LISTERIA SELECTIVE AGAR (OXFORD FORMULATION)

Code: CM0856

A selective and diagnostic medium for the detection of Listeria monocytogenes, when prepared from Listeria Selective Agar Base and Listeria Selective Supplement SR0140 or Modified Listeria Selective Supplement (Oxford) SR0206

Typical Formula*	**gm/litre**
Columbia Blood Agar Base	39.0
Aesculin	1.0
Ferric ammonium citrate	0.5
Lithium chloride	15.0
pH 7.0 ± 0.2 @ 25°C	

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

LISTERIA SELECTIVE SUPPLEMENT (OXFORD FORMULATION)

Code: SR0140

Vial contents (each vial is sufficient for 500ml of medium)	**per vial**	**per litre**
Cycloheximide	200mg	400mg
Colistin sulphate	10.0mg	20.0mg
Acriflavine	2.5mg	5.0mg
Cefotetan	1.0mg	2.0mg
Fosfomycin	5.0mg	10.0mg

MODIFIED LISTERIA SELECTIVE SUPPLEMENT (OXFORD)

Code: SR0206

Vial contents (each vial is sufficient for 500ml of medium)	**per vial**	**per litre**
Amphotericin B	5.0mg	10.0mg
Colistin sulphate	10.0mg	20.0mg
Acriflavine	2.5mg	5.0mg
Cefotetan	1.0mg	2.0mg
Fosfomycin	5.0mg	10.0mg

Directions

Suspend 27.75 of the Listeria Selective Agar Base (Oxford Formulation) in 500ml of distilled water. Bring gently to the boil to dissolve. Sterilise by autoclaving at 121°C for 15 minutes. Cool to 50°C and aseptically add the contents of one vial of Listeria Selective Supplement (Oxford Formulation) or Modified Listeria Selective Supplement (Oxford) SR0206, SR0140 reconstituted with 5ml of 70% ethanol. Mix well and pour into sterile Petri dishes.

Description

Listeria Selective Medium (Oxford Formulation) is based on the formulation described by Curtis *et al*.[9] and is recommended for the detection of *Listeria monocytogenes* from clinical and food specimens.

The medium utilizes:

(i) the selective inhibitory components lithium chloride, acriflavine, colistin sulphate, cefotetan, cycloheximide or amphotericin B and fosfomycin,

(ii) the indicator system aesculin and ferrous iron for the isolation or differentiation of *Listeria monocytogenes*.

Listeria monocytogenes hydrolyses aesculin, producing black zones around the colonies due to the formation of black iron phenolic compounds derived from the aglucon.

Gram-negative bacteria are completely inhibited. Most unwanted Gram-positive species are suppressed, but some strains of *enterococci* grow poorly and exhibit a weak aesculin reaction, usually after 40 hours incubation. Some *staphylococci* may grow as aesculin-negative colonies.

Typical *Listeria monocytogenes* colonies are almost always visible after 24 hours, but incubation should be continued for a further 24 hours to detect slow-growing strains.

Techniques for isolation vary with the author and the material under examination[10,11]. For all specimens selective enrichment and cold enrichment have been shown to increase isolation rates significantly [12,13,14]. The efficacy of Listeria Selective Medium (Oxford Formulation) has been confirmed for various foods[15,16] following the methodology and using selective enrichment media described in the literature[16,17,18,19]. Oxford agar is a specified plating medium in the FDA/BAM isolation procedure[20] and in the standardised testing methods of other national and international bodies [21].

Oxford agar base was used by Al-Zoreki and Sandine as the basal medium for their ASLM agar which incorporates ceftazidime, moxalactam and cycloheximide as selective agents [22].

Technique

Faecal and biological specimens

The sample is homogenised in 0.1% Peptone Water CM0009 (1 part to 9 parts peptone water).

Direct surface plate method

1. Inoculate 0.1ml of the homogenised specimen onto the Listeria Selective Medium plates.

2. Incubate at 35°C for up to 48 hours.

3. Examine for typical colonies of Listeria after 24 and 48 hours incubation.

Selective Enrichment Method

1. Add the homogenised specimen to the selective enrichment broth and incubate at 30°C for up to 7 days.
2. Inoculate 0.1ml of the selective enrichment broth, after 24 hours, 48 hours and 7 days, onto the Listeria Selective Medium plates.
3. Incubate the plates at 35°C for up to 48 hours.
4. Examine for typical colonies of Listeria after 24 and 48 hours incubation.

Food and Environmental Samples

Techniques for isolation vary with the author, material and authorities. For detection of *Listeria monocytogenes* when present in small numbers, the test samples must be inoculated into an enrichment broth to allow multiplication before isolation and identification. Depending on the type of sample under test, an appropriate method and selective enrichment broth should be chosen prior to inoculation onto the Listeria Selective Medium plates.

1. Inoculate 0.1ml of the selective enrichment broth onto the Listeria Selective Medium plates.
2. Incubate at 35°C for up to 48 hours.
3. Examine for typical colonies after 24 and 48 hours incubation.

Colonies presumptively identified as *Listeria monocytogenes* must be confirmed by biochemical and serological testing [23].

Note

Differences in susceptibility of *Listeria monocytogenes, Listeria seeligeri* and *Listeria ivanovii* to β-lactam antibiotics and fosfomycin have been observed dependent on whether incubation is at 30°C or 35-37°C [24].

Storage conditions and Shelf life

Store the dehydrated medium at 10-30°C and use before the expiry date on the label.
Store the prepared plates at 2-8°C in the dark.

Appearance

Dehydrated medium: Straw-coloured, free-flowing powder
Prepared medium: Pale green-coloured gel

Quality control

Positive control:	**Expected results**
Listeria monocytogenes ATCC® 7644 *	Good growth; brown coloured colonies with aesculin hydrolysis
Negative control:	
Entercoccus feacalis ATCC® 29212 *	No growth

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

Precautions

Listeria monocytogenes is in ACDP Group 2 i.e. `might be a hazard to laboratory workers' and should be handled in a suitable environment only. It is also recommended that pregnant staff should be excluded from working with known cultures of *Listeria.*

Listeria media containing acriflavine should be protected from light because photo-oxidation makes it inhibitory to *Listeria.*

Supplement SR0140 used in this medium contains a toxic concentration of cycloheximide. Note the precautions to be taken under HAZARDS.

References

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