**TECHNICAL DATA SHEET**

# COMPASS® *LISTERIA* AGAR

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

## 1 INTENDED USE

**COMPASS® Listeria** constitutes a method constitutes a method for the detection of Listeria monocytogenes and of Listeria spp, and a method for the enumeration of Listeria monocytogenes in food products, and in environmental samples even heavily contaminated.

- **Alternative rapid method for the detection of *Listeria monocytogenes* and of *Listeria* spp.**

The method COMPASS® *Listeria* is used in the context of the alternative rapid method of detection of *L. monocytogenes*, in human food products and environmental samples.

It is characterized by one sole step in selective enrichment ½ Fraser broth, followed by a re-inoculation onto COMPASS *Listeria* Agar. The enrichment will be realized at 37°C during 18 to 24 hours or at 30°C during 22 to 28 hours.

The method is certified NF VALIDATION, according to the validation protocol of NF EN ISO 16140-2 of 2016 for all human food products and samples of the industrial production environment. The reference method used for validation is the NF EN ISO 11290-1 standard of 2017.
The term of validity is 28 November 2023.

**BKR 23/02-11/02,**
**METHODES ALTERNATIVES D'ANALYSE**
**POUR L'AGROALIMENTAIRE**
Certifié par AFNOR Certification http://nf-validation.afnor.org

In the context of the label NF VALIDATION, sampling sizes greater than 25 g were not tested.

- **Rapid alternative method for the enumeration of *Listeria monocytogenes***

The method COMPASS® *Listeria* agar can also be used within the context of the alternative rapid method of enumeration of *L. monocytogenes* for all human foods and for environmental samples, by inoculation of one plate either on the surface or as a pour plate.
It is officially certified by AFNOR Certification, under the reference number BKR 23/05-12/07, of which its period of validity runs until December 4th, 2023.

**BKR 23/05-12/07**
**METHODES ALTERNATIVES D'ANALYSE**
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In the context of the label NF VALIDATION, sampling sizes greater than 25 g were not tested.

- **Normalized method for the detection and enumeration of *Listeria***

The formulation of the COMPASS *Listeria* Agar corresponds to that advised in the international standards NF EN ISO 11290-1 and NF EN ISO 11290-2.
COMPASS® *Listeria* Agar represents the first mandatory isolation media in the operating protocol for the detection of *L. monocytogenes* and *Listeria spp,*as well as the sole media in the operating protocol for the enumeration of *L. monocytogenes* and *Listeria spp*.

## 2 HISTORY

In 1991, Mengaud et al. identified a specific phospholipase C phosphatidyl-inositol (PI-PLC) produced by the two pathogenic species of *Listeria* : *Listeria monocytogenes* and *Listeria ivanovii*, the former being the sole human pathogen They suggested that this enzyme could be a virulence factor in these species. 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-phosphosphatidylinositol. 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 media. In this fashion, the presence of esculinase (β-glucosidase) can be demonstrated by the formation of a blue precipitate in the center of the colony. A judicious use of selective mixture successfully inhibits nearly all other contaminating bacteria.

By the association of these three 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 in the center of 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 are 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**
For 1 liter of media:

- 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
- Cycloheximide ..... 0.05 g
- Bacteriological agar ..... 12.00 g

pH of the ready-to-use media 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 selective supplement per 100 mL of base and mix well.
- Just prior to the moment when the complete medium is to be used, add 3 mL of enrichement supplement (BS070) previously brought to room temperature.
- Mix thoroughly and pour into plates.

**Kit media 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 enrichment supplement (R2) by adding 2 mL of sterile distilled water.
- Into each 200 mL vial of base media, add aseptically 2 mL of the selective supplement and mix well.
- Just prior to the use of the complete media, add 6 mL of enrichment supplement (R3) brought back to room temperature.
- Mix well and pour into plates.

**NOTE:**
The complete media can be maintained in molten state for 4 hours at 44-47 °C.
It is nevertheless recommended to prepare the media progressively as needed and to use immediately after preparation so that the media can maintain a clear appearance for easy colony reading.
After maintaining the complete media in a molten state, insure a vigorous homogenization before use.

## 6 QUALITY CONTROL

**Aspect:** beige powder, free-flowing and homogeneous.
**Aspect, color of the complete media**: 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 00020 | $P_R \geq 50\%$ | 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 RAPID ALTERNATIVE METHOD FOR THE DETECTION OF *LISTERIA MONOCYTOGENES* AND *LISTERIA* SPP. (CERTIFIED NF VALIDATION)

Always respect Good Laboratory Practices.

Refer to the recommendations in the Directive NF EN ISO 7218.

**Instructions for Use**

- Prepare a primary dilution of the sample to be analyzed in Half Fraser broth, taking care to respect the initial 1:10 ratio (sample to enrichment media).
- Incubate this suspention at 30 ± 1 °C for 22 to 28 hours.

**Or (Short protocol)**

- Prepare a primary dilution of the sample to be analyzed in a preheat Half Fraser broth, taking care to respect the initial 1:10 ratio..
- **Incubate this suspention at 37 ± 1 °C for 18 to 24 hours.**

✓ **Enrichment:**
**1 :10 in ½ Fraser broth.**
**22 h at 30 °C or 18 h at 37°C**

✓ **Detection:**
**100 µL on surface.**

- Inoculate 100 µL of the above culture on a prepared or pre-poured plate of COMPASS® *Listeria* Agar using a loop or Pasteur pipette.
- Incubate at 37 ± 1 °C for 24± 2 to 48 heures. Reading can be done after only 22 hours of incubation.

**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 halos.

NOTES
- After enrichment, for organizational reasons in the laboratory, Half Fraser broth can be kept up to 3 days at 2-8°C before being inoculated onto **COMPASS® *Listeria* Agar**.
- The agar plates can be kept 48 days at 2-8°C before performing the confirmation tests.

## 8 RAPID ALTERNATIVE METHOD FOR THE ENUMERATION OF *LISTERIA MONOCYTOGENES* (CERTIFIED NF VALIDATION)

Always respect Good Laboratory Practices.
Refer to the recommendations in the Directive NF EN ISO 7218.

**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 one single plate (one plate by dilution) of prepared or pre-poured.
- Spread the inoculum on the surface with the aid of a sterile triangle or “hockey stick”.

**or**

- Transfer 1 mL of the suspension, and if necessary, any serial dilutions into an empty sterile Petri dish. Pour approximately 15 mL of the molten, complete media into the plate. Homogenize well by swirling and let solidify on a cool surface.
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- Incubate the plates at 37 ± 1 °C for 24 to 48 ± 2 hours.

✓ **Inoculation:**
**0.1 mL on surface or**
**1 mL in pour plates.**

✓ **Incubation:**
**48 h at 37 °C.**

**Results :**

Characteristic colonies of *Listeria monocytogenes* appear blue to blue-green and are surrounded by an opaque halo. Certain strains of *Listeria ivanovii* can also display the same characteristics.

An initial reading may be performed after 24 hours of incubation for a more simple and quick detection of samples that are heavily contaminated, however the final results is given only after 48 hours.

If colonies are characteristic after only 24 hours of incubation, the confirmations can be performed at this time.

Perform the definitive count at 48 ± 2 hours of incubation.

NOTE
The plates can be kept at 72 hours at 2-8°C before performing the confirmation tests.

## 9 CONFIRMATION OF *LISTERIA MONOCYTOGENES* AND *LISTERIA* SPP (NF VALIDATION)

**Confirmation of *Listeria monocytogenes* (detection or enumeration method)**

In the context of the method **COMPASS® *Listeria* Agar**, when the presence of *Listeria monocytogenes* has already been confirmed during the detection phase, it is possible to skip the confirmation step when performing the enumeration in the event of positive results. Inversely, when the presence of *Listeria monocytogenes* has been confirmed during an enumeration, it is possible to skip the confirmation step if running a detection method.

In the context of NF VALIDATION, all samples identified as positive must be confirmed by one of the following means:

Option 1**:** According to classical tests described in methods standardized by CEN or ISO (including a purification step) from characteristics colonies (blue to blue-green with halo) isolated from COMPASS® *Listeria* Agar.

Option 2**:** use of **CONFIRM' *L. mono* Agar®**(BM139), from a characteristic colony.
Sample a characteristic colony from the surface of COMPASS® *Listeria* Agar and inoculate by streaking (up to 6 radial streaks per plate).
Incubate at 37 ± 1 °C for 24 ± 3 hours.
The presence of a characteristic colony is the result of growth on the plate, with a yellow discoloration and the appearance of an opaque halo.

Option 2: Use of **CONFIRM' *L. mono broth*** (BM162), from a characteristic colony.
Sample one colony per tube of broth.
Incubate at 37 ± 1 °C for 6 to 24 hours.
The color change to yellow in the tube confirms the presence of *Listeria monocytogenes*.

**NOTES**
A negative result or a brownish coloration after 6 hours is considered discordant. The laboratory should proceed with additional tests to verify the validity of the results obtained, for example by pursuing the incubation until 24 hours.

In the event of doubtful results after 24 hours of incubation, proceed with another confirmation tests (biochemical gallery, for example).

Option 2: Use of biochemical identification gallery API LISTERIA, from a characteristic colony

Option 3**:** use of another certified NF Validation method, using a different principle. In this case, the validated protocol of the second method must be followed in its entirety, and all the steps prior to the intermediary step from which the confirmation is taken should have common ties between the two methods (for instance a common selective enrichment with the same media). The two validated methods (one for detection, the other for confirmation) should therefore have one common procedure.

**NOTE :**
In case of discordant results (positive by the alternative method, not confirmed by one of the options described above), the laboratory must implement sufficient means to ensure the validity of the result.

**Confirmation of *Listeria* spp (detection method)**

In the context of the certified NF VALIDATION, all positive results must be confirmed by one of the following tests:

Option 1: According to classical tests described in methods standardized by CEN or ISO, including a purification step (for example Gram stain or catalase test) from characteristics colonies (blue to blue-green, surrounded or not by an opaque halo) isolated from COMPASS® *Listeria* Agar.

Option 2: Use of **PALCAM** agar

Sample a characteristic colony from the surface of COMPASS® *Listeria* Agar (blue to blue-green colony with or without halo) and inoculate by pricking onto PALCAM agar (up to 15 picks per plate).
Incubate at 37 ± 1 °C for 24 ± 3 hours.
The presence of a characteristic colony (olive green surrounded by a black halo) confirms the identity to the genus *Listeria*.

Option 2: Use a biochemical identification gallery, taken from one isolated colony.

Option 3: use of another certified NF Validation method, using a different principle. In this case, the validated protocol of the second method must be followed in its entirety, and all the steps prior to the intermediary step from which the confirmation is taken should have common ties between the two methods (for instance a common selective enrichment with the same media). The two validated methods (one for detection, the other for confirmation) should therefore have one common procedure.

**NOTE**

In the event of discordant results (positive by the alternative method, without confirmation from one of the options mentioned above), the laboratory must perform the necessary steps to assure the validity of the results.

To not confirm 5 colonies in enumeration implies a risk to render an overestimated result in light of the eventual presence of characteristic colonies that may not be that of *Listeria monocytogenes*.

## 10 STORAGE / SHELF LIFE

**Dehydrated base media:** 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 media in vials (*):** 180 days at 2-8 °C.
**Prepared complete media in vials (*):** 4 hours at 44-47 °C
**Rehydrated freeze-dried supplements (*):** 15 days at 2-8 °C, shielded from light.
**Complete media in plates, with supplements (*):** 15 days at 2-8 °C.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

## 11 PACKAGING

**Pre-poured media in Petri plates (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 media:**
500 g bottle ..... BK192HA

**Enrichment supplement:**
8 vial pack to prepare 8 x 1 L of base media..... BS07008

**Freeze-dried selective supplement:**

8 vial pack to prepare 8 x 1 L of base media ..... BS07108

**CONFIRM' *L.mono* broth:**
18 x 1 mL vials ..... BM16208

**CONFIRM' *L.mono* Agar:**
10 plates ..... BM13908

**PALCAM agar:**
20 plates ..... BM02008
500 g dehydrated media bottle ..... BK145HA
Freeze-dried supplement Qs 500 mL ..... BS00408
Freeze-dried supplement Qs 2.5 L ..... BS04908

## 12 BIBLIOGRAPHY


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## 13 ADDITIONAL INFORMATION

**COMPASS®** is a registered trademark of SOLABIA S.A.S.

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.

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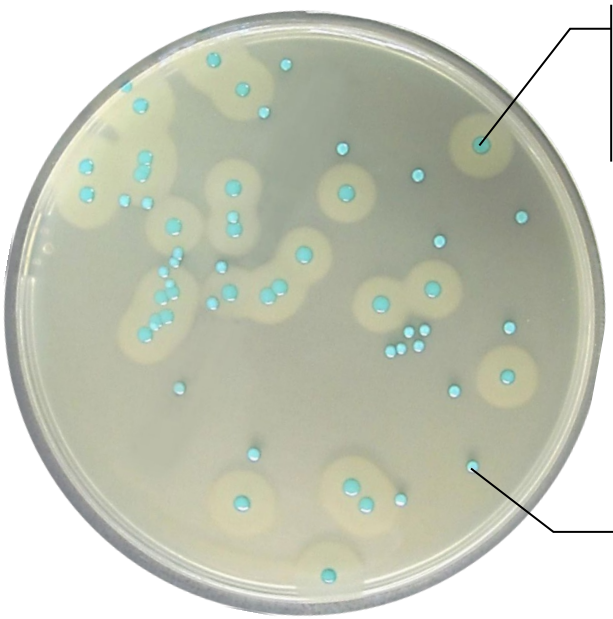
# ANNEX 1: PHOTO SUPPORT

## 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*.