

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 monocytogenes* 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 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,
METHODES ALTERNATIVES D'ANALYSE
POUR L'AGROALIMENTAIRE
Certifié par 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 food products and environmental samples, by surface or deep plating.



BKR 23/05-12/07
METHODES ALTERNATIVES D'ANALYSE
POUR L'AGROALIMENTAIRE
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- 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.

COMPASS® *Listeria* Agar is the first mandatory isolation medium in the *L. monocytogenes* and *Listeria spp.* test 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* : *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 medium. 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 antibiotics 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 excellent 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 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 \pm 0,2.

5 PREPARATION

Dehydrated and associated supplements

- 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 prior to the moment when the complete medium is to be used, add 3 mL of enrichment supplement (BS070) previously brought to room temperature.
- Mix thoroughly and pour into plates.

✓ **Reconstitution:**
71,9 g/L

✓ **Sterilization:**
15 min at 121°C

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 enrichment 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 prior to the use of 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 immediately after preparation so that the medium 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: beige powder, free-flowing and homogeneous.

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
<i>Listeria monocytogenes</i>	WDCM 00021	$P_R \geq 50\%$	Blue-green colonies surrounded by an opaque halo
<i>Listeria monocytogenes</i>	WDCM 00020	$P_R \geq 50\%$	Blue-green colonies surrounded by an opaque halo
<i>Listeria innocua</i>	WDCM 00017	Good	Blue-green colonies without halo
<i>Enterococcus faecalis</i>	WDCM 00087	Inhibited	-
<i>Escherichia coli</i>	WDCM 00013	Inhibited	-

7 RAPID ALTERNATIVE METHOD FOR THE DETECTION OF *LISTERIA MONOCYTOGENES* AND *LISTERIA* SPP.

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 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 above culture onto a 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.

✓ **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**.

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* (NF VALIDATION CERTIFIED)

Always 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 1 ml on the surface of 3 plates.
- 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 (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.
- 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.

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 EN ISO 11290-1 and EN 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 EN 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 (biochemical gallery for example).

- 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.
- Failure to confirm 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:

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 EN ISO 11290-1 and EN 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), 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 streaks 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, that is to say that 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.

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 medium 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

PALCAM agar:

20 plates BM02008

500 g dehydrated medium bottle BK145HA

Freeze-dried supplement Qs 500 mL BS00408

Freeze-dried supplement Qs 2.5 L BS04908

13 BIBLIOGRAPHY

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14 ADDITIONAL INFORMATION

COMPASS® is a registered trademark of BOKAR DIAGNOSTICS (division 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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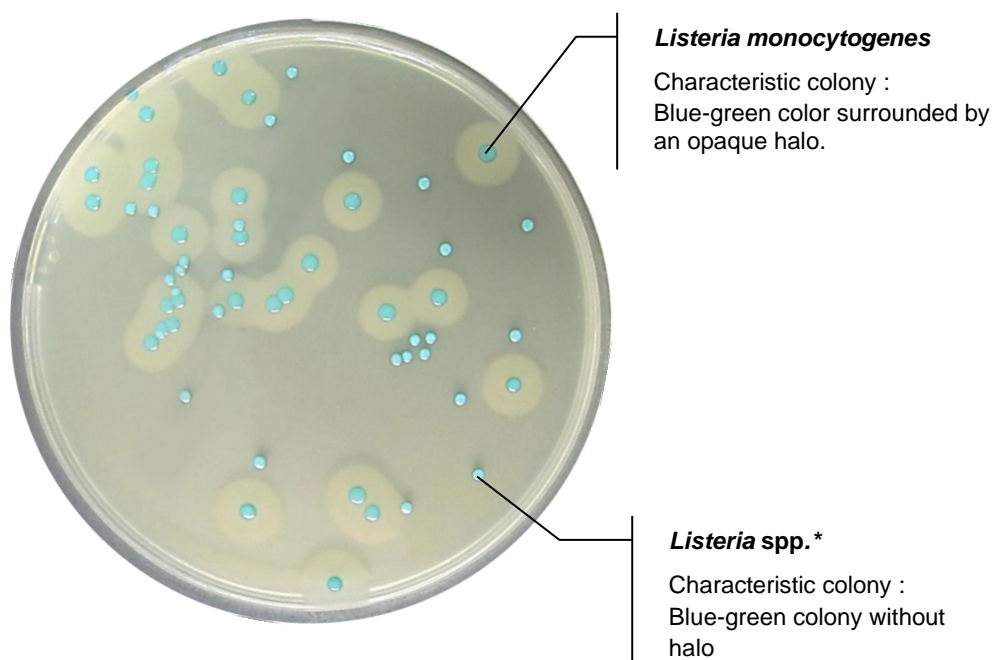
ANNEX 1: PHOTO SUPPORT

COMPASS® *Listeria* Agar

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

Results:

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



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