (Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of *Listeria monocytogenes* - part 1, Incorporating Amendment 1.)
2. Cummins, A.J., Fielding, A.K. and McLauchlin, J. (1994) *Listeria ivanovii* infection in a patient with AIDS. Journal of Infection 28, p89-91
3. Data on file at Oxoid.