TECHNICAL DATA SHEET

OXFORD AGAR

DETECTION OF *LISTERIA*

1 INTENDED USE

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

The media can be used as the second media of choice in the context of the method of detection of *Listeria monocytogenes* in food microbiology NF EN ISO 11290-1.

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

Polypeptone 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.

Contaminating microflora is inhibited by lithium chloride, cycloheximide, colistin, cefotetan, fosfomycin and acriflavin).

4 TYPICAL COMPOSITION

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

For 1 liter of complete media :

- Polypeptone ..... 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 BS003

- Cycloheximide	200,0 mg
- Colistine (sulfate)	10,0 mg
- Cefotetan	1,0 mg
- Fosfomycin	5,0 mg
- Acriflavin	2,5 mg

For 58,5 g of dehydrated base media BK110

- Polypeptones ..... 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
- Bacteriological agar..... 13,0 g

5 PREPARATION

- Dissolve 58,5 g od dehydrated media (BK110) in 1 liter of distilled or demineralized water.
- Slowly bring to boiling, stirring with constant agitation until complete dissolution.
- Dispense 100 mL in 150 mL vials.
- 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

- Rehydrate the supplement BS003 with 5 mL of a 1 :1 solution of ethanol / sterile distilled water.
- Mix or vortex to insure 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 1 :1 ethanol / 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 : yellow pellet, giving rise after reconstitution to a yellow-green fluorescent 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 00020	$P_R \geq 50$ %	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 media : 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 media : Oxford agar base
500 g bottle BK110HA

CCCFA Selective Supplement for Oxford agar :
10 vials qsp 500 mL BS00308

11 BIBLIOGRAPHY

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

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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_ENv10.
Creation date : 01-2003
Updated : 02-2018
Origin of revision : Bibliography

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.