DEFINITION
Selective chromogenic medium for detection and enumeration of *Listeria monocytogenes* and other species of *Listeria* in food products for human consumption and in environmental samples.

NF VALIDATION by AFNOR CERTIFICATION as per EN ISO 16140 protocol
The RAPID’L.mono method has been certified by NF Validation as an alternative to the reference standard NF EN ISO 11290-1, according to the ISO 16140 protocol, for the detection of *Listeria monocytogenes* and other species of *Listeria* spp in all food products for human consumption and in environmental samples.

AOAC VALIDATION
The RAPID’L.mono method is validated by the AOAC Research Institute under the “Performance Methods Tested” status for detecting *Listeria monocytogenes* (brie cheese, surimi, mixed salad and deli turkey). This validation is for qualitative analysis only. Positive results on RAPID’L.mono agar are not confirmed colonies according to AOAC guidelines. These colonies should be considered presumptive positive and should be confirmed according to FDA-BAM procedures (US Food and Drug Administration, Center for Food Safety and Applied Nutrition, Bacterial Analytical Manual 8th Edition (Revision A). Chapter 10 – *Listeria monocytogenes*. January 2003.)
Certificate n°030406

NORDVAL VALIDATION
The RAPID’L.mono method is NORDVAL validated as an alternative method to the reference standard NF EN ISO 11290-1 for detecting *Listeria monocytogenes* in all food products for human consumption and in environmental samples without confirmation of positive colonies.

STANDARD REFERENCES

NF EN ISO 11290-1/A1 (February 2005)
Food microbiology – Horizontal method for detection and enumeration of *Listeria monocytogenes* - Part 1 Detection method (IC: V08-028-1)

NF EN ISO 11290-2/A1 (February 2005)
Food microbiology – Horizontal method for detection and enumeration of *Listeria monocytogenes* - Part 2 Enumeration method (IC: V08-028-2)
PRINCIPLE
The principle behind the RAPID’L.mono (RLM) medium relies on the specific detection of \textit{L. monocytogenes} phospholipase C (PIPLC) and on the inability of this species to metabolise xylose. After 24 hours of incubation, \textit{Listeria monocytogenes} forms characteristic blue (pale blue, grey-blue to dark blue) colonies without a yellow halo. Colonies formed by other species of \textit{Listeria} are white, with or without a yellow halo. The particularity of the species \textit{Listeria ivanovii}, infrequently found in food matrices, should be noted: they present blue-green colonies with a yellow halo (xylose positive character). This halo can appear after 24 to 48 hours of incubation. The selective solution in the medium permits inhibition of most interfering flora (Gram-positive and Gram-negative bacteria, yeasts and moulds). Thus RAPID’L.mono permits rapid and specific identification of \textit{Listeria monocytogenes} in 24 hours and of other \textit{Listeria} species in 24 and 48 hours, after preparing samples in compliance with standards:
- enrichment in Fraser ½ broth for 24 hours (detection)
- revivification in Buffered Peptone Water or Fraser ½ broth without selective agents for 1 hour (enumeration of \textit{Listeria monocytogenes}).

PRESENTATION
- Pre-poured
  - 20 dishes x 90 mm code 356-3694
  - 120 dishes x 90 mm code 356-3964
- Ready-to-use
  - 1 kit code 355-5294

The kit contains:
- 190 ml bottle (Base)
- 6 ml bottle (Supplement 1)
- 14 ml bottle (Supplement 2 – freeze-dried)
- Instructions
- Dehydrated + supplements
  - 500 g code 356-4293
  - Supplement 1 code 356-4294
  - Supplement 2 code 356-4746

STORAGE / SHELF-LIFE / BATCH
- +2 °C, protected from light.
- Expiration date and batch number are shown on the package.
- Medium prepared by the user: 1 week at +2 °C protected from light. (non-dried dishes, wrapped in plastic pack or equivalent).

THEORETICAL FORMULA
Peptones 30 g
Meat extract 5 g
Yeast extract 1 g
Lithium extract 9 g
Xylose 10 g
Phenol red 120 mg
Agar B 13 g
Growth activators 2 g
Chromogenic solution 1 ml
Selective solution 20 ml
Distilled water 1,000 ml

Final pH = 7.2 ± 0.2

OTHER PRODUCTS REQUIRED (NOT SUPPLIED)
- Tryptone-Salt Diluent:
  - 9 ml x 25T (e.g.* code 355-5754)
  - 500 g (e.g.* code 356-4544)
- 4 x 3 l bags (e.g.* code 355-5796)
- Sterile distilled water (e.g.* code 355-4154):
  - 9ml x 25T for reconstituting RLM supplement2
- Rhamnose test (e.g.* code 355-3669)

Detection method:
- Fraser ½ broth:
  - Ready-to-use (complete):
    - 6 x 225 ml bottles (e.g.* code 355-5797)
    - 4 x 3L bags (e.g.* code 355-5794)
  - Dehydrated (base):
    - 500 g (e.g.* code 356-4604)
  - Freeze-dried selective supplement:
    - Pack of 10 bottles (e.g.* code 356-4616)
  - \textit{Agar Listeria} according to Ottaviani and Agosti.
  - Ready-to-use:
    - 20 x 90 mm dishes (e.g.* code 356-3695)
    - 120 x 90 mm dishes (e.g.* code 356-3965)
  - Base in bottles:
    - 6 x 237.5 ml (e.g.* Code 355-5200)
  - Dehydrated (base + suppl.):
    - 500g (e.g.* code 356-4043)
    - supplement 1 (e.g.* code 356-4041)
    - supplement 2 (capsule) (e.g.* code 356-4201)
    - supplement 2 (e.g.* code 356-4042)
  - Rhamnose test (e.g.* code 355-3669)

Enumeration method:
- Fraser ½ broth without selective agents with Ferric (III) ammonium citrate
  - Buffer peptone water:
    - 6 x 225 ml bottles (e.g.* code 355-4170)
    - 500 g (e.g.* code 356-4684)
    - 4 x 3 l bags (e.g.* code 355-5795)
    - 2 x 5 l bags (e.g.* code 355-5790)
  - \textit{Agar Listeria} according to Ottaviani and Agosti.
  - Ready-to-use:
    - 20 x 90 mm dishes (e.g.* code 356-3695)
120 x 90 mm dishes (e.g.* code 356-3965)
Base in bottles:
6 x 237.5 ml (e.g.* Code 355-5200)
Dehydrated (base + suppl.):
500g (e.g.* code 356-4043)
supplement 1 (e.g.* code 356-4041)
supplement 1 (capsule) (e.g.* code 356-4201)
supplement 2 (e.g.* code 356-4042)
• Rhamnose test (code 355-3669)
See corresponding Technical Sheet(s)
*e.g.: for exemple

MATERIALS REQUIRED NOT PROVIDED
(non-exhaustive)
• Scales
• Sterile weighing bags
• Mill
• Stirrer-homogeniser
• Sterile Petri dishes (Ø = 90 or 140 mm)
• Sterile pipettes (0.1 ml; 1 ml…..)
• Sterile spreaders
• Sterile swabs
• Sterile Pasteur pipettes
• Water-bath
• Thermostatically-controlled incubator or incubation room precise to ± 1°C
• All usual laboratory equipment

RECONSTITUTION OF RLM SUPPLEMENT Ready-to-use kit (code 355-5294)
- Under aseptic conditions, reconstitute the freeze-dried RLM supplement 2 by adding 14 ml of sterile distilled water to the bottle.
- Homogenize until freeze-dried product is completely dissolved.

Supplement 1 RAPID’L.mono (code 356-4294) used with the dehydrated base
- Under aseptic conditions, reconstitute the freeze-dried RLM supplement 1 by adding 25 ml of sterile distilled water to the bottle.
- Homogenize until freeze-dried product is completely dissolved.

PREPARATION OF COMPLETE MEDIUM Ready-to-use kit (code 355-5294)
- In the boiling water bath, melt 190 ml of RAPID’L mono agar. Stir the bottle by hand on removal, in order to resuspend the white deposit.
- Cool the bottle to 44-47°C, stir the bottle by hand and add, under sterile conditions
- 6 ml of RLM supplement 1
- 14 ml of reconstituted RLM supplement 2
- Mix thoroughly avoiding frothing.
- Immediately pour the complete medium into a Petri dish (Ø = 90 or 140 mm)
- Leave to set on a cool, level surface.

Do not stack the dishes.

Note: 1 bottle of complete medium can be used to prepare approximately 13 Petri dishes of Ø= 90 mm or 7 Petri dishes of Ø= 140 mm.

Dehydrated + supplements (code 356-4293, 356-4294, 356-4746)
Always shake before use
- Dissolve 68,1g of powder in 950 ml of distilled water.
- Warm slowly, and bring to the boiling point, stirring carefully.
- Do not over heat (boiled max 2 minutes)
- Cool down at 47/50°C
- Add 2 vials of reconstituted supplement 1 (code 356-4294)
- Homogenize the mixture.
- Add 2 vials of supplement 2 (code 356-4746)
- Homogenize the mixture.
- Pour in Petri dishes.

500g of powder make 7.4 liters of medium.

PROTOCOLS
Detection of Listeria monocytogenes and other Listeria spp. in η g or η ml of sample:

Preparation of sample
- Dilute η g or η ml of sample in 9 x η ml of Fraser ½ broth.
Example: dilute 25g or 25 ml of sample in 225 ml of Fraser½ broth to prepare a 1/10 dilution.
- Incubate at 30°C (∓ 1°C) for 24 hours (∓ 2 h).

After incubation, the Fraser ½ selective enrichment broth may be stored in a refrigerator (3°C ± 2°C) for 72 hours, before inoculating RAPID’L Mono.

Isolation and Incubation
- At the end of incubation, remove 0.1 ml of Fraser ½ broth using a sterile pipette, and place drops on the outside edge of half of the surface of the RAPID’L mono agar (diagram n°2).
- Using a sterile swab, spread over half the surface by means of to-and-fro movements (diagram n°2).
- Using a sterile Pasteur pipette, isolate on the other half of the agar surface (diagram n°3) by spreading the deposit in relatively close streaks over the whole dish, starting from the edge of the previous spread
- Incubate the upturned dishes at 37°C (∓ 1°C) for 24 h (∓ 2h)
Diagram n°1:
0.1 ml deposit

Diagram n°2:
Swab
Starting point

Diagram n°3:
Starting point
End point
pipette

Reading
- Take a reading of the Listeria monocytogenes after 24 hours (± 2 h) of incubation. Listeria monocytogenes strains will have formed characteristic colonies.
- Take a reading of the Listeria spp other than Listeria monocytogenes after 24 hours and 48 hours of incubation. Between 24 h and 48 h, the incubation can be stopped at any moment Listeria genus is confirmed. (see INTERPRETATION section).

Note 1: It is possible to take a reading of the Listeria monocytogenes after 48 hours of incubation.
Note 2: After incubation, the RAPID’ L mono dishes can be stored in a refrigerator (3°C ± 2°C) for 48 hours, before reading and confirmation.
Note 3: In the context of NF VALIDATION mark, no sample of over 25 g was tested.
Note 4: In case of a positive result with the RAPID’ L mono enumeration validated method, it is not necessary to make a confirmation if the sample has been already confirmed as positive for Listeria monocytogenes in the detection method with RLM.

INTERPRETATION
Detection / Enumeration of Listeria monocytogenes colonies (CFU)
- After the incubation, and depending on the method, perform the detection or enumeration of Listeria monocytogenes. These colonies are typically blue* without a yellow halo, round and smooth, with an average diameter of 1 to 2 mm.
*Depending on the food matrix, the blue of the colony may vary in depth of colour (pale blue, grey-blue, mid blue, with a grey-white contour…)

Detection / Enumeration of Listeria spp. colonies (CFU)
- After the incubation, and depending on the method, perform the detection or enumeration of Listeria spp.. These colonies are typically white or pale yellow with or without a yellow halo, forming a round shape, with a smooth, convex appearance, average diameter 1 to 2 mm. Listeria ivanovii, infrequently found in food matrices, present blue-green colonies with a yellow halo (xylose positive character). Listeria monocytogenes forms characteristic...
blue (pale blue, grey-blue to dark blue) colonies without a yellow halo.

Some interfering species may grow forming white colonies. These species can be differentiated from Listeria by their characteristic morphology (e.g.: bacillus, spread out and notched, enterococcus, very small). Gram staining will prove that they are not Listeria.

Note 1:
- Enumeration of Listeria spp. others than Listeria monocytogenes are not in the scope of NF VALIDATION Mark.

Note 2:
- Count only dishes containing a maximum of 150 characteristic colonies and a maximum of 300 colonies in total.

CONFIRMATION (Detection or Enumeration)

Confirmation of positive Listeria monocytogenes results

When RAPID’L.mono is used outside the context of the protocol certified by AFNOR Certification or AOAC-certified protocol, confirmation of positive results is unnecessary.

Confirm less than five colonies involves a risk of making an overestimation because of the presence of typical colonies that would not be Listeria monocytogenes.

In the context of NF VALIDATION Mark, only one colony confirmation is required, and must be confirmed in one of the following way:

- Using the conventional tests described in the standardised CEN or ISO reference methods (with purification step)
- Using nucleic probes as described in ISO 7218 standard, using isolated colonies (with or without purification step), for example: iQ-Check™ Listeria monocytogenes PCR method (code 357-8124)
- A colony isolated on RAPID’L.mono can be confirmed using a Rhamnose test (code 355-3669),
- Using spot sub-culture of an isolated colony on Agar Listeria according to Ottaviani and Agosti (ex. A.L. code 356-3695)

Note 1: For spot confirmation on Agar Listeria according to Ottaviani and Agosti after 24 hours of incubation of RAPID’L.mono agar, carry out a spot sub-culture of a part of the colony and, in parallel, prolong incubation of the RAPID’L.mono agar for a further 24 hours to verify that a yellow halo appears for Listeria ivanovii, which ferment xylose slowly.

Note 2: It is possible to confirm up to 12 colonies on a dish of Agar Listeria according to Ottaviani and Agosti.
- Using any other NF VALIDATION certified method based on a different principle from that of RAPID’L.mono. The validated second method protocol should be respected in its entirety, i.e. all stages preceding the intermediary stage from which confirmation is sought must be common to the two methods. In this way, it is possible to confirm with a PCR technique using enrichment broth. (iQ-Check™ Listeria monocytogenes code 357 8124)

Confirmation of positive Listeria results other than Listeria monocytogenes

In the context of NF VALIDATION Mark, positive results must be confirmed in one of the following way:

- Using the conventional tests described in the standardised CEN or ISO reference methods (with purification step)
- Using nucleic probes as described in ISO 7218 standard, using isolated colonies (with or without purification step), for example: iQ-Check™ Listeria spp. PCR method (code 357 8113) using isolated colonies (with or without purification step).
- Using any other NF VALIDATION certified method based on a different principle from that of RAPID’L.mono. The validated second method protocol should be respected in its entirety, i.e. all stages preceding the intermediary stage from which confirmation is sought must be common to the two methods.

For each target, Listeria monocytogenes and Listeria spp., in the event of discrepant results (positive with RAPID’L.mono method, negative with confirmation method), the laboratory should take sufficient measures to ensure the validity of its findings. This must be
Expression of results / Calculations
- Refer to Standards 11290-1 and 2, ISO 7218 and XP V08-102

PRECAUTIONS
- Standard precautions concerning the handling of potentially-contaminated products in a microbiology laboratory should be observed.
- Before using the RAPID’L mono dishes, according to Standard ISO 7218, at 25°-50°C until the drops on the surface of the medium have disappeared. Prolonged drying can alter the medium’s performance, and should be avoided.
- When using the enumeration method, impregnate the medium well using the spreader. After spreading, in order to permit the inoculum to be correctly absorbed by the agar, the dishes can be left as they are on the work surface for 15 to 30 minutes before being incubated.
- RLM (base) bottle:
  - A white deposit at the bottom of the bottle is normal. To ensure resuspension and satisfactory homogeneity, it is important to stir the bottle by hand when it is taken out of the boiling and melting water baths, and to pour it immediately after adding the supplements.
  - Avoid prolonged overheating of the product during melting.
  - A strain of Listeria Ivanovii with a slow xylose metabolism can be find in sheep milk. This a typical strain is difficult to differentiate with a Listeria monocytogenes strain, even after 48h on RAPID’L mono. Therefore, AL confirmation isn’t advised when sheep milk products are tested.
  - Comply with Good Laboratory Practice (EN ISO 7218)

QUALITY CONTROL
Every product manufactured and marketed by Bio-Rad is subject to a quality-assurance procedure at all stages, from reception of raw materials through to marketing of the finished products.
Each batch of finished product undergoes quality control and is marketed only if it satisfies the acceptability criteria.
Documentation relative to the production and quality control of each batch is kept on file.

TEST QUALITY AND PERFORMANCE

<table>
<thead>
<tr>
<th>STRAINS</th>
<th>Appearance of colonies after 24-48 hours of incubation at 37 °C</th>
<th>GROWTH</th>
<th>COLOUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Listeria</td>
<td></td>
<td></td>
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<tr>
<td>monocytogenes</td>
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<tr>
<td>4b ATCC 13932</td>
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<td>+</td>
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KEY WORDS
RAPID’L mono / Listeria monocytogenes
Detection / Enumeration / Food products
Environment / Fraser / Phospholipase
Chromogenic / Medium

BIBLIOGRAPHY
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