

Oxford/Agar

356-3664

DEFINITION

A selective medium for the isolation of *Listeria* spp. in clinical sample of food.

STANDARDS

FOOD MICROBIOLOGY

- **NF EN ISO 11290-1/A1 (February 2005):** Food microbiology - Horizontal method for the detection and enumeration of *Listeria monocytogenes* - Method of detection (IC: V 08-028-1).
- **FIL 143A standard (1995):** Milk and dairy products - Detection of *Listeria monocytogenes*.
- **Method for the enumeration of *Listeria monocytogenes* in vegetable products or products of vegetable origin** (BOCCRF of 30 May 1992, amended 5 September 1992).

PRINCIPLE

OXFORD medium is used as a selective isolation medium for the detection of *Listeria* spp. in food products and clinical samples. Due to the combined action of lithium chloride and the selective supplement, this medium inhibits non-*Listeria* flora.

On this medium, at 37°C, in 24 hours *Listeria* spp. form shiny gray or gray-green colonies of about 1 mm diameter, surrounded by a brown-black halo (esculin hydrolysis). After 48 hours incubation, typical colonies with a diameter of approximately 2 mm are encrusted in the agar and present a central depression.

PRESENTATION

Pre-poured

20 dishes x 90 mm

code 356-3664

STORAGE

- Pre-poured: + 2°C to 8°C in the dark.
- Dehydrated base: +15°C to 25°C, in carefully-sealed bottles in a cool, dry place.
- Freeze-dried selective supplement: + 2°C to 8°C in the dark.
- Expiration date and batch number are shown on the package.

THEORETICAL FORMULA

Complete medium

Peptone	23 g
Starch	1 g
Sodium chloride	5 g
Esculin	1 g
Ferric ammonium citrate (II)	500 mg
Lithium chloride	15 g
Agar	12 g
Distilled water	1,000 ml
Cycloheximide	400 mg
Colistin sulfate	20 mg
Acriflavine HCl	5 mg
Cefotetan	2 mg
Phosphomycin	10 mg

Final pH (25°C) = 7.0 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)

- Diluent(s)
- Distilled water

EQUIPMENT REQUIRED (NOT SUPPLIED) (non-exhaustive)

- Scales
- Sterile weighing bags
- Grinder
- Sterile Petri dishes (Ø= 90 mm)
- Vortex-type shaker
- Sterile, disposable platinum loop or inoculating loop
- Water-bath precise to ± 1°C
- Thermostatically-controlled incubator or incubation room
- Autoclave

PROTOCOL

Inoculation and incubation

Using a sterile inoculating loop, remove a drop of enrichment broth at the end of incubation and isolate on the surface of the Oxford agar. Incubate at 37°C ± 1°C for 18-24 hours, and if necessary prolong incubation to 48 hours.

READING AND INTERPRETATION

After incubation, *Listeria* spp. forms shiny gray or gray-green colonies surrounded by a brown-black halo, of about 1 mm diameter (24 hours incubation) or of 2 mm diameter (48 hours incubation).

To verify membership of the genus *Listeria* and to determine the species, select 5 typical colonies (if there are fewer, select them all) and proceed with the identification tests summarized in Tables I and II.

	GRAM	CATALASE	MOBILITY
GENUS <i>Listeria</i>	+	+	+

Table I: Test of adherence to the genus *Listeria*.

	1	2	3	4	5	6	7
<i>L. monocytogenes</i>	+	-	+	-	+	-	-
<i>L. innocua</i>	-	-	-	-	V	-	-
<i>L. ivanovii</i>	+	+	-	+	-	-	-
<i>L. welshimeri</i>	-	-	-	+	V	-	-
<i>L. seeligeri</i>	+	-	+	+	-	-	-
<i>L. grayi</i>	-	-	-	-	-	+	-
<i>L. murrayi</i>	-	-	-	-	-	+	+

Table II : Identification of *Listeria* species.

Legend:

- 1: HEMOLYSIS
- 2: C.A.M.P. TEST *R. equi*
- 3: C.A.M.P. TEST *S. aureus*
- 4: D-XYLOSE
- 5: L-RHAMNOSE
- 6: MANNITOL
- 7: REDUCTION OF NITRATES

V: variable reaction.
 +: over 90% positive reactions.
 -: no reaction.

PRECAUTIONS

- The selective supplement contains toxic products. It is recommended that protective clothing be worn during its preparation (gloves and safety glasses).
- Comply with Good Laboratory Practice.

QUALITY CONTROL OF MANUFACTURER

Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from the reception of raw materials to the marketing of the end-product. Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.

Documentation relative to the production and control of each batch is kept on file.

PERFORMANCES / QUALITY CONTROL OF THE TEST

The growth performances of the media are verified with the following strains:

STRAINS	Results after 24h culture at 37°C
<i>Listeria monocytogenes</i> 1/2a ATCC 19111	Gray to black colonies with black halo PR ≥ 0.5
<i>Listeria monocytogenes</i> 4b ATCC 13932	Gray to black colonies with black halo PR ≥ 0.5
<i>C. albicans</i> ATCC 10231	No culture
<i>Escherichia coli</i> ATCC 25922	No culture
<i>Enterococcus faecalis</i> ATCC 19433	No culture

KEY WORDS

Oxford / *Listeria* / Food products / Detection / Medium.

BIBLIOGRAPHY

- **CURTIS G.D.W., MITCHELL R.G., KING A.F. and GRIFFIN E.J. (1989):** J. Lab. Clin. Méd 44: 301.
- **CURTIS G.D.W., NICHOLS W.W. and FALLA T.J. (1989):** *Letters in Applied Microbiology*. 8: 169-172
- **PRENTICE G.A. and NEAVES P. (1988):** *Bulletin of the International Dairy Federation*: 223
- **VAN NETTEN P., VAN DE VEN A., PEDERALE I. and MOSSEL D.A.A. (1988):** *International Journal of Food Microbiology*. 6: 187-198
- **LOVETT J., FRANCIS D.W. and HUNT J.M. (1987):** *Journal of Food Protection*.50: 188-192.