COMPASS *Listeria* Agar

INTENDED USE

COMPASS *Listeria* Agar is a selective medium used for the differentiation, isolation and enumeration of *Listeria monocytogenes* in food products, environmental samples, and animal-based pathological samples regardless of background contamination. Its formulation corresponds to that prescribed in the detection and enumeration protocols of the EN ISO 11290-1 and 11290-2 standards including Amendment A1.
COMPASS *Listeria* Agar is also used in the context of the alternative rapid method of detection of *L. monocytogenes and Listeria* spp, in human food products and environmental samples, shortened to one selective preenrichment step : it is officially certified by AFNOR Certification, under the reference number BKR 23/2-11/02, of which its period of validity runs until November 28th, 2014.
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, 2011.

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 esculine 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 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*.

PRINCIPLES

- The peptones and growth factors (yeast extract, sodium pyruvate and magnesium sulfate) favor the excellent growth of *Listeria monocytogenes.*
- Yeast extract is also a source of vitamin B complex.
- Sodium chloride maintains the osmotic equilibrium of the media.
- *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.

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Tél : + 33 (0)3 44 14 33 33 – Fax : + 33 (0)3 44 14 33 34 – www.biokar-diagnostics.com

PREPARATION OF COMPLETE MEDIUM FROM THE DEHYDRATED BASE MEDIUM (BK192) AND SUPPLEMENTS (BS070+BS071)

- Dissolve 71,9 g of dehydrated base medium (BK192) in 1 liter of distilled or deionized water.
- Slowly stir until complete dissolution.
- Dispense in flasks of 1 L.
- 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.
- Into each 1 L vial of base media, first aseptically add the 10 mL of the reconstituted selective supplement, followed by the 30 mL of enrichment supplement (BS070).
- Mix thoroughly after the addition of each supplement.
- Pour into sterile Petri dishes, for use in the context of either detection or enumeration by surface inoculation.
- Dry the plates in an incubator, with the covers partially removed.

PREPARATION OF COMPLETE MEDIUM FROM THE BT008 KIT

- Melt the base medium (Reagent R1, BM12800 from the BT008 kit) for the minimum amount of time necessary in order to achieve total liquefaction.
- Cool and maintain at 44-47°C.
- Aseptically reconstitute the freeze-dried selective supplement (Reagent R2, BS06708 from the BT008 kit) by adding 2 mL of sterile distilled water.
- Into each 200 mL vial of base media, first aseptically add 2 mL of the reconstituted selective supplement, followed by 6 mL of enrichment supplement (Reagent R3, BS06808 from the BT008 kit).
- Mix thoroughly after the addition of each supplement.
- Pour into sterile Petri dishes, for use in the context of either detection or enumeration by surface inoculation.
- Dry the plates in an incubator, with the covers partially removed.

INSTRUCTIONS FOR USE

Comply with Good Laboratory Practices (NF EN ISO 7218).

♦ Rapid alternative detection method

- Prepare a primary dilution of the sample to be analyzed in **Half Fraser broth** [BK133 +(BS030 or BS032) or BK173 + (BS059 or BS062) or BM016 for example], taking care to respect the initial 1:10 ratio (sample to enrichment media).
- Incubate this initial suspension at **(30 ± 1)°C for (24 ± 2) hours**.
- Inoculate 100 µL of the above culture onto a plate of **COMPASS *Listeria* Agar** using a loop or Pasteur pipette of the above culture.
- Incubate at **(37 ± 1)°C for 24 to 48 hours**. Reading may be performed as early as after 24 hours of incubation.

NOTE 1 :

During the validation study, for 2 milk product samples, halos appeared after 48 hours of incubation.

NOTE 2 :

After enrichment, for organizational reasons in the laboratories, Half Fraser broths may be kept for up to 3 days at 2-8°C before being restruck onto COMPASS® *Listeria* Agar.

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♦ **Rapid alternative enumeration method**

- Prepare a primary dilution of the sample to be analyzed in **Buffered peptone water** [BK018, BK131, BM010, BL056 or BM057 for example].
- Incubate the suspension at **(20 ± 2)°C for (60 ± 5) minutes**.
- 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 **COMPASS *Listeria* Agar**.
- Spread the inoculum on the surface with the aid of a sterile triangle or "hockey stick".

or

- Transfer 0.1 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 media into the plate.
- Homogenize well by swirling and let solidify on a cool surface.
- Incubate at **(37 ± 1)°C for (24 ± 2) hours**.
- In the absence of characteristic colonies (absence of colonies or blue colonies with halos only partially visible at 24 hours), prolong the incubation for an additional 24 hours.
- The expression of the results should be made conform to the recommendations established in ISO 7218 (August 2007).

NOTE 3 :

COMPASS *Listeria* Agar can also be used as the primary mandatory isolation media in the detection of *Listeria monocytogenes* as described in the EN ISO 11290-1/A1 standard, as well as the sole media for the enumeration of *L. monocytogenes* according to EN ISO 11290-2/A1.

NOTE 4 :

In the context of NF VALIDATION, test portions weighing more than 25 g have not been tested.

RESULTS

Characteristic colonies of *Listeria monocytogenes* 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. It should be noted certain strains of *Listeria ivanovii* can sometimes produce characteristic colonies, however usually of much smaller size.

CONFIRMATION

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

♦ ***Listeria monocytogenes* :**

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

Option 2: use of **CONFIRM' *L. mono* Agar**.

Option 3: use of another NF Validation validated method, using a different principle that that found in COMPASS *Listeria* Agar. 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.

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♦ ***Listeria spp*** **:**

Option 1 : According to **classical tests described in methods standardized by CEN or ISO (including a purification step)** from characteristics colonies isolated from **COMPASS *Listeria* Agar.** Examples might include (but not limited to) a Gram stain or catalase test.

Option 2 : Use of PALCAM agar according to the specified protocol
or
Use of a biochemical identification gallery from an isolated colony.

Option 3 : Use of another NF Validation validated method, using a different principle that that found in COMPASS *Listeria* Agar. 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.

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.

NOTE 5 :

When the presence of *Listeria monocytogenes* has already been confirmed following detection protocols, it is possible to skip the confirmation step after performing the enumeration procedure, in the event of positive results.

TYPICAL FORMULA

(may be adjusted in order to achieve optimal results)

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
- Polymyxin B (sulfate).....76700 IU
- Cycloheximide.....0.05 g
- Bacteriological agar.....12.00 g

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

QUALITY CONTROL

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- Dehydrated base medium: beige powder, free-flowing and homogeneous.
- Prepared media in plates : opalescent, amber agar.
- Typical cultural response after 24 - 48 hours incubation at 37°C :

Microorganisms		Growth (Productivity ratio P_R)	Characteristics
Listeria monocytogenes	CIP 59.53	$P_R \geq 50\%$	blue-green colonies surrounded by an opaque halo
Listeria monocytogenes	CIP 78.31	$P_R \geq 50\%$	blue-green colonies surrounded by an opaque halo
Listeria innocua	ATCC® 33090	good, score 2	blue-green colonies without a halo
Enterococcus faecalis	ATCC 29212	inhibited, score 0	
Escherichia coli	ATCC 25922	inhibited, score 0	

STORAGE / SHELF LIFE

Dehydrated base medium:
- Store between 2-30°C, shielded from light
- The expiration date is indicated on the label

Complete, prepared medium with supplements (benchmark value*****) :
- Prepared from kit components : 15 days at 2-8°C.

Pre-poured media in Petri dishes or kit reagents :
- Store between 2 – 8°C, shielded from light.
- The expiration date is indicated on the label.

PACKAGING

Pre-poured media in Petri dishes (Ø 90 mm) :	Code
- 20 plates	BM12308
- 120 plates	BM12408
COMPASS *Listeria* Agar kit :	
- Composed of 6 x 200 mL vials of base media (R1) ; 6 freeze-dried selective supplement vials (R2) ; and 6 vials of liquid enrichment supplement (R3)	BT00808
Dehydrated medium:	
- 500 g bottle	BK192HA
Enrichment Supplement :	
- 8 vial pack for 8 x 1 L of base medium	BS07008
Selective Supplement :	
- 8 vial pack for 8 x 1 L of base medium	BS07108

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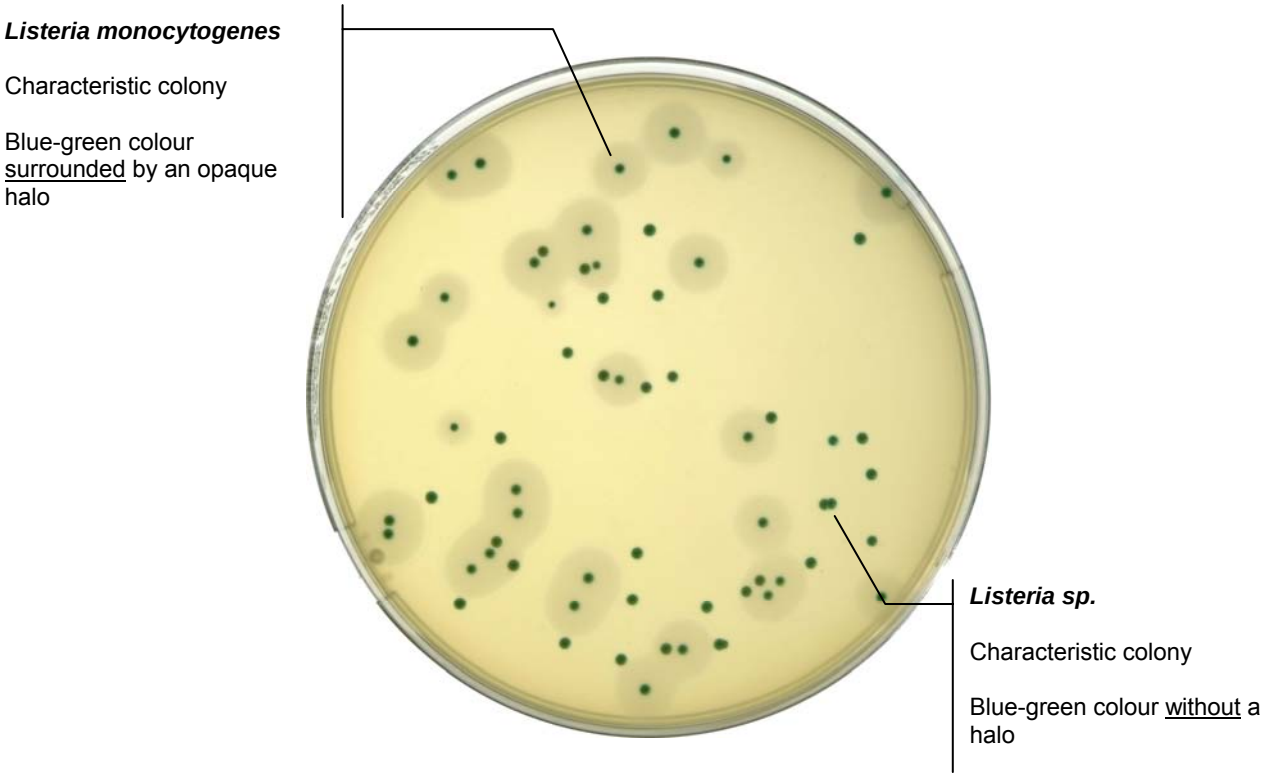
PHOTO SUPPORT :

Product reference : BK192HA + [BS07008 + BS07108], BM12308/12408, BT00808

Media used for: The differentiation, isolation and enumeration of *Listeria monocytogenes.*



COMPASS *Listeria* Agar

Ref : BM12308

Incubation 24 hours at 37°C (surface)

Characteristic *Listeria monocytogenes*: blue-green colonies surrounded with an opaque halo (hydrolysis of X-β-glucoside and degradation of L-α-phosphatidyl-inositol)

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The information provided on the package take precedence over the formulations or instructions described in this document.
The information and specifications contained in this technical data sheet date from 2011-07-01.
They are susceptible to modification at any time, without warning.
Code document : BM123/A/2007-05 : 8.

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