RAPID'*Listeria* spp./Agar

356-3950
356-4744

APPLICATIONS

Selective **chromogenic** medium for identification and enumeration of ***Listeria spp*** in human food products and environmental samples.

PRINCIPLE

Identification of *Listeria* spp using RAPID'*Listeria* spp. medium is based on the detection of β-D-glucosidase activity by a chromogenic substrate. Listeria colonies are blue to bluish-green.
The medium is made selective by the combined action of lithium chloride and an antibiotic mixture.

NF VALIDATION by AFNOR CERTIFICATION as per EN ISO 16140 protocol

The RAPID'*L.spp* method has been certified by AFNOR Certification as an alternative to the reference standard NF EN ISO 11290-1, according to the ISO 16140 protocol, for the **detection of *Listeria spp*** in all food products for human consumption and in environmental samples.
End of certificate validity: 15/12/2014

BRD 07/12 – 12/06
ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS
Certified by AFNOR Certification
www.afnor-validation.com

AOAC-RI VALIDATION

RAPID'*Listeria* spp. is validated by the AOAC Research Institute under the "Performance Methods Tested" status.
certificate n°080701.

NORMATIVE REFERENCES

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

NF EN ISO 11290-1/A1 (February 2005)
Food Microbiology – Horizontal method for the 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 the detection and enumeration of Listeria monocytogenes – Part 2 Counting method (IC: V08-028-2)

PRESENTATION

Dehydrated

- 500g **code 356-4744**
- Supplement 1 (10 flasks, to make 500 ml of basic agar) **code 356-4745**
- Supplement 2 (10 flasks, to make 500 ml of basic agar) **code 356-4746**

Pre-poured

- 90 mm x 20 dishes **code 356-3950**

STORAGE/VALIDITY/BATCH

- Pre-poured and supplements: + 2-8 °C in the dark.
- Dehydrated: 15-25°C, securely closed flask in a cool dry place.
- The expiration date and the batch number are printed on the packaging.

THEORETICAL FORMULA

Peptones	20 g
Yeast extract	1 g
Sodium pyruvate	2 g
Iron ammonium citrate	0.5 g
Maltose	1 g
Sodium chloride	4 g
Lithium chloride	10.5 g
Silica	20 g
Growth activators	2 g
Chromogenic mixture	75 mg
Antibiotic mixture	40 mg
Agar	12 g
Distilled water to make	1000 ml

Final pH (25°C) = 7 - 7.5

OTHER REQUIRED PRODUCT(S) (NOT SUPPLIED)

- Tryptone salt diluent:

9 ml x 25T	(ex. **code 355-5754)**
500 g	(ex. **code 356-4544)**
5 x 2.3-l packs	(ex. **code 355-5791)**

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• 1/2 FRASER broth:
Ready to use (complete):
6 x 225 ml Bottles (ex. **code 355-5797)**
5 x 2.3-l packs (ex. **code 355-5788)**
Dehydrated (base):
500 g (ex. **code 356-4604)**
Freeze-dried selective supplement 10 flasks (ex. **code 356-4616)**

• PALCAM agar:
20 x 90 mm dishes (ex. **code 356-3674)**
500 g dehydrated (ex**. code 356-4754)**
Supplement (ex. **code 356-4752)**

• RAPID'*Lmono* agar:
20 x 90mm dishes **(code 356-3694)**
Ready to use 190 ml flask + supplement **(code 355-5294)**

See corresponding technical datasheet(s)

REQUIRED EQUIPMENT (NOT SUPPLIED)

(non-exhaustive list)

- Scale
- Sterile weighing boats
- Grinder
- Mixer-homogenizer
- Sterile Petri dishes (Ø = 90 or 140 mm)
- Sterile pipettes (0.1 ml; 1 ml…..)
- Sterile swabs
- Sterile Pasteur pipettes
- Precision water bath (± 1 ℃)
- Precision incubator or thermostatically controlled warm room (± 1 ℃)
- Routine laboratory equipment

PREPARATION OF THE DEHYDRATED MEDIUM

CONDITIONS FOR USE
"Always agitate before use"
Dissolve 71.5 g of powder in 950 ml of distilled water. Sterilize in the autoclave at 121℃ for 15 minutes. Cool to 47-50℃. For 1 liter of medium, aseptically add two flasks of supplement 1 (code 356 4745), each reconstituted with 25 ml of sterile water. Mix well. For 1 liter of medium, add two 5-ml flasks of supplement 2 (code 356 4746). Mix well. pH 7 - 7.5. Pour into sterile Petri dishes.
Store the securely closed flask in a cool dry place.

Reconstitution concentration: 71.5 g /L
500 g of powder makes 7 liters of medium

PROTOCOL

• Detection of *Listeria spp.* in η g or η ml of sample:

• Sample preparation / Selective enrichment

- Seed η g or η ml of the sample in 9 x η ml of 1/2 FRASER broth.
- Incubate at 30 ℃ (± 1 ℃) for 24 hours (± 2 h).

Note: In the context of NF VALIDATION mark, no samples of over 25 g were tested.

After incubation, the ½ FRASER selective enrichment broth can be kept in the cold (3℃±2℃) for 72h before seeding on RAPID'*Listeria* spp..

• Isolation and incubation
- Collect 0.1 ml of ½ Fraser broth at the end of incubation using a sterile pipette and place as a drop on the outer edge of half of the RAPID'*Listeria* spp. agar surface *(Diagram 1).*
- Using a sterile swab, spread back and forth over half of the dish (*Diagram 2*).
- Using a sterile Pasteur pipette and starting from the end of the previous spread, isolate in "relatively tight" streaks over the entire plate. (*diagram 3).*
- Incubate the plates upside down at 37℃ (± 1℃) for 24h (± 2 h).

Diagram 1:

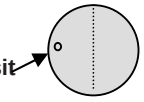


Diagram 2:

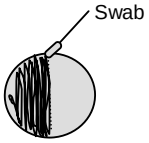
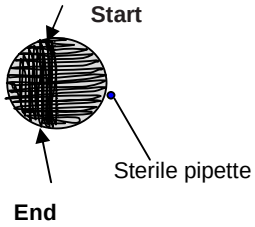


***Diagram 3*:**



• Reading
- Take one reading after incubation.
Listeria spp. appear as characteristic blue to bluish green colonies on RAPID'*Listeria* spp..

Note: It is possible to take the reading up until 48 hours of incubation of the RAPID'*Listeria* spp. agar**.**

After incubation, RAPID'*Listeria* spp. agar plates may be kept in the cold (3℃±2℃) for

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48h before reading and confirmation if applicable.

Note 1:
If no characteristic colonies are observed on RAPID'*Listeria* spp., it may be concluded that *Listeria spp.* is absent (and therefore that *Listeria monocytogenes* is absent).

Note 2:
If characteristic colonies are present on RAPID'*Listeria* spp., confirmation and identification of the species involved can be performed.

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

1- Using the **conventional tests** described in the standardised CEN or ISO reference methods (with purification step) or using **validated nucleic probes**, for example: **IQ-Check™** *Listeria spp.* PCR method (code **357 8113)** using isolated colonies (with or without purification step).

2- **Repicking and spotting** of at least one colony isolated from RAPID'*Listeria* spp. onto **RAPID'*L.mono*** agar, together with a Gram test and a catalase test. Up to 15 colonies can be confirmed on a single plate of RAPID'*L.mono* agar.
Or **Repicking and spotting** at least one isolated colony on a **PALCAM** agar plate. Up to 15 colonies can be confirmed on a single PALCAM agar plate.

3- Using any other **NF VALIDATION certified method** based on a **different principle** from that of RAPID'*L.spp*. 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 using the RAPID'*Listeria* spp. method but unconfirmed by one of the methods described above), the laboratory shall use whatever means are necessary to ensure that the result delivered is valid.

• Expression of results/Calculations
Please refer to ISO 11290-1 and 2, and ISO 7218 standards.

PRECAUTIONS FOR USE

- Usual precautions for handling potentially contaminated products in a microbiology laboratory must be observed.
- Before using RAPID'*Listeria* spp. plates, allow drying at 25-50 °C according to ISO standard 7218, until droplets disappear from the surface of the medium. However, avoid prolonged drying so as not to modify the efficiency of the medium.
- Respect Good Laboratory Practices (EN ISO 7218)

MANUFACTURER'S QUALITY CONTROL

All products manufactured and marketed by the Bio-Rad Company are monitored by a quality assurance system right from reception of the raw materials through to marketing of the finished products.
Every batch of finished product is subject to quality control and will only be marketed if it complies with acceptance criteria.
The documents on the production and control of each batch are kept for reference.

QUALITY CONTROL AND EFFICIENCY OF THE TEST

Culture efficiency is checked using the following strains:

MICRO-ORGANISMS	Aspect of colonies after incubating 24 hr at 37 °C	
	GROWTH	COLOR
Listeria monocytogenes ATCC 35152	+	Blue
Listeria innocua ATCC 33090	+	Blue
Escherichia coli ATCC 25922	Inhibited	NA
Enterococcus faecalis ATCC 19433	Inhibited	NA

NA = Not applicable

KEYWORDS

RAPID'*Listeria* spp. / Listeria / *Listeria monocytogenes* / Detection / Enumeration / Food products / Environment / Fraser / Glucosidase / Chromogenic / Medium.

BIBLIOGRAPHY

• **OTTAVIANI F., OTTAVIANI M., AGOSTI M. (1997b):** Differential agar medium for Listeria monocytogenes. Quimper Refrigeration Symposium Proceedings p6. ADRIA, Quimper, France. **16-18** June 1997.

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