TECHNICAL DATA SHEET

OXFORD AGAR

DETECTION OF *LISTERIA*

1 INTENDED USE

Oxford Agar is a selective medium used for the isolation and the enumeration of *Listeria monocytogenes* and other *Listeria* in food samples, even highly contaminated.

2 HISTORY

The medium was prepared by Curtis *et al.* in 1988 for the isolation of *Listeria monocytogenes* from clinical samples containing considerable contaminating microflora. In most cases, the authors observed that *Listeria* colonies appeared within 24 hours of incubation and that associated microorganisms were inhibited. The studies were based on the work of Rodriguez (1984), who was the first to use esculin and iron salts to visualize *Listeria monocytogenes* by its esculinase-positive character. Many selective media for *Listeria* containing esculin, however, also enable enterococci to grow. Curtis *et al.* showed that secondary microflora was inhibited by lithium chloride, acriflavin, cycloheximide, colistin, cefotetan and fosfomycin.

3 PRINCIPLES

Peptone favors the excellent growth of *Listeria*. Yeast

extract is a source of vitamin B complex.

Starch is the energy source for microbial development.

Sodium chloride maintains osmotic balance.

Listeria hydrolyze esculin to glucose and esculetin, the latter compound forming a black complex with ferric ions supplied by ferric citrate.

The inhibitor mixture consists of three antibiotics (colistin, cefotetan, and fosfomycin), an antifungal agent, lithium chloride, and an antiseptic dye (acriflavine).

4 TYPICAL COMPOSITION

The composition can be adjusted / supplemented in order to achieve optimal performance.

For 1 liter of complete media :

- Peptone ..... 23.0 g
- Starch ..... 1.0 g
- Sodium chloride ..... 5.0 g
- Esculin ..... 1.0 g
- Ferric ammonium citrate ..... 0.5 g
- Lithium chloride ..... 15.0 g
- Cycloheximide* ..... 400.0 mg
- Colistine sulfate ..... 20.0 mg
- Cefotetan ..... 2.0 mg
- Fosfomycin ..... 10.0 mg
- Acriflavin ..... 5.0 mg
- Bacteriological agar ..... 13.0 g

pH of the ready-to-use media at 25 °C : 7.0 ± 0.2.

For a vial of supplement BS106

- Colistine (sulfate) ..... 10.0 mg
- Cefotetan ..... 1.0 mg
- Fosfomycin ..... 5.0 mg

For 58,5 g of dehydrated base media BK250

- Peptones ..... 23.0 g
- Starch ..... 1.0 g
- Sodium chloride ..... 5.0 g
- Esculin ..... 1.0 g
- Ferric ammonium citrate ..... 0.5 g
- Cycloheximide* ..... 400.00 mg
- Acriflavin ..... 5 mg
- Lithium chloride ..... 15.0 g
- Bacteriological agar ..... 13.0 g

*Cycloheximide or another equivalent molecule.

5 PREPARATION

Preparation of base medium

- Dissolve 58.5 g of dehydrated media (BK250) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense 100 mL per vial.
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain in a molten state at 44-47 °C

✓ **Reconstitution:**
58.5 g/L

✓ **Sterilization:**
15 min at 121 °C

Preparation of complete medium

- Rehydrate the supplement BS106 with 5 mL of sterile distilled water.
- Mix or vortex to ensure complete dissolution, avoiding the formation of foam.
- Aseptically add 1 mL of the supplement per 100 mL of base.
- Mix well.
- Pour into sterile Petri plates.
- Let solidify on a cold, flat surface.

✓ **Rehydratation:**
5 mL water

✓ **Add to base:**
1 mL / 100 mL

6 INSTRUCTIONS FOR USE

- Dry the plates in an incubator with the covers partially removed.
- Streak for isolation on the surface of the plates, using a loop of selective enrichment broth.
- Incubate at 37 ± 1 °C for 24 to 48 hours.

✓ **Inoculation:**
Surface streaking

✓ **Incubation:**
24 h to 48 h at 37 °C

Note:
For foods only slightly contaminated by a secondary flora, the media can be incubated at 30°C or at 35°C.

7 RESULTS

After 24 hours of incubation, *Listeria monocytogenes* forms olive-green colonies surrounded by a black halo. After 48 hours, they become darker with a hollow black center and are surrounded by black zones.
Oxford Agar is a highly selective medium, but it is sometimes possible to observe colonies of staphylococci or enterococci (which grow slowly, giving a weak yellow or black color, generally after 30 to 40 hours of incubation).

See ANNEX 1: PHOTO SUPPORT

8 QUALITY CONTROL

Dehydrated base media: beige powder, free-flowing and homogeneous.
Supplement: cream color, freeze-dried, giving after reconstitution a clear, colorless solution.
Prepared (complete) media: yellow-green agar with slight blue reflections.

Typical culture response after 24-48 hours of incubation at 37 °C:

Microorganisms		Growth (Productivity Ratio : P_R)	Characteristics
Listeria monocytogenes 4b	WDCM 00021	$P_R \geq 50$ %	Green-olive colonies with black halo
Listeria monocytogenes	WDCM 00109	$P_R \geq 50$ %	Green-olive colonies with black halo
Escherichia coli	WDCM 00013	Inhibited, score 0	-
Enterococcus faecalis	WDCM 00087	Inhibited, score 0	-

9 STORAGE / SHELF LIFE

Dehydrated base medium: 2-30 °C.
Selective supplement: 2-8 °C.
The expiration dates are indicated on the labels.

Prepared base media in vials (*): 180 days at 2-8 °C.
Prepared (complete) media in plates (*): 30 days at 2-8 °C.
Reconstituted freeze-dried supplement (*): 7 days at 2-8 °C, shielded from light.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated medium : Oxford agar base
500 g bottle..... BK250HA

Selective Supplement for Oxford agar:
10 vials qsp 500 mL BS10608

11 BIBLIOGRAPHY

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Tiwari, N.P. and Aldenrath S.G. 1990. Isolation of *Listeria monocytogenes* from Food Products on Four Selective Plating Media. Journ. of Food Protection, 53: 382-385.

Journal Officiel du 7 Avril 1992. Contrôle microbiologique des produits végétaux ou d'origine végétale. (Arrêté du 13 mars 1992).

NF EN ISO 11290-1. July 2017. Microbiology of the food chain — Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp.Part 1: Detection method

12 ADDITIONAL INFORMATION

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

Document code : OXFORD AGAR_BK250_BS106_v1(en)
Creation date : 01-2026
Updated :
Origin of revision :

ANNEX 1 : PHOTO SUPPORT

OXFORD Agar

Detection and enumeration of *Listeria*

Results :

Growth obtained after 48 hours of incubation at 37 °C.

Listeria monocytogenes

Characteristic colony :
Green-olive color with a black, concave center, surrounded by a black halo.