**TECHNICAL DATA SHEET**

# COMPASS® *LISTERIA* AGAR (ISO 11290-1 & -2)

**DETECTION AND ENUMERATION OF *LISTERIA* SPP. AND *LISTERIA MONOCYTOGENES***

## 1 INTENDED USE

The formulation of the **COMPASS® *Listeria* Agar** corresponds to **Agar *Listeria* according to Ottaviani and Agosti** defined in the international standards ISO 11290-1 and ISO 11290-2 as well as in FDA's Bacteriological Analytical Manual (**FDA-BAM),** Chapter 10.

COMPASS® *Listeria* Agar is the first mandatory isolation medium in the *L. monocytogenes* and *Listeria* spp. detection protocol (ISO 11290-1), and the only medium in the *L. monocytogenes* and *Listeria* spp. enumeration protocol (ISO 11290-2).

COMPASS® *Listeria* Agar meets the requirements of NF V45-008 and NF V45-009 and can be used according to the protocol described in this standard.

Also, the COMPASS® *Listeria* Agar method is certified NF Validation according to the NF EN ISO 16140-2: 2016 validation protocol as a rapid alternative method for:

- Detection of *Listeria monocytogenes* and *Listeria* spp. in food products and environmental samples by comparison to NF EN ISO 11290-1 :2017.
- Enumeration of *Listeria monocytogenes* in food products and environmental samples by surface or deep plating by comparison to NF EN ISO 11290-2 :2017.

Please refer to the COMPASS® *Listeria* Agar Technical data sheet (alternative methods).

## 2 HISTORY

In 1991, Mengaud *et al*. identified a specific phospholipase C phosphatidyl-inositol (PI-PLC) produced by the two pathogenic species of *Listeria: L. ivanovii* and *L. monocytogenes*, only the latter is pathogenic for humans. They suggested this enzyme could be a virulence factor. The same year, Notermans *et al*. developed a double layer method for the detection of the PI-PLC in a solid agar medium by using L-α-phosphatidylinositol. Under these conditions, the two pathogenic species form colonies surrounded by an opaque halo, while colonies of non-pathogenic species did not have this characteristic. The use of a chromogenic substrate, 5-bromo-4-chloro-3-indolyl-β-D-glucoside (X-glucoside), allowed the replacement of esculin previously used in Oxford and PALCAM medium. The presence of esculinase (β-glucosidase) can be detected by the formation of a blue precipitate on the colony. The selective mixture contained in the medium inhibits nearly all other contaminating bacteria.

By the association of these principles, **COMPASS® *Listeria* Agar** allows the detection of blue colonies surrounded by an opaque halo, typical of *Listeria monocytogenes* and certain strains of *Listeria ivanovii*, and of blue colonies without a halo, characteristic of other species belonging to the genera *Listeria*.

## 3 PRINCIPLES

The peptones and growth factors (yeast extract, sodium pyruvate and magnesium sulfate) favor the growth of *Listeria monocytogenes.*

*Listeria* hydrolyze the 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside (or X-β-glucoside). The resulting product is subjected to an oxidative dimerization that forms a blue precipitate on the colonies.

Phosphatidyl-inositol is used as a substrate for the detection of phospholipase C of *Listeria monocytogenes*. When it is degraded, an opaque precipitate is formed around the colonies.

Secondary microflora is inhibited by the association of lithium chloride and a judicious mixture of selective agents that include several antibiotics and an antifungal agent.

## 4 TYPICAL COMPOSITION

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

**COMPASS® *Listeria* Agar** in accordance with Agar *Listeria* according to Ottaviani and Agosti
For 1 liter of medium:

- Peptic digest of meat ..... 18.00 g
- Tryptone ..... 6.00 g
- Yeast extract..... 10.00 g
- Sodium pyruvate..... 2.00 g
- Glucose ..... 2.00 g
- Magnesium glycerophosphate..... 1.00 g
- Magnesium sulfate, anhydrous..... 0.50 g
- Sodium chloride ..... 5.00 g
- L-α-phosphatidyl-inositol..... 2.00 g
- Disodium hydrogenphosphate, anhydrous ..... 2.50 g
- Lithium chloride..... 10.00 g
- 5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside ..... 0.05 g
- Nalidixic acid..... 0.02 g
- Ceftazidime..... 0.02 g
- Polymyxine B (sulfate) ..... 76700 UI
- Amphotericin..... 0.01 g
- Bacteriological agar ..... 12.00 g

pH of the ready-to-use medium at 25 °C: 7.2 ± 0.2.

## 5 PREPARATION

**Dehydrated and associated supplements**

✓ **Reconstitution: 71.9 g/L**

✓ **Sterilization: 15 min at 121°C**

- Dissolve 71.9 g of dehydrated base medium (BK192) in 1 liter of distilled or demineralized water.
- Slowly boil under stir and maintain it for the necessary time for its dissolution.
- Dispense into vials (100 mL or multiples of 100 mL).
- Sterilize in an autoclave at 121°C for 15 minutes.
- Cool and maintain at 44-47 °C.
- Aseptically reconstitute the freeze-dried selective supplement (BS071) by adding 10 mL of sterile distilled water.
- Aseptically add 1 mL of reconstituted selective supplement per 100 mL of base and mix well.
- Just before using the complete medium, add 3 mL of enrichment supplement (BS070) previously brought to room temperature.
- Homogenize carefully and pour into plates.

**Kit medium to reconstitute (BT008):**

- Melt the 200 mL vials of base medium (R1) for the minimum amount of time necessary in order to achieve total liquefaction.
- Cool and maintain at 44-47 °C.
- Aseptically reconstitute the selective supplement (R2) by adding 2 mL of sterile distilled water.
- Into each 200 mL vial of base medium, first aseptically add 2 mL of the reconstituted selective supplement each 200 mL vial of base medium and mix well.
- Just before using the complete medium, add 6 mL of enrichment supplement (R3) brought back to room temperature.
- Mix well and pour into plates.

**NOTE:**

- It is highly recommended to prepare the medium and to pour into plates immediately to ensure that it retains a clear appearance for easy colony reading.
- The complete medium can be maintained in molten state for 4 hours at 44-47 °C. After maintaining the complete medium in a molten state, insure a vigorous homogenization before use.

## 6 QUALITY CONTROL

**Aspect, color of the complete medium**: opalescent, amber agar.

- Typical cultural response after 48 hours incubation at 37°C (NF EN ISO 11133):

| Microorganisms | | Growth (Productivity Ratio: $P_R$) | Characteristics |
|---|---|---|---|
| *Listeria monocytogenes* | WDCM 00021 | $P_R \geq 50\%$ | Blue-green colonies surrounded by an opaque halo |
| *Listeria monocytogenes* | WDCM 00109 | $P_R \geq 50\%$ | Blue-green colonies surrounded by an opaque halo |
| *Listeria innocua* | WDCM 00017 | Good | Blue-green colonies without halo |
| *Enterococcus faecalis* | WDCM 00087 | Inhibited | - |
| *Escherichia coli* | WDCM 00013 | Inhibited | - |

## 7 DETECTION OF *LISTERIA MONOCYTOGENES* AND *LISTERIA* SPP. (ISO 11290-1)

Respect Good Laboratory Practices.
Refer to the recommendations in the Directive ISO 7218.

✓ **Primary enrichment:**
**25 ± 1h at 30 ± 1°C**

✓ **Secondary enrichment:**
**24 ± 2h at 37 ± 1°C**

✓ **Inoculation:**
**On surface**

✓ **Incubation:**
**Up to 48 ± 2h in total, at 37± 1°C**

**Instructions for Use (see flowchart in Annex 1)**

- Prepare an initial suspension of the sample to be analyzed in half-Fraser broth (broth of primary enrichment), taking care to respect the initial 1:10 ratio (sample to enrichment medium).
- Homogenize well, use a paddle blender if needed.
- Incubate this suspension at 30 ± 1 °C for 25 ± 1 hours.

- Inoculate 0.1 mL of half-Fraser broth (primary enrichment broth) in 10 mL of Fraser broth (secondary enrichment broth).
- Incubate the Fraser broth at 37 ± 1 °C for 24 ± 2 hours.

- After incubation of half-Fraser and Fraser broths (primary and secondary enrichment broths):

  - Inoculate on prepared plates or pre-poured medium COMPASS® *Listeria* Agar (BM123, BM124) brought to room temperature with a sterile loop. Incubate at 37 ± 1 °C for up to 48 ± 2 hours. Stop the incubation if the presumptive colonies are evident at 24 ± 2 hours.
  - Inoculate on another selective medium, for example PALCAM agar or Oxford agar, with a sterile loop.

Refer to ISO 11290-1 for more detail.

**NOTE:**
- After primary enrichment, the half-Fraser broth can be stored at 5°C for up to 72 hours before inoculation in Fraser broth.
- Before streaking on plates, the half-Fraser and Fraser broths can be stored at 5 °C for up to 72 hours.
- For the detection of *Listeria* spp. other than *Listeria monocytogenes*, an extra 24 hours of incubation of secondary enrichment may allow more species to be recovered.
- After incubation, the plates can be stored at 5°C for up to 48 hours before reading.

**Results**

Characteristic colonies of *Listeria monocytogenes* and certain strains of *Listeria ivanovii* appear blue to blue-green and are surrounded by an opaque halo. Other species of *Listeria* can form blue to blue-green colonies, but without the halo.

## 8 ENUMERATION OF *LISTERIA MONOCYTOGENES* AND *LISTERIA* SPP. (ISO 11290-2)

Respect Good Laboratory Practices.

Refer to the ISO 7218 standard for plating, colony counting and for calculations and expression of results.

**Instructions for Use**

- Prepare a primary dilution of the sample to be analyzed in half-Fraser broth (with antibiotics) or in Buffered peptone water in a 1:10 dilution ratio.
- Transfer 0.1 mL of the suspension, and if necessary, any serial dilutions onto the surface of each plate required of prepared or pre-poured COMPASS® *Listeria* Agar (BM123, BM124). It is also possible to inoculate 1mL on the surface of 3 plates (Ø 90 mm) or 1 large plate (Ø 140 mm).
- Spread the inoculum on the surface with the aid of a sterile triangle or "hockey stick".
- Incubate the plates at 37 ± 1 °C for 24 to 48 ± 2 hours.

NOTE: For the detection of small number, refer to ISO 7218.

✓ **Inoculation:**
**0.1 mL on surface.**

✓ **Incubation:**
**48 ± 4 h at 37 ± 1 °C.**

**Results:**

Characteristic colonies of *Listeria monocytogenes* and certain strains of *Listeria ivanovii* appear blue to blue-green and are surrounded by an opaque halo. Other species of *Listeria* can form blue to blue-green colonies, but without the halo.

An initial reading may be performed after 24 hours of incubation for an earlier detection of positive samples that are heavily contaminated, however the final result is given only after 48 hours.

Count all the colonies presumed to be *L.monocytogenes* on each plate (Ø 90 mm) containing less than 150 *L.monocytogenes* characteristic colonies, or less than 360 *L.monocytogenes* characteristic colonies on large plates (Ø 140 mm).

Count all the colonies presumed to be *Listeria* spp. on each plate (Ø 90 mm) containing less than 150 *Listeria* spp. characteristic colonies, or less than 360 *Listeria* spp. characteristic colonies on large plates (Ø 140 mm).

In case of mixed cultures of blue-green colonies with or without opaque halo, or when blue-green colonies with large opaque halos overlap, count the colonies on each plate (Ø 90 mm) containing less than 100 *Listeria* spp. characteristic colonies, or less than 240 *Listeria* spp. characteristic colonies on large plates (Ø 140 mm).

## 9 CONFIRMATION OF *LISTERIA MONOCYTOGENES*

All positive results must be confirmed.

As the **COMPASS® *Listeria* Agar** formulation is in accordance with the **Agar *Listeria* according to Ottaviani and Agosti** described in ISO 11290-1 and ISO 11290-2, carry out the classic tests described in CEN or ISO standardized methods, including the purification step, for example on a TSYEA agar, from characteristic colonies.

## 10 STORAGE / SHELF LIFE

**COMPASS® *Listeria* Agar:**

**Dehydrated media (base):** 2-30 °C.
**Enrichment supplement:** 2-25 °C.
**Selective supplement:** 2-8 °C.
**Pre-poured media in Petri plates:** 2-8 °C.
**Kit:** 2-8 °C.
The expiration dates are indicated on the labels.

**Prepared base medium in vials (*):** 180 days at 2-8 °C.
**Prepared complete medium in vials (*):** 4 hours at 44-47 °C.
**Rehydrated freeze-dried supplements (*):** 15 days at 2-8 °C, shielded from light.
**Complete medium in plates, with supplements (*):** 15 days at 2-8 °C.

(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

## 11 PACKAGING

**COMPASS® *Listeria* Agar:**

**Pre-poured media in Petri plates COMPASS® *Listeria* Agar (Ø 90 mm):**
20 plates..... BM12308
120 plates..... BM12408

**Kit COMPASS® *Listeria* Agar:**
Kit containing 6 x 200 mL vials (R1), and 6 vials of freeze-dried selective supplement (R2)
and 6 vials of liquid enrichment supplement (R3)..... BT00808

**Dehydrated base medium COMPASS® *Listeria* Agar:**
500 g bottle..... BK192HA

**Enrichment supplement for COMPASS® *Listeria* Agar:**
8 vial pack to prepare 8 x 1 L of base medium..... BS07008

**Freeze-dried selective supplement COMPASS® *Listeria* Agar:**
8 vial pack to prepare 8 x 1 L of base medium..... BS07108

**Primary (half-Fraser) and secondary (Fraser) enrichment broth:**
Available in different formats and packaging, please refer to the corresponding technical data sheets

**Diluent (Buffered peptone water):**
Available in different formats and packaging, please refer to the corresponding technical data sheet

**PALCAM as the 2nd selective medium:**
Available in different formats and packaging, please refer to the corresponding technical data sheet

**Oxford as the 2nd selective medium:**
Available in different formats and packaging, please refer to the corresponding technical data sheet

**Medium used in confirmation TSYEA:**
Available in different formats and packaging, please refer to the corresponding technical data sheet

## 12 BIBLIOGRAPHY


OTTAVIANI, F., OTTAVIANI, M., AGOSTI, M.. 1997. Esperienza su un agar selettivo e differenziale per *Listeria* monocytogenes. Industrie Alimentari, **36:** 1-3.

OTTAVIANI, F., OTTAVIANI, M., and AGOSTI, M.. 1997. Differential agar medium for *Listeria monocytogenes*. Quimper Froid Symposium Proceedings p6. ADRIA Quimper France. 16-18 June 1997.

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.

NF EN ISO 11290-2. May 2017. Microbiology of the food chain - Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria spp*. - Part 2: enumeration method.

NF V045-008. March 2009. Processed fish - Method for enumeration of low level contamination Listeria monocytogenes in smoked salmon and trout.

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NF EN ISO 7218. October 2007. Microbiology of food and animal feeding stuffs. General requirements and guidance for microbiological examinations. Modified in October 2013 by the amendment A1.

PR NF EN ISO 7218. Scheduled publication in February 2024. Microbiology of the food chain - General requirements and guidance for microbiological examinations.

United States Food and Drug Administration. Bacteriological Analytical Manual. Chapter 10: Detection of Listeria monocytogenes in Foods and Environmental Samples, and Enumeration of Listeria monocytogenes in Foods. https://www.fda.gov/media/157717/download?attachment Accessed March 2024.

## 13 ADDITIONAL INFORMATION

**COMPASS®** is a registered trademark of BIOKAR DIAGNOSTICS (division of SOLABIA S.A.S.)

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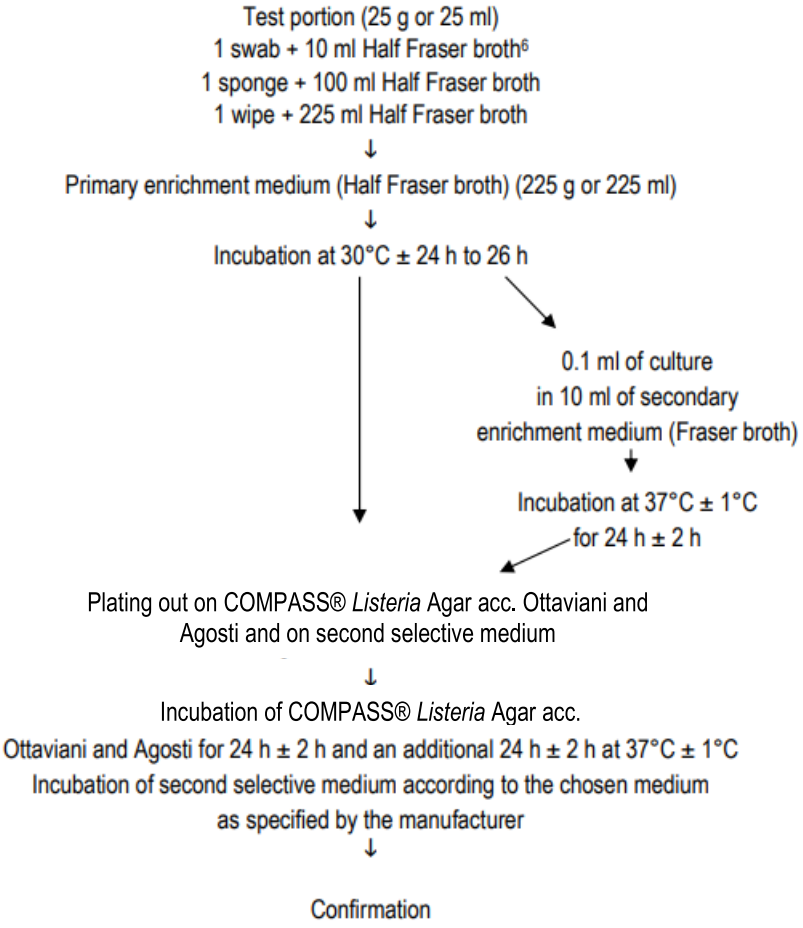
# ANNEX 1: Detection of *Listeria monocytogenes* et *Listeria spp.* (ISO 11290-1)



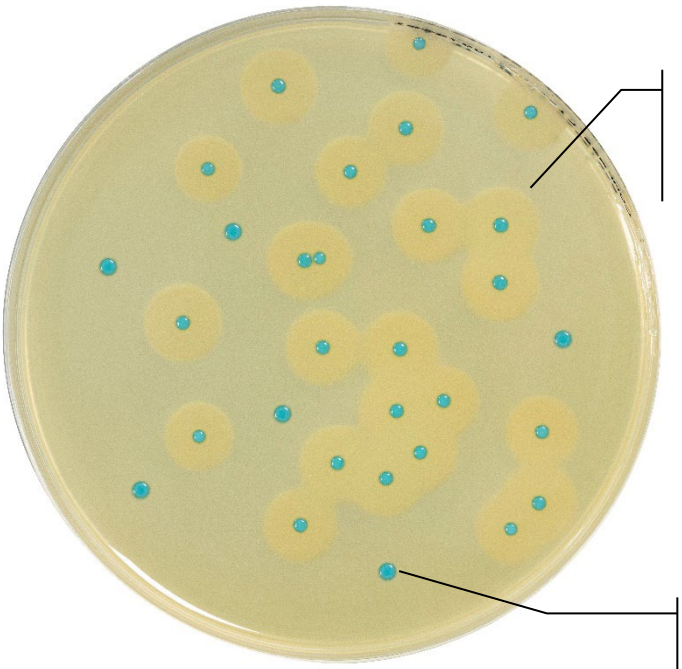
# ANNEX 2: PHOTO SUPPORT

## COMPASS® *Listeria* Agar

Detection and enumeration of *Listeria* spp. & *Listeria monocytogenes*.

**Results:**

Growth obtained after 24 hours of incubation at 37 °C.



***Listeria monocytogenes***

Characteristic colony:
Blue-green color surrounded by an opaque halo.

***Listeria* spp.***

Characteristic colony:
Blue-green colony without halo

*other than *Listeria monocytogenes* and certain strains of *Listeria ivanovii.*