TECHNICAL DATA SHEET

COMPASS® *LISTERIA* AGAR

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

1 INTENDED USE

EN ISO 16140-2 validated alternative method for the detection of *Listeria monocytoge*nes and *Listeria spp.*

COMPASS® *Listeria* is a rapid alternative method used for the detection of *Listeria monocytogenes* and of *Listeria* spp, and also for the enumeration of *Listeria monocytogenes* in food products, and in environmental samples.

It features a single step of selective enrichment in Fraser ½ broth, followed by subculture onto COMPASS® *Listeria* Agar. Enrichment can be performed at 37°C for 18 to 24 hours, or at 30°C for 22 to 28 hours.

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

BKR 23/02-11/02,
ALTERNATIVE ANALYSIS METHOD
FOR FOOD INDUSTRY
Certified by AFNOR Certification http://nf-validation.afnor.org

Refer to the certificate available on the NF VALIDATION website for the validity end date of the method.
In the context of the label NF VALIDATION, sampling sizes greater than 25 g were not tested.

EN ISO 16140-2 validated alternative method for the enumeration of *Listeria monocytogenes*

The COMPASS® *Listeria* method is also used as a rapid alternative method for the enumeration of *L. monocytogenes* in human food products and environmental samples, by surface or deep plating.

BKR 23/05-12/07
ALTERNATIVE ANALYSIS METHOD
FOR FOOD INDUSTRY
Certified by AFNOR Certification http://nf-validation.afnor.org

Refer to the certificate available on the NF VALIDATION website for the validity end date of the method.

- Normalized method for the detection and enumeration of *Listeria monocytogenes* et *Listeria* spp.

The formulation of the COMPASS *Listeria* Agar corresponds to that recommended in the international standards NF EN ISO 11290-1 and NF EN ISO 11290-2. (Refer to COMPASS® *Listeria* (ISO11290-1 & -2) data sheet.)
COMPASS® *Listeria* Agar is the first mandatory isolation medium in the *L. monocytogenes* and *Listeria* spp. ~~test~~ detection protocol, and the only medium in the *L. monocytogenes* and *Listeria* spp. enumeration protocol.

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- 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 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 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 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
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
- Cycloheximide ..... 0.05 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:
The complete medium can be maintained in molten state for 4 hours at 44-47 °C.
It is nevertheless recommended to prepare the medium progressively as needed and to use it immediately after preparation to ensure it can maintain a clear appearance for easy colony reading.
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 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. (NF VALIDATION CERTIFIED)

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 medium).
- Incubate this suspension 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 (sample to enrichment medium).
- **Incubate this suspension at 37 ± 1 °C for 18 to 24 hours.**
- Inoculate 100 µL of the obtained culture onto a plate of COMPASS® *Listeria* Agar by streaking using a loop or Pasteur pipette.
- Incubate at 37 ± 1 °C for 24± 2 to 48 hours. Reading can be done after only 22 hours of incubation.

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

✓ **Detection:**
100 µL on surface.

NOTE:

- The short protocol (18 hours incubation at 37°C) is only applicable to Fraser 1/2 broths of BIOKAR DIAGNOSTICS.

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.

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**.

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

Respect Good Laboratory Practices.
Refer to the NF EN 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. It is also possible to inoculate 1mL on the surface of 3 plates.
- Spread the inoculum on the surface with the aid of a sterile triangle or “hockey stick”.

Or

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

✓ **Incubation:**
48 h at 37 °C.

- Transfer 1 mL of the suspension, and if necessary, any serial dilutions into an empty, sterile Petri dish (one dish per dilution). Pour approximately 15 mL of the molten, complete medium into the plate. Homogenize well by swirling and let solidify on a cool surface.

  NOTE:

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

- Incubate the plates at 37 ± 1 °C for 24 to 48 ± 2 hours.

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 result 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 agar plate can be stored for 72 hours at 2-8 °C before counting.

9 CONFIRMATION OF *LISTERIA MONOCYTOGENES* AND *LISTERIA* SPP

All positive results must be confirmed in one of the following ways:

Standardised methods or NF EN ISO 16140-6 validated methods

As the COMPASS® *LISTERIA* formulation is in accordance with the *Listeria* agar according to Ottaviani and Agosti described in ISO 11290-1 and ISO 11290-2, the following methods can be used:

- Implementation of the classical tests described in the CEN or ISO standard methods (including the purification step), starting from the characteristic colonies (blue to blue-green surrounded by an opaque halo) isolated on COMPASS® *Listeria* Agar.

- Implementation of methods ISO 16140-6 certified, starting from the characteristic colonies (blue to blue-green surrounded by an opaque halo) isolated on COMPASS® *Listeria* Agar.

NF VALIDATION certified methods

Agar plates can be stored for 72 hours at 2-8°C before performing the confirmation tests.

- Implementation of the classical tests described in the CEN or ISO standardized methods (including the purification step), starting from the characteristic colonies (blue to blue-green surrounded by an opaque halo) isolated on COMPASS® *Listeria* Agar.

- Implementation of **CONFIRM' *L. mono* Agar®,** from a characteristic colony.

- Pick a characteristic colony from the surface of COMPASS® *Listeria* Agar and streak the agar (up to 6 radial streaks per plate).
- Incubate at 37 ± 1 °C for 24 ± 3 hours.
- The presence of a characteristic colony is indicated by growth on the agar, with yellow discoloration and appearance of an opacification halo.

- Implementation of **CONFIRM' *L. mono* broth**, starting from a characteristic colony

- Transfer one colony per broth tube.
- Incubate at 37 ± 1 °C for 6 to 24 hours.
- Yellow turn of the tube confirms the presence of *Listeria monocytogenes*.

NOTES:
- A negative result or brownish staining after 6 hours is discordant. The laboratory should perform additional tests to verify the validity of the result, e.g., by continuing the incubation for up to 24 hours.
- In case of doubtful reaction after 24 hours of incubation, implement another confirmatory test (*e.g.*, biochemical gallery).

- Implementation of a **biochemical identification gallery**, from an isolated colony (type 2 option).

- Use of **any other NF VALIDATION certified method**, of different principle (type 3 option).

The validated protocol of the second method must be respected as a whole, i.e., all the steps prior to the intermediate step from which we start again for the confirmation must be common to both methods. The two validated methods (one used for detection and the other for confirmation) must therefore have a common core.

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.

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.
- The absence of confirmation of 5 colonies in enumeration implies a risk of overestimating the result due to the possible presence of characteristic colonies that are not *Listeria* monocytogenes.

10 CONFIRMATION OF *LISTERIA* SPP

All positive results must be confirmed in one of the following ways:

Standardized methods or NF EN ISO 16140-6 validated methods

As the COMPASS® *Listeria* formulation is in accordance with the *Listeria* agar according to Ottaviani and Agosti described in EN ISO 11290-1 and EN ISO11290-2, the following methods can be used:

- Implementation of the classical tests described in the CEN or ISO standard methods (including the purification step), e.g., Gram and Catalase tests, starting from the characteristic colonies isolated on COMPASS® *Listeria* Agar.

- Implementation of methods certified according to EN ISO 16140-6 starting from characteristic colonies isolated on COMPASS® *Listeria* Agar

NF VALIDATION certified methods

Agar plates can be stored for 72 hours at 2-8°C before performing the confirmation tests.

- Implementation of the classical tests described in the CEN or ISO standardized methods (including the purification step), for example, Gram and Catalase tests, starting from the characteristic colonies isolated on COMPASS® *Listeria* Agar.

- Implementation of **PALCAM agar (BM145) (Formula described in ISO 11290 standards).**

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

- Implementation of a biochemical identification gallery, from an isolated colony.

- Use of **any other NF VALIDATION certified method**, of different principle.

The validated protocol of the second method must be respected in its entirety, i.e., all the steps prior to the intermediate step from which we start again for the confirmation must be common to both methods. The two validated methods (one used for detection and the other for confirmation) must therefore have a common core.

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.

11 STORAGE / SHELF LIFE

Dehydrated base medium: 2-30 °C.
Enrichment supplement: 2-25 °C.
Selective supplement: 2-8 °C.
Pre-poured medium in Petri plates: 2-8 °C.
Kit: 2-8 °C.
Ready-to-use media in flexible bag: 2-8 °C shielded from light.
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.

12 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 medium:
500 g bottle BK192HA

Enrichment supplement:
8 vial pack to prepare 8 x 1 L of base medium BS07008

Freeze-dried selective supplement:
8 vial pack to prepare 8 x 1 L of base medium BS07108

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

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

Ready-to-use Half-FRASER in bottle:
10 x 225 mL BM01608

Ready-to-use Half-FRASER in flexible bags:
3 x 3 L BM13308
2 x 5 L BM13408
40 x 5 L BM18808

Ready-to-use Half-FRASER + Tween® in flexible bags:
3 x 3 L BM21208

13 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 16140-2. July 2016. Microbiology of the food chain — Method validation — Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method

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

NF V045-009. April 2023. Processed fish made from fishery and aquaculture - Method for the enumeration of Listeria monocytogenes at low contamination levels in smoked fish (by the pour plate technique)

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

NF EN ISO 16140-6. December 2019. Microbiology of the food chain - Method validation - Part 6: protocol for the validation of alternative (proprietary) methods for microbiological confirmation and typing procedures

14 ADDITIONAL INFORMATION

Document code : COMPASS LISTERIA_EN_V17
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Origin of revision : Reconduction, minor modifications

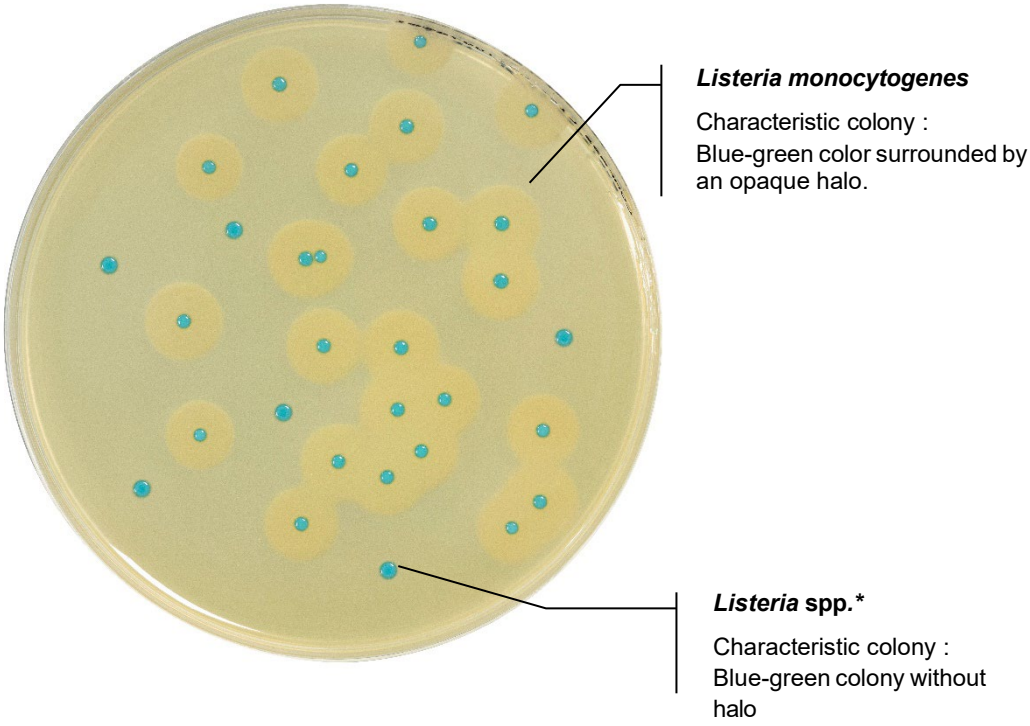
ANNEX 1: PHOTO SUPPORT

COMPASS® *Listeria* Agar

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

Results:

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



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