

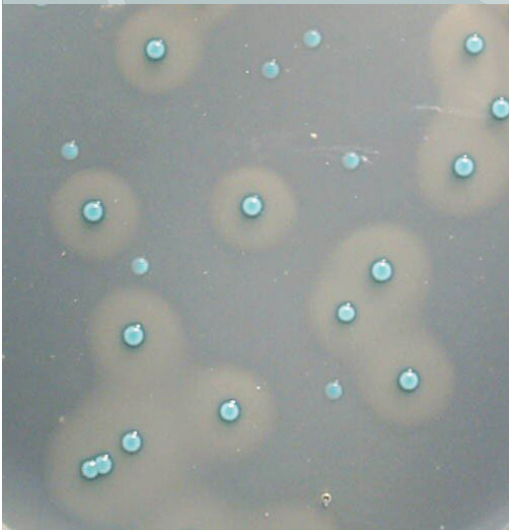


Rapid Culture Method

Listeria Precis

A quick and easy method for the enrichment, detection, enumeration and confirmation of *Listeria monocytogenes* from food, animal feed and environmental samples.

- Validated by AFNOR to ISO 16140 standard
- Simple and easy procedure – no specialized equipment required
- Single 24-hour enrichment
- Single sample transfer
- Single 24-hour plate incubation
- Quick and convenient confirmation: O.B.I.S. mono test or ISO 11290 standard tests
- Reduced time to result: 2 days compared with up to 7 days for standard culture and confirmation



Introduction

The Oxoid Listeria Precis™ method combines the benefits of ONE Broth-Listeria, *Brilliance*™ Listeria Agar and the O.B.I.S. mono test to reduce time to result over conventional culture methods.

ONE Broth-Listeria is a highly nutritious medium for the recovery and growth of *Listeria* while inhibiting competing organisms. The balance between selective and nutritive properties allows recovery of even stressed *Listeria* cells while inhibiting the growth of most competing microflora that may be present in the sample.

Brilliance Listeria Agar is a chromogenic medium incorporating X-glucoside. This chromogen is cleaved by the enzyme-glucosidase, common to all *Listeria* species, giving rise to blue-green colonies. Other organisms that are positive for this enzyme are inhibited by the selective agents in the medium: lithium chloride, polymyxin B and nalidixic acid. Inclusion of amphotericin inhibits the growth of any yeasts and moulds present.

Listeria monocytogenes and pathogenic *L. ivanovii* are then further differentiated by their ability to produce the phospholipase enzyme, lecithinase. This enzyme hydrolyses lecithin in the medium, producing an opaque, white halo around the colony.

Protocol for Listeria Precis Method

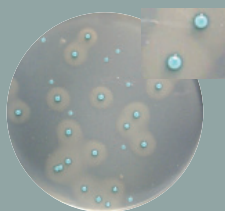
Day 0: Enrichment

25g or 25mL of sample + 225mL ONE Broth-Listeria
Incubate for 24 ± 2 hours at 30°C



Day 1: Plating

Using a 10µL microbiological loop inoculate a single *Brilliance* Listeria plate. Incubate for 22–26 hours at 37°C



(for meat samples re-incubate plates that show no blue colonies with halos for a further 22–26 hours at 37°C)

Select green-blue colonies with halos for confirmation

Day 2: Results

If present, confirm blue/green colonies with halos as *L. monocytogenes* using the O.B.I.S. mono test. Alternatively, confirm using standard ISO methods.**



For *Listeria monocytogenes* enumeration:

Resuscitate any organisms present in the sample by adding 25g or 25mL to 225mL of Buffered Peptone Water and incubate for 1 hour at 20°C. Inoculate a single *Brilliance* Listeria plate with 100µL and incubate for 45–51 hours at 37°C. Inspect the plate for characteristic blue/green colonies with halos and count. Confirm using O.B.I.S. mono or alternatively, confirm using standard ISO methods.** Calculate CFU/g or CFU/mL of sample.

**If there is insufficient material to carry out an O.B.I.S. mono test, or if a mixed culture of *L. monocytogenes* and other *Listeria* species is suspected, first purify suspect colonies by sub-culture onto a second *Brilliance* Listeria plate.

Enzyme targeted	Colony colour/appearance		
	Blue	Blue + halo	Colourless or inhibited
<i>Listeria</i> spp.		<i>L. monocytogenes</i> and pathogenic <i>L. ivanovii</i>	Non- <i>Listeria</i>
β-glucosidase	+	+	-
Lecithinase	-	+	-

The O.B.I.S. mono test allows rapid differentiation of *L. monocytogenes* from other *Listeria* species. All *Listeria* species, with the exception of *L. monocytogenes*, possess the enzyme D-alanyl aminopeptidase. Its presence can be detected using the substrate, D-alanyl-7-amido-4-methylcoumarin (DALA), and colour developer, dimethylamino-cinnamaldehyde. O.B.I.S. mono produces a deep purple reaction if this enzyme is present.

AFNOR Validation

The Listeria Precis method has been validated and approved by AFNOR according to ISO 16140 standard against the reference methods ISO 11290 Part 1:1997 and Part 2:1997 incorporating Amendment 1:2004 for the detection and enumeration of *L. monocytogenes* in food, animal feed and environmental samples. AFNOR validation certificates **UNI 03/04 - 04/05** (Detection) and **UNI 03/05 - 09/06** (Enumeration) are available in PDF format from the AFNOR website www.afnor-validation.com

For flexibility, confirmation was validated using either the O.B.I.S. mono test or tests outlined in ISO 11290. Alternatively, biochemical panels, such as Microbact™ 12L or Remel RapID™ CB Plus Panel, may be used.

Enrichment/resuscitation media	SIZE/FORMAT	ORDER CODE
ONE Broth-Listeria in Bottles	10x225mL	BO1066S*
ONE Broth-Listeria in ReadyBags	3x3 litres	FR60031*
ONE Broth-Listeria Base	500g	CM1066B
ONE Broth-Listeria Supplement for 500mL	10 vials	SR0234E
ONE Broth-Listeria Supplement for 2.25 litres	10 vials	SR0234B
Buffered Peptone Water (ISO) in Bottles	10x225mL	BO1067S*
Buffered Peptone Water (ISO) in ReadyBags	4x3 litres	BM1104T*
Buffered Peptone Water	500g	CM0509B

Plating media

<i>Brilliance</i> Listeria (ready to use 90mm plates)	10 plates	PO1102A*
<i>Brilliance</i> Listeria Agar Base	500g	CM1080B
<i>Brilliance</i> Listeria Agar Base 2 litre pack	134.4g	CM1080E
<i>Brilliance</i> Listeria Selective Supplement for 500mL	10 vials	SR0227E
<i>Brilliance</i> Listeria Differential Supplement for 500mL	10 vials	SR0228E

Confirmation

O.B.I.S. mono Kit	60 tests	ID0600M
Microbact 12L	20 tests	MB1128A
Remel RapID CB Plus Panel	20 panels	R8311008*
Gram Stain Kit	250mLx4	R40080*
Microbact Oxidase Strips	50 tests	MB0266A
Tryptone Soya Broth	500g	CM0129B
Yeast Extract Powder	500g	LP0021B
Agar Bacteriological	500g	LP0011B
Blood Agar Base No. 2	500g	CM0271B
Defibrinated Sheep Blood	25mL	SR0051B*

Culti-Loops™ for CAMP Test

<i>Staphylococcus aureus</i> ATCC® 25923™†	5 loops	CL7010*
<i>Rhodococcus equis</i> ATCC® 6939™†	5 loops	CL5400*

Quality Control Organisms – Culti-Loops™

<i>Listeria monocytogenes</i> ATCC® 7644™†	5 loops	CL3970*
<i>Listeria innocua</i> ATCC® 33090™†	5 loops	CL9005*
<i>Escherichia coli</i> ATCC® 25922™†	5 loops	CL7050*
<i>Enterococcus faecalis</i> ATCC® 29212™†	5 loops	CL7030*

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* Check code and availability with your local Oxoid Representative

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