RAPID'*L.mono*/Agar

356-3694 / 356-3964
355-5294 / 356-4293
356-4294 / 356-4746

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.

BRD 07/04 - 09/98
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification
www.afnor-validation.com

The RAPID'*L.mono* method has also been certified by NF Validation as an alternative to the reference standard NF EN ISO 11290-2, according to the ISO 16140 protocol, for the **enumeration of *Listeria monocytogenes* in all food products for human consumption** and in environmental samples.

BRD 07/05 -09/01
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification
www.afnor-validation.com

End of NF VALIDATION: please see the certificate BRD : 07/04 - 09/98 for the detection protocol and BRD : 07/05 - 09/01 for the enumeration protocol. These certificates are available from Bio-Rad representative or AFNOR Certification

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

U.S. Department of Health and Human Services, U. S. Food and Health Administration, Center for Food Safety & Applied Nutrition, Bacteriological Analytical, Online Manual, January 2003.

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)

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RAPID'*L.mono*

PRINCIPLE

The principle behind the RAPID'*L.mono* (RLM) medium relies on the specific detection of *L. monocytogenes* phospholipase C (PIPLC) and on the inability of this species to metabolise xylose.

After 24 hours of incubation, *Listeria monocytogenes* forms characteristic blue (pale blue, grey-blue to dark blue) colonies without a yellow halo. Colonies formed by other species of *Listeria* are white, with or without a yellow halo. The particularity of the species *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 *Listeria monocytogenes* in 24 hours and of other *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 *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 ˜ 8°C, protected from light.
- Expiration date and batch number are shown on the package.
- Medium prepared by the user: 1 week at + 2 ˜ 8°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

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)**

- 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	**(e.g.* code 355-3669**)

Enumeration method:

- Fraser ½ broth without selective agents with Ferric (III) ammonium citrate

Buffered 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)**

- Agar *Listeria* according to Ottaviani and Agosti.

Ready-to-use:

20 x 90 mm dishes	**(e.g.* code 356-3695)**

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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 **(e.g.* 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 (∅ = 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 (∅ = 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°1).*
- 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)

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Diagram n°1:

***Diagram n°2*:**

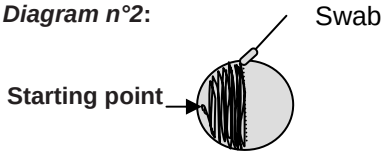
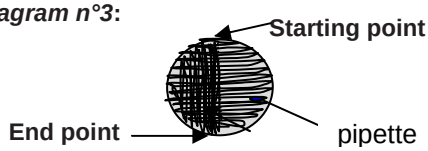


***Diagram n°3*:**



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 RAPID' *L.mono* 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 samples of over 25 g were tested.

Note 4:, In case of a positive result with the RAPID'L.mono detection validated method, it is not necessary to make a confirmation if the sample has been already confirmed as positive for *Listeria monocytogenes* in the enumeration method

<u>Enumeration of *Listeria monocytogenes* in η g or η ml of sample:</u>

Preparation of sample / Revivification

- Dilute η g or η ml of sample in 9 x η ml of buffered peptone water or Fraser ½ broth without selective agents with Ferric (III) ammonium citrate.
- Incubate at 20°C (± 2°C) for 1 h (± 5 min)

Spreading and Incubation

Using stock solution:

- Spread 0.1 ml on 1 dish of RAPID'*L.mono*
- If necessary, prepare a 1/10 dilution (or more) in η Tryptone-Salt diluents or Buffered Peptone water as per the ISO 6887-1 standard, and spread 0.1 ml of each dilution on 1 dish of RAPID'*L.mono*
- Incubate the upturned dishes at 37°C (± 1°C) for 24 hours (± 2h).

If it is necessary to estimate small numbers for some products, spread 1 ml of stock solution over 3 dishes of Ø= 90 mm (~ 0.33 ml/dish) or over 1 dish of Ø= 140 mm (NF VALIDATION certified protocols) or over 2 dishes of Ø=90 mm (0.5ml/dish) of RAPID'*L.mono* (NF VALIDATION non-certified protocol).

Reading

- Take a reading after 24 h (± 2h)

(see INTERPRETATION section).

Note 1: It is possible to take a reading of the RAPID' *L.mono* dishes after 48 hours of incubation

Note 2: 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

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 colonies (CFU) other than *Listeria monocytogenes*

- After the incubation, and depending on the method, perform the detection or enumeration of *Listeria* spp. other than *Listeria monocytogenes.* 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.

Some interfering species may grow forming

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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

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.

For an AFNOR Certification certified enumeration method, following the NF EN ISO 16140 protocol, in the event of a positive result using RAPID'*L.mono* for enumeration, confirmation is not necessary when the presence of *Listeria monocytogenes* has been confirmed during detection.

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**)
Note1: 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.

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.

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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 metabolisation 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

STRAINS	Appearance of colonies after 24-48 hours of incubation at 37 °C	
	GROWTH	COLOUR
Listeria monocytogenes 4b ATCC 13932	+	Blue No halo
Listeria ivanovii SDP 905398	+	Blue-green Yellow halo*
Listeria welschimeri SDP 907	+	White Yellow halo
Escherichia coli ATCC 25922	Inhibition	NA
Enterococcus faecalis ATCC 19433	Inhibition	NA

* Yellow halo at 48 hours
NA = Not applicable

KEY WORDS

RAPID'*L.mono* / *Listeria monocytogenes* Detection / Enumeration / Food products Environment / Fraser / Phospholipase Chromogenic / Medium

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