Thermo Scientific™ Listeria Precis™ Methods

Publication Number MAN0026538 Revision K

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. SDSs are available from **thermofisher.com/support**.

Intended use

Thermo Scientific™ Oxoid™ *Brilliance*™ Listeria Agar (ISO) complies with the formulation described by Ottaviani and Agosti (see "References" on page 22).

- Thermo Scientific™ Listeria Precis™ Detection Method allows the simultaneous detection of presumptive positive samples for *Listeria* species and *L. monocytogenes* within two days. The method enables negative products to be released at least 24 hours earlier than the reference methods.
- Thermo Scientific™ Listeria Precis™ Enumeration Method allows the enumeration of *Listeria* species and *L. monocytogenes* within two days. The method enables negative products to be released at least 24 hours earlier than the reference methods.

Flexibility is offered with the possible use of (1) two enrichment broths in the detection workflow, (2) surface and pour plating in the enumeration workflow, and (3) multiple confirmations. Note that it is possible to confirm presumptive results the same day or the next day after the recovery of characteristic colonies.

Both detection and enumeration workflows are meant for use in laboratories undertaking microbiological analysis.

Summary

Oxoid™ *Brilliance*™ Listeria Agar (ISO) is a chromogenic medium incorporating X-glucoside. This chromogen is cleaved by the enzyme beta-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, ceftazidime, and nalidixic acid. Inclusion of amphotericin inhibits the growth of any yeasts and molds present. *Listeria monocytogenes* colonies are then further differentiated by their ability to produce the phospholipase enzymes PIPLC and PCPLC which hydrolyse phosphatidylinositol or lethicin in the medium, producing an opaque white halo around the colony. Note that some pathogenic *L. ivanovii* strains can also produce halos, but these halos are smaller than the halos around *L. monocytogenes* colonies.

The Listeria Precis™ Detection Method combines the benefits of one single enrichment to the benefit of Oxoid™ *Brilliance*™ Listeria Agar (ISO). Simply streak 10 µL of the enrichment broth on the Oxoid™ *Brilliance*™ Listeria Agar (ISO) using a basic inoculation loop, with no need of pipetting a higher volume.

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The Listeria Precis™ Detection Method offers two enrichment broth options—the Thermo Scientific™ Oxoid™ ONE Broth–Listeria and the Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB).

This method reduces the time to result over standard culture methods for both negative and positive screening.

The Listeria Precis™ Enumeration Method offers flexibility and ease-of-use. Surface and pour plating can be run to facilitate the handling depending on the contamination levels.

The confirmation procedures can be performed on isolated characteristic colonies without the need of a purification step. Same-day results can be generated thanks to the Thermo Scientific™ PrecisCheck™ L. monocytogenes Kit and Thermo Scientific™ PrecisCheck™ Listeria species Kit, the Thermo Scientific™ Oxoid™ Biochemical Identification System (O.B.I.S.) Mono, the Thermo Scientific™ SureTect™ Listeria species PCR Assay, or the Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay (or equivalent) as described in the ISO 7218:2024 standard. The use of the Thermo Scientific™ Oxoid™ Microbact™ Listeria 12L Kit (or equivalent) or Rhamnose Test[1] provides next-day results. It is also possible to use any appropriate ISO 16140-6:2019 validated confirmation methods or any appropriate reference confirmation procedure (e.g. ISO 11290-1:2017, FDA BAM Chapter 10, USDA/FSIS MLG 8.13).

Media composition

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

The following compositions are for typical formulae. Adjustments might be required to meet performance standards.

Table 1 Thermo Scientific™ Oxoid™ ONE Broth–Listeria Base (Cat. No. CM1066B 500 g, CM1066R 2.5 kg)

Reagents	Concentration
Peptone	28.0 g/L
Salt mix	10.0 g/L
Carbohydrate mix	6.0 g/L (anhydrous)
pH 7.4 ± 0.2 at 25°C	

Table 2 Thermo Scientific™ Oxoid™ ONE Broth–Listeria Selective Supplement (Cat. No. SR0234B 10 vials each for 2.25 L, SR0234E 10 vials each for 500 mL)

Reagents	Concentration
The formulation of Oxoid™ ONE Broth–Listeria Selective Supplement is not published.	

[1] See note regarding the Rhamnose Test in “Confirm positive results”.

Table 3 Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Base (Cat. No. CM1107B)

Reagents	Concentration
Peptone	23.4 g/L
Yeast extract	5.0 g/L
Lithium chloride	10.0 g/L
Ferric ammonium citrate	0.1 g/L
Sodium chloride	5.0 g/L
pH 7.4 ± 0.2 at 25°C	

Table 4 Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Selective Supplement (Cat. No. SR0243E)

Vial contents	SR0243E (1 vial per 500 mL medium)	Per litre before sample addition
Polymyxin	5.0 mg	10.0 mg
Quinolone antimicrobials	17.5 mg	35.0 mg

Table 5 Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Complete (Cat. No. CM1154B)

Reagents	Concentration
Peptone	23.4 g/L
Yeast extract	5.0 g/L
Lithium chloride	10.0 g/L
Ferric ammonium citrate	0.1 g/L
Sodium chloride	5.0 g/L
Polymyxin	10.0 mg
Quinolone antimicrobials	35.0 mg
pH 7.4 ± 0.2 at 25°C	

Table 6 Thermo Scientific™ Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement (Cat. No. BO1339E; BO1204M)

Item	Source
Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement[1] • 24 × 10 mL • 10 × 100 mL	BO1339E BO1204M Available through the Thermo Fisher Microbiology ordering process

[1] This product may crystallize during storage. If crystals are present, place the tube in a 37°C water bath for 5–10 minutes, or until all of the crystals are dissolved.

Table 7 Thermo Scientific™ Oxoid™ *Brilliance*™ Listeria Agar (ISO) Base (Cat. No. CM1212)

Reagents	Concentration
Enzymatic digest of animal tissues	18.0 g/L
Enzymatic digest of casein	6.0 g/L
Yeast extract	10.0 g/L
Sodium pyruvate	2.0 g/L
Glucose	2.0 g/L
Magnesium glycerophosphate	1.0 g/L
Magnesium sulphate (anhydrous)	0.5 g/L
Sodium chloride	5.0 g/L
Lithium chloride	10.0 g/L
Disodium hydrogen phosphate (anhydrous)	2.5 g/L
5-Bromo-4-chloro-3-indolyl-ß-d-glucopyranoside	0.05 g/L
Agar	12.0 g/L
pH 7.2 ± 0.2 at 25°C	

Table 8 Thermo Scientific™ Oxoid™ *Brilliance*™ Listeria Agar (ISO) Selective Supplement (Cat. No. SR0257E; SR0257B)

Typical formulation	SR0257E (1 vial per 500 mL medium)	SR0257B (1 vial per 200 mL BO1370Z medium)	Per litre
Nalidixic acid sodium salt	10.0 mg	4.0 mg	20.0 mg
Polymyxin B sulphate	38,350 IU	15,340 IU	76,700 IU
Ceftazidime	10.0 mg	4.0 mg	20.0 mg
Amphotericin B	5.0 mg	2.0 mg	10.0 mg

Table 9 Thermo Scientific™ Oxoid™ *Brilliance*™ Listeria Agar (ISO) Differential Supplement (Cat. No. SR0258E; SR0258B)

Typical formulation	SR0258E (1 vial per 500 mL medium)	SR0258B (1 vial per 200 mL BO1370Z	Per litre
L-α-phosphatidylinositol solution	15.0 mL	6.0 mL	30.0 mL

Table 10 Thermo Scientific™ Oxoid™ prepared media and other media

Item	Source
Oxoid™ ONE Broth–Listeria Base in Bottles (10 × 225 mL)	BO1066S[1]
Oxoid™ ONE Broth–Listeria Base in Ready Bags (3 × 3 L)	FR60031[1]
Oxoid™ *Brilliance*™ Listeria Agar (ISO) (10 ready-to-use 90 mm plates)	PO1298A[1] or PO5332A[1]
Oxoid™ *Brilliance*™ Listeria Agar (ISO) (10 x 200 mL bottles)	BO1370Z[1] SR0257B[1] SR0258B [1]
Oxoid™ 24 Listeria Enrichment Broth (24 LEB) (10 × 225 mL in 250 mL PET sirop bottles)	BO1205S[1,2]
Oxoid™ 24 LEB Complete (Oxoid™ 24 Listeria Enrichment Broth (24 LEB) (Dehydrated) plus Oxoid™ 24 LEB Selective Supplement)	CM1154B
Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement[3] • 24 × 10 mL • 10 × 100 mL	BO1339E BO1204M
Oxoid™ Buffered Peptone Water ISO Formulation, Dehydrated	CM1211B, CM1049B, or equivalent
Thermo Scientific™ FitBag™ 24 Listeria Enrichment Broth • 30 × 2.7 L • 20 × 4.5 L • 10 × 9 L	DF1107A DF1107B DF1107C
Thermo Scientific™ QuickBag™ 24 Listeria Enrichment Broth • 3 × 2.7 L • 2 × 4.5 L	DQ1107A DQ1107B
Thermo Scientific™ Dry-Bags™ 24 Listeria Enrichment Broth (5 × 20 L)	DB1107V

[1] Check code and availability with your local Oxoid™ Representative.
[2] Includes 24 LEB Selective Supplement (SR0243E). Do not use in the Listeria Precis™ Enumeration Method.
[3] This product may crystallize during storage. If crystals are present, place the tube in a 37°C water bath for 5–10 minutes, or until all of the crystals are dissolved.

Table 11 Materials for confirmation testing

Item	Source
Thermo Scientific™ PrecisCheck™ L. monocytogenes Kit	LF0200A
Thermo Scientific™ PrecisCheck™ Listeria species Kit	LF0100A
Thermo Scientific™ Oxoid™ Biochemical Identification System (O.B.I.S.) Mono	ID0600M
Thermo Scientific™ Oxoid™ Microbact™ Listeria 12L Kit	MB1128A
Thermo Scientific™ Oxoid™ PALCAM Medium	PO5104A
Thermo Scientific™ SureTect™ Listeria monocytogenes PCR Assay	A56843
Thermo Scientific™ SureTect™ Listeria species PCR Assay	A56842

Required materials not supplied

- Inoculating loops, swabs, collection containers
- Incubators
- Quality control organisms

Prepare the materials

Note: Ready-to-use Thermo Scientific™ Oxoid™ prepared media can be used as well. See Table 10.

Prepare the Oxoid™ ONE Broth–Listeria

1. Suspend 22 g of Oxoid™ ONE Broth–Listeria Base (Cat. No. CM1066) in 500 mL (44 g/L) of distilled water.
2. Mix well, then sterilize by autoclaving at 121°C for 15 minutes.
3. Cool the medium to below 50°C.
4. Aseptically, add the contents of one vial of Oxoid™ ONE Broth–Listeria Selective Supplement (Cat. No. SR0234E), reconstituted as directed.

Prepare the Oxoid™ 24 Listeria Enrichment Broth (24 LEB)

1. Completely dissolve 21.75 g of Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Base (Cat. No. CM1107B) per 500 mL of distilled water.
2. Sterilize by autoclaving at 121°C for 15 minutes.
3. Cool to 50°C.
4. Aseptically, add the contents of one vial of Oxoid™ 24 LEB Selective Supplement (Cat. No. SR0243E) per 500 mL medium, reconstituted as directed.
5. Cool to room temperature before use.
   Store the prepared medium at 2–8°C in the dark.

Prepare the Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Complete

1. Completely dissolve 43.5 g of Oxoid™ 24 LEB Complete (Cat. No. CM1154B) in 1,000 mL of distilled water.
2. Mix well, then sterilize by autoclaving at 121°C for 15 minutes.
3. Cool to room temperature (23±5°C) before use.
   Once made, store out of direct sunlight.

Prepare the Oxoid™ *Brilliance*™ Listeria Agar (ISO) Base

1. Suspend 34.5 g of Oxoid™ *Brilliance*™ Listeria Agar (ISO) Base (Cat. No. CM1212) in 480 mL of distilled water.
2. Mix well and sterilize by autoclaving at 121°C for 15 minutes.
3. Cool the medium to 46°C.
4. Add one vial of Oxoid™ *Brilliance*™ Listeria Agar (ISO) Selective Supplement (Cat. No. SR0257E), reconstituted as directed, then add one vial of Oxoid™ *Brilliance*™ Listeria Agar (ISO) Differential Supplement (Cat. No. SR0258E).
5. Mix well and pour into sterile petri dishes.

Prepare the Oxoid™ *Brilliance*™ Listeria Agar (ISO) Bottle Media

1. Place the bottles containing 200 mL Oxoid™ *Brilliance*™ Listeria Agar (ISO) Base (Cat. No. BO1370Z) in a steamer for approximately 40–45 minutes to completely melt the agar until fully liquified.
2. Cool and maintain the molten agar in a water bath at 46±2°C.
3. Aseptically supplement each bottle with 1 vial of Oxoid™ *Brilliance*™ Listeria Agar (ISO) Selective Supplement (Cat. No. SR0257B), reconstituted as directed, then add one vial of Oxoid™ *Brilliance*™ Listeria Agar (ISO) Differential Supplement (Cat. No. SR0258B).
4. Thoroughly mix the supplements with the molten agar by capping the bottle, then gently invert 2 or 3 times until the colour is homogeneous.
5. After melting and/or supplementation, the molten agar can be maintained in the water bath at 44–46°C for up to 4 hours, after which time the media must be discarded.

Isolate *Listeria* species and *L. monocytogenes* from a broad range of foods and from environmental samples

Method certified EN ISO 16140-2:2016 by NF VALIDATION™ Certificate UNI 03/04-04/05 and UNI 03/14-06/22

Comply with Good Laboratory Practices (see EN ISO 7218:2024 standard).

The EN ISO 6887 series and the ISO 11290-1:2017 standard technical rules are optional for the preparation of the initial suspensions.

1. Enrich the samples as follows:

Target	Matrices	Media	Incubation
L. monocytogenes	Broad range of foods (25 g)	Add up to 25 g of sample to 225 mL (1-in-10) of Oxoid™ ONE Broth–Listeria.	30±1°C for 25±3 hours
		Add up to 25 g of sample to 225 mL (1-in-10) of Oxoid™ 24 Listeria Enrichment Broth (24 LEB). Then add 10 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement.[1]	37±1°C for 23±3 hours
	Up to 125 g dairy products (raw and pasteurized) and multi component foods (RTE, RTRH, pastries, and egg-based products)	Prepare 1-in-10 ratio of sample to pre-warmed media (37±1°C). • Add up to 125 g of sample to 1,125 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB). Then add 50 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement.[1]	37±1°C for 23±3 hours

(continued)

Target	Matrices	Media	Incubation
L. monocytogenes	Environmental samples	Prepare 1-in-10 ratio of sample to media. • Add up to 25 g of sample to 225 mL of Oxoid™ ONE Broth–Listeria. • Add one swab to 10 mL of Oxoid™ ONE Broth–Listeria. • Add one sponge to 100 mL of Oxoid™ ONE Broth–Listeria.	30±1°C for 25±3 hours
		Prepare 1-in-10 ratio of sample to media. • Add up to 25 g of sample to 225 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB). Then add 10 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement[1]. • Add one swab to 10 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB). Then add 0.44 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement[1]. • Add one sponge to 100 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB). Then add 4.4 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement[1].	37±1°C for 23±3 hours
Listeria species	Select foods (25 g): meat products (e.g. raw, ready-to-cook, RTE/RTRH), dairy products (e.g. raw and pasteurized), vegetables (raw, processed, cooked, frozen), fish and seafood products (e.g raw, processed, ready-to-eat)	Add up to 25 g of sample to 225 mL (1-in-10) of Oxoid™ ONE Broth–Listeria.	30±1°C for 25±3 hours
	Broad range of foods (25 g)	Add up to 25 g of sample to 225 mL (1-in-10) of Oxoid™ 24 Listeria Enrichment Broth (24 LEB). Then add 10 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement[1].	37±1°C for 23±3 hours

(continued)

Target	Matrices	Media	Incubation
Listeria species	Up to 125 g dairy products (raw and pasteurized) and multi component foods (RTE, RTRH, pastries, and egg-based products)	Prepare 1-in-10 ratio of sample to pre-warmed media (37±1°C). • Add up to 125 g of sample to 1,125 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB). Then add 50 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement.[1]	37±1ºC for 23±3 hours
	Environmental samples	Prepare 1-in-10 ratio of sample to media. • Add up to 25 g of sample to 225 mL of Oxoid™ ONE Broth–Listeria • Add one swab to 10 mL of Oxoid™ ONE Broth–Listeria • Add one sponge to 100 mL of Oxoid™ ONE Broth–Listeria	30±1ºC for 25±3 hours
		Prepare 1-in-10 ratio of sample to media. • Add up to 25 g of sample to 225 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB). Then add 10 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement[1]. • Add one swab to 10 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB). Then add 0.44 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement[1]. • Add one sponge to 100 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB). Then add 4.4 mL of Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement[1].	37±1ºC for 23±3 hours

[1] This product may crystallize during storage. If crystals are present, place the tube in a 37°C water bath for 5–10 minutes, or until all of the crystals are dissolved.

2. (Optional) Store enrichment broths or plates after incubation at 5±3ºC for up to 72 hours before streaking or reading.

Note: Storage of Oxoid™ ONE Broth–Listeria for the *L. monocytogenes* target was not covered in the validation; the user may validate the storage of the enrichment broth internally.

3. Gently agitate the bag, then, using a microbiological loop, inoculate 10 µL of the broth onto a plate of Oxoid™ *Brilliance*™ Listeria Agar (ISO) using a diminishing sweep technique to produce single colonies.

4. Incubate the plates at 37±1°C as described for 24±2 hours when using Oxoid™ ONE Broth–Listeria and at 37±1°C for 24±2 hours when using Oxoid™ 24 Listeria Enrichment Broth (24 LEB) (except for meat products when enriched in Oxoid™ ONE Broth–Listeria).

   Note: For meat samples enriched in Oxoid™ ONE Broth–Listeria, re-incubate the plates that show no blue-green colonies at 37±1°C for a further 24±2 hours.

Listeria species colonies grow as blue-green colonies. *Listeria monocytogenes* colonies will include the addition of a typical halo. Non-target organisms are either inhibited or grow as straw or white colonies.

Note: Some *L. ivanovii* strains can as well show a typical halo due to a positive lecithinase activity, but these halos are smaller than the halos around *L. monocytogenes* colonies.

Blue-green colonies are presumptive positive *Listeria* species, and blue-green colonies with halos are presumptive positive *L. monocytogenes*.

See “Confirm positive results” to confirm the observed characteristic colonies.

Listeria Precis™ Detection Method workflow

Broad range of food and production environmental samples

1-in-10 dilution in Oxoid™ ONE Broth–Listeria

- Up to 25 g of sample to 225 mL of Oxoid™ ONE Broth–Listeria
- Add one swab to 10 mL of Oxoid™ ONE Broth–Listeria
- Add one sponge to 100 mL of Oxoid™ ONE Broth–Listeria

▼

30±1°C for 25±3 hours

or

1-in-10 dilution in Oxoid™ 24 LEB

- Add up to 25 g of sample to 225 mL of Oxoid™ 24 LEB. Then add 10 mL of Oxoid™ 24 LEB Buffer Supplement.
- Add one swab to 10 mL of Oxoid™ 24 LEB. Then add 0.44 mL of Oxoid™ 24 LEB Buffer Supplement.
- Add one sponge to 100 mL of Oxoid™ 24 LEB. Then add 4.4 mL of Oxoid™ 24 LEB Buffer Supplement.

▼

37±1°C for 23±3 hours

Possibility to store for 72 hours at 5°C±3°C

▼

Streak 10 µL onto Oxoid™ *Brilliance*™ Listeria Agar (ISO)

▼

Incubate the plates at 37±1°C for 24±2 hours

For meat samples enriched in Oxoid™ ONE Broth–Listeria, re-incubate the plates that show no blue-green colonies at 37±1°C for a further 24±2 hours

▼

Possibility to store for 72 hours at 5°C±3°C

▼

Typical colonies

Blue-green colonies are presumptive positive *Listeria* species

Blue-green colonies with halo are presumptive positive *L. monocytogenes*

▼

PrecisCheck™ L. monocytogenes Kit or PrecisCheck™ Listeria species Kit

(pick depending on colony characteristic)

or

Oxoid™ Microbact™ Listeria 12L Kit biochemical galleries or equivalent

or

Oxoid™ Biochemical Identification System (O.B.I.S.) Mono

or

(continued)

Broad range of food and production environmental samples
Rhamnose Test for *L. monocytogenes* presumptive colonies. See note regarding the Rhamnose Test in "Confirm positive results".
or
EN ISO 11290-1:2017 confirmation procedure
or
Spot on Oxoid™ PALCAM for *Listeria* spp. presumptive colonies
or
Molecular hybridization test as described in EN ISO 7218:2024 (e.g. SureTect™ Listeria species PCR Assay and/or SureTect™ Listeria monocytogenes PCR Assay)
or
Any appropriate EN ISO 16140-6:2019 validated confirmation method (or any equivalent reference method procedure, e.g. FDA BAM, USDA/FSIS)

Enumerate *Listeria* species and *L. monocytogenes* from a broad range of foods and from environmental surfaces

Method certified EN ISO 16140-2:2016 by NF VALIDATION™ Certificate UNI 03/05-09/06 and UNI 03/15-12/22

Comply with Good Laboratory Practices (see EN ISO 7218:2024 standard).

For preparation of initial suspensions, it is recommended to follow the instructions of EN ISO 6887 series and EN ISO 11290-2:2017 standard.

1. Dilute the samples as follows:

Matrices	Media
10 g or 10 mL food samples and environmental samples	• Follow EN ISO 6887 series instructions and ISO 11290-2:2017 or • 1-in-10 ratio of sample to Oxoid™ 24 LEB (without selective supplement) with 4 mL of Oxoid™ 24 LEB Buffer Supplement[1]
25 g or 25 mL food samples and environmental samples	• Follow EN ISO 6887 series instructions and ISO 11290-2:2017 or • 1-in-10 ratio of sample to Oxoid™ 24 LEB (without selective supplement) with 10 mL of Oxoid™ 24 LEB Buffer Supplement[1]
Swab	• Follow EN ISO 6887 series instructions and ISO 11290-2:2017 or • Add one swab to 10 mL Oxoid™ 24 LEB (without selective supplement) with 0.44 mL of Oxoid™ 24 LEB Buffer Supplement[1]
Sponge	• Follow EN ISO 6887 series instructions and ISO 11290-2:2017 or • Add one sponge to 100 mL Oxoid™ 24 LEB (without selective supplement) with 4.4 mL of Oxoid™ 24 LEB Buffer Supplement[1]

[1] 24 LEB Buffer Supplement may crystallize during storage. If crystals are present, place the tube in a 37°C water bath for 5–10 minutes, or until all of the crystals are dissolved.

2. Gently agitate the bag, then transfer 100 µL onto one Oxoid™ *Brilliance*™ Listeria Agar (ISO) plate or transfer 1 mL of the enrichment onto three Oxoid™ *Brilliance*™ Listeria Agar (ISO) plates. Alternatively, transfer 1 mL of sample preparation into a 90 mm sterile petri dish and pour 20±2 mL of molten (45±1°C) Oxoid™ *Brilliance*™ Listeria Agar (ISO) into the petri dish.

3. Repeat this step for each dilution.

4. Incubate the plates at 37±1°C for 24–48 hours ± 3 hours. The plates can be read in 24 hours.

Note: It is possible to store the plates after incubation for 72 hours at 5°C±3°C before reading.

Listeria species colonies grow as blue-green colonies. *Listeria monocytogenes* colonies will include the addition of a typical halo. Non-target organisms are either inhibited or grow as straw or white colonies.

Note: Some *L. ivanovii* strains can as well show a typical halo due to a positive lecithinase activity, but these halos are smaller than the halos around *L. monocytogenes* colonies.

Blue-green colonies are presumptive positive *Listeria* species, and blue-green colonies with halos are presumptive positive *L. monocytogenes*.

See “Confirm positive results” to confirm the observed characteristic colonies.

Listeria Precis™ Enumeration Method workflow

Broad range of food and environmental samples

Follow EN ISO 6887 series instructions and ISO 11290-2:2017 instructions

or

1-in-10 ratio of sample to buffered Oxoid™ 24 LEB (without selective supplement)

- Add one swab to 10 mL of diluent
- Add one sponge to 100 mL of diluent

▼

1 mL onto three Oxoid™ *Brilliance*™ Listeria Agar (ISO) plates

or

1 mL into a 90mm sterile petri dish and add 20±2 mL of molten (45±1°C) Oxoid™ *Brilliance*™ Listeria Agar (ISO)

100 µL onto one Oxoid™ *Brilliance*™ Listeria Agar (ISO) plate

Repeat for each dilution

▼

Incubate the plates at 37±1ºC for 48±3 hours

Plates can be read at 24 hours

▼

Typical colonies

Blue-green colonies are presumptive positive *Listeria* species

Blue-green colonies with halo are presumptive positive *L. monocytogenes*

▼

Oxoid™ Microbact™ 12L biochemical galleries or equivalent

or

Oxoid™ Biochemical Identification System (O.B.I.S.) Mono

or

Rhamnose Test for *L. monocytogenes* presumptive colonies. See note regarding the Rhamnose Test in “Confirm positive results”.

or

PrecisCheck™ L. monocytogenes Kit or PrecisCheck™ Listeria species Kit

or

Spot on Oxoid™ PALCAM for *Listeria* spp. presumptive colonies

or

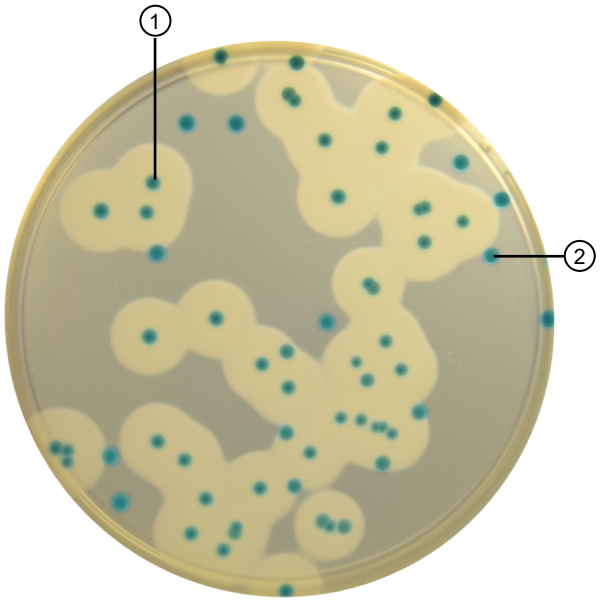
EN ISO 11290-1:2017 confirmation procedure

or

(continued)

Broad range of food and environmental samples
Molecular hybridization test as described in EN ISO 7218:2024 (e.g. SureTect™ Listeria species PCR Assay and/or SureTect™ Listeria monocytogenes PCR Assay)
or
Any appropriate EN ISO 16140-6:2019 validated confirmation method

Example results



① *Listeria monocytogenes* colony
② *Listeria* species (non-*L. monocytogenes* colony)

Figure 1 Example results—mixed culture

Confirm positive results

In the context of NF VALIDATION™, all samples identified as presumptive for *Listeria* species and/or *L. monocytogenes* shall be confirmed.

Confirmation is performed from isolated characteristic colonies on Oxoid™ *Brilliance*™ Listeria Agar (ISO) and running one of the five options below:

IMPORTANT! The instructions of the manufacturer must be followed for the selected confirmation test.

- Option 1: PrecisCheck™ L. monocytogenes Kit (Cat. No. LF0200A) or PrecisCheck™ Listeria species Kit (Cat. No. LF0100A) (pick depending on colony characteristic). See Appendix A, “Confirmation of presumptive colonies using PrecisCheck™ Listeria Kits” for more details.
- Option 2: Biochemical galleries, e.g. Oxoid™ Microbact™ Listeria 12L Kit (Cat. No. MB1128A).
- Option 3: Oxoid™ Biochemical Identification System (O.B.I.S.) Mono (Cat. No. ID0600M) that rapidly differentiates *Listeria monocytogenes* from other *Listeria* species in 6 minutes.
- Option 4: Perform a Rhamnose Test according to ISO 11290:2017 part 1 and part 2 standards. In most cases (particularly for microvolume tubes), positive reactions indicated by a color usually occur within 24–48 hours, but can take up to 5 days.

  Note: Within the 48 hours of plate incubation, some rare strains of *Enterococcus* present slow phosphatidylinositol activity and ferment the rhamnose as *L. monocytogenes*. A catalase test using an isolated blue colony with a halo, allows to differentiate *L. monocytogenes* and *L. ivanovii* (catalase positive) from *Enterococcus spp.* (catalase negative). For more information, the atypical characteristics of these *Listeria* strains are detailed in ISO 11290-1 (Appendix D).

- Option 5: For presumptive *Listeria* species colonies only, pick a well-isolated colony using a sterile loop and spot it onto a PALCAM agar plate by stabbing the PALCAM agar. Incubate the PALCAM plate at 37°C ± 1°C for 24 ± 2 hours.

In the context of ISO general rules, it is also possible to confirm the colonies with one of the following options:

1. Any appropriate EN ISO 16140-6:2019 validated method.
2. EN ISO 11290:2017 confirmation procedure.
3. Molecular hybridization as described in EN ISO 7218:2024 using for instance the SureTect™ Listeria species PCR Assay A56842) and SureTect™ Listeria monocytogenes PCR Assay (Cat. No. A56843) that are validated according to the ISO 16140-2:2016 standard (respectively NF VALIDATION™ certificate UNI 03/08-11/13 and NF VALIDATION™ certificate UNI 03/09-11/13).

In the event of discordant results (positive with the Listeria Precis™ Methods or non-confirmed by one of the means described above), the laboratory must follow the necessary steps to ensure the validity of the result obtained. See “Troubleshooting” on page 21.

Performance validation

Table 12 NF VALIDATION™ certification of the methods

Certification	Scope and expiration
BY AFNOR CERTIFICATION NF VALIDATION EN ISO 16140 UNI 03/04–04/05, UNI 03/14–06/22, UNI 03/05–09/06, UNI 03/15–12/22 ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS http://nf-validation.afnor.org/en	The NF VALIDATION™ certificate can be obtained from our Technical Support team. • (Europe: email: microbiology.techsupport.uk@thermofisher.com telephone: +44 (0)1256 694238) • AFNOR Certification (nf-validation.afnor.org/en) For more information about the validity of the NF VALIDATION™ certification, please see the certificates UNI 03/04-04/05 and UNI 03/14-06/22 for the detection method and UNI 03/05-09/06 and UNI 03/15-12/22 for the enumeration method available at nf-validation.afnor.org/en or obtain from our technical support team.

In the context of NF VALIDATION™, the Listeria Precis™ methods have been certified as alternative methods for the analysis of a broad range of foods and environmental samples. This validation has been obtained in comparison with the applicable reference method described in the international standard EN ISO 11290 according to EN ISO 16140-2:2016.

Appendix A Confirmation of presumptive colonies using PrecisCheck™ *Listeria* Kits

PrecisCheck™ L. monocytogenes Kit (Cat. No. LF0200A)

Confirmation is performed from isolated characteristic colonies on Oxoid™ *Brilliance*™ Listeria Agar (ISO):

1. Select 1 to 5 isolated presumptive colonies on the Oxoid™ *Brilliance*™ Listeria Agar (ISO) plate. For small colonies, select up to 5 isolated presumptive colonies.
2. Emulsify the colony (or colonies) in 300 µL of diluent (e.g. saline, BPW) inside a cluster tube.
3. Place the PrecisCheck™ *L. monocytogenes* test strip into inoculated cluster tube with the arrow facing down.
4. Incubate for 20 minutes at 20 to 25°C. Although some reactions may take up to 20 minutes, it is possible to obtain a positive result in 10 minutes.
5. Read the results as shown in Figure 2.

PrecisCheck™ Listeria species Kit (Cat. No. LF0100A)

Confirmation is performed from isolated characteristic colonies on Oxoid™ *Brilliance*™ Listeria Agar (ISO):

1. Select 1 to 5 isolated presumptive colonies on the Oxoid™ *Brilliance*™ Listeria Agar (ISO) plate. For small colonies, select up to 5 isolated presumptive colonies.
2. Emulsify the colony (or colonies) in 300 µL of diluent (e.g. saline, BPW) inside a cluster tube.
3. Heat for 5 to 15 minutes at 95–100°C (using a dry bath incubator or equivalent).
4. Cool to room temperature.
5. Place the PrecisCheck™ *Listeria* species test strip into inoculated, heat-treated, cluster tube with the arrow facing down.
6. Incubate for 20 minutes at 20 to 25°C. Although some reactions may take up to 20 minutes, it is possible to obtain a positive result in 10 minutes.
7. Read the results as shown in Figure 2.

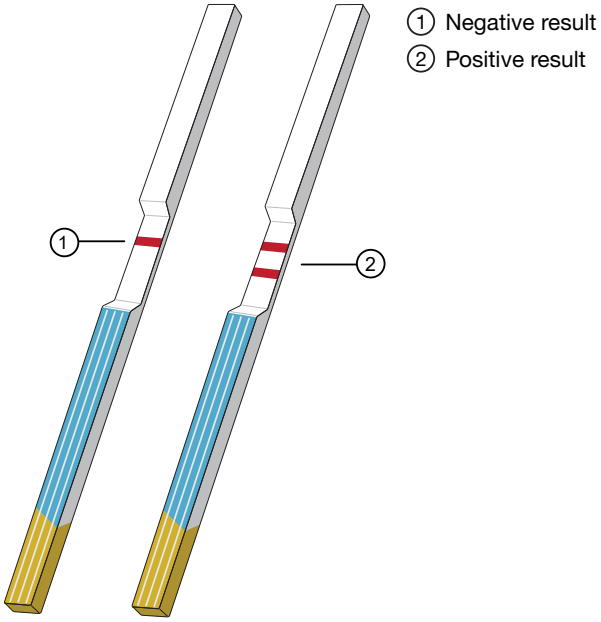


Figure 2 PrecisCheck™ Listeria test strip results

- No color line: the quality control is negative; something went wrong; repeat the test.
- One single color line: the quality control assesses the validity of the test and the result is negative.
- Two color lines: the quality control assesses the validity of the test and the result is positive.

Troubleshooting

Observation	Possible cause	Recommended action
The selected confirmation test does not match the *Brilliance*™ Listeria Agar (ISO) result.	The colony selected for the confirmation test may have shared phenotypic characteristics with typical *Listeria* colonies.	Pick another well-isolated suspect colony from the *Brilliance*™ Listeria Agar (ISO) plate and perform one of the alternative confirmation tests listed. If the colony fails to confirm as expected, follow the confirmation procedure outlined in ISO 11290-1:2017.
Suspect colonies on *Brilliance*™ Listeria Agar (ISO) are too small to conduct a confirmation test.	The isolate was sensitive to selective components in the medium or the lower limit of the incubation time was used.	Purify the well-isolated, suspect colony on a non-selective plating medium to increase biomass before continuing with confirmation.
Suspect colonies on *Brilliance*™ Listeria Agar (ISO) are not well isolated.	The enriched sample contained high levels of background flora that were not inhibited on *Brilliance*™ Listeria Agar (ISO).	Purify the suspect colonies on a second *Brilliance*™ Listeria Agar (ISO) plate.
There is an overgrowth of background microflora on *Brilliance*™ Listeria Agar (ISO) that may conceal target colonies.	Background microflora that were not susceptible to selective agents in the enrichment or plate due to species resistance or concentration of cells grew on the agar.	Sub-culture 100 µL of the retained enrichment into 10 mL of suitable selective *Listeria* enrichment broth (One Broth Listeria or 24 LEB or Fraser Broth). Incubate at 37°C for 24–28 hours before continuing with confirmation.

References

EN ISO 7218:2024. Microbiology of the food chain—General requirements and guidance for microbiological examinations.

EN ISO 6887-1:2017. Microbiology of the food chain—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—Part 1: General rules for the preparation of the initial suspension and decimal dilutions.

EN ISO 6887-2:2017. Microbiology of the food chain—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—Part 2: Specific rules for the preparation of meat and meat products.

ISO 6887-3:2017. Microbiology of the food chain—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—Part 3: Specific rules for the preparation of fish and fishery products.

EN ISO 6887-4:2017. Microbiology of the food chain—Preparation of test samples, initial suspension and decimal dilutions for microbiological examination—Part 4: Specific rules for the preparation of miscellaneous products.

EN ISO 6887-5:2020. Microbiology of the food chain— Preparation of test samples, initial suspension and decimal dilutions for microbiological examination— Part 5: Specific rules for the preparation of milk and milk products.

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

ISO 11290:1-2017. Microbiology of the food chain— Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp.— Part 1: Detection method. Appendix D - Reactions for the identification of *Listeria* species.

ISO 11290:2-2017. Microbiology of the food chain— Horizontal method for the detection and enumeration of Listeria monocytogenes and of Listeria spp.— Part 2: Enumeration method.

EN ISO 16140-2:2016. Microbiology of food and animal feed— Method validation— Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.

EN ISO 16140-6:2019. Microbiology of food and animal feed— Method validation— Part 6: Protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures.

FDA *Bacteriological Analytical Manual* (BAM), Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples and Enumeration of Listeria monocytogenes in Foods.

Ottaviani F., Ottaviani M., Agosti M., Differential agar medium for Listeria monocytogenes. Quimper Froid Symposium Proceedings, P6 ADRIA Quimper, France, 16–18 June 1997.

Ottaviani F., Ottaviani M., Agosti M., Esperienza su un agar selettivo e differenziale per L. mono. Industrie Alimentari, 1997.

USDA/FSIS *Microbiology Laboratory Guidebook* (MLG) 8.13 Isolation and Identification of Listeria monocytogenes from Red Meat, Poultry, Ready-To-Eat Siluriformes (Fish) and Egg Products, and Environmental Samples.

Revision history: MAN0026538 K (English)

Revision	Date	Description
K	13 May 2025	• Up to 125 g dairy products and multi-component foods was added to “Isolate Listeria species and L. monocytogenes from a broad range of foods and from environmental samples”. • Clarifications were added to the dates and reference methods for NF VALIDATION™.
J	17 April 2025	The matrices were separated by target in “Isolate Listeria species and L. monocytogenes from a broad range of foods and from environmental samples”.
H	17 January 2025	• A note was added in regard to the Rhamnose Test for *L. monocytogenes.* • The incubation temperature was added to the PrecisCheck™ L. monocytogenes Kit and PrecisCheck™ Listeria species Kit instructions. • A note was added to “Confirm positive results” that the manufacturer's instructions must be followed for all confirmation tests. • Instructions for PALCAM Medium were added to “Confirm positive results”. • These changes do not impact the certified workflow for AFNOR.
G	19 November 2024	A revision was made concerning the Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement. The AFNOR validation was not affected.
F	18 October 2024	A footnote was added for the Oxoid™ 24 Listeria Enrichment Broth (24 LEB) Buffer Supplement.
E	30 July 2024	Instructions were added for preparing Oxoid™ 24 LEB Complete.
D00	21 March 2024	A footnote was added for 24 Listeria Enrichment Broth (24 LEB) (10 × 225 mL in 250 mL PET sirop bottles).
C.0	23 October 2023	Instructions were added for preparing Oxoid™ *Brilliance*™ Listeria Agar (ISO) Bottle Media.
B.0	22 May 2023	An amendment was made to the confirmation section.
A.0	12 January 2023	New document created for NF VALIDATION™ study of Thermo Scientific™ Listeria Precis™ Methods.

The information in this guide is subject to change without notice.

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