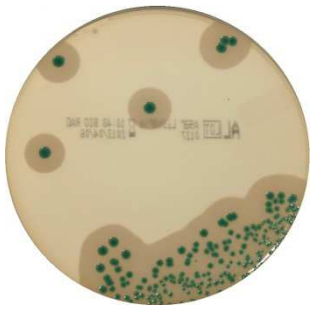
AL



Ref#	Description
Pre-poured	
3563695	90 mm x 20 dishes
3563965	90 mm x 120 dishes
Base in bottle	
3555200	237.5 ml x 6 bottles
Dehydrated base	
3564043	500 g
Supplements	
3564041	Supplement 1 (Freeze dried, 10 vials)
3564042	Supplement 2 (Liquid, 25 ml x 10 vials)

FIELD OF APPLICATION

Selective chromogenic medium used for the detection and enumeration of *Listeria monocytogenes* and other species of *Listeria* in food products for human and animal consumption, and in environmental samples. This medium may be used in standardized protocol or in an alternative validated short protocol.

PRINCIPLE

The principle of AL medium (Agar *Listeria* according to Ottaviani and Agosti) is based on the simultaneous detection of 2 enzyme activities: β-D-glucosidase and phosphatidylinositol-specific phospholipase C (PI-PLC). The β-D-glucosidase activity, common to all *Listeria* genus bacteria is detected using a chromogenic substrate (X-glucoside). Its hydrolysis induces the formation of a blue to blue-green colour in all *Listeria* colonies.
PI-PLC is an enzyme only detected in pathogenic *Listeria* species: *Listeria monocytogenes* and *Listeria ivanovii*. AL medium contains phosphatidylinositol which, when it breaks down, produces an opaque halo around colonies of bacteria of these 2 species.
This halo generally appears after 24 hours of incubation in *L. monocytogenes* and after only 48 hours of incubation in *L. ivanovii*.
Selectivity of the medium is achieved by the combined action of lithium chloride, antibiotics and the anti-fungal.

STANDARDS & VALIDATIONS

Bacteriological Analytical Manual Chapter 10 Detection and Enumeration of *Listeria monocytogenes* in Foods
MFHPB-30: Isolation of *Listeria monocytogenes* and Other *Listeria* spp. from Foods and Environmental Samples
ISO 11290-1 & 2 Microbiology of the food chain -- Horizontal method for the detection and enumeration of *Listeria monocytogenes* and other *Listeria* spp.

The AL has been certified NF VALIDATION (EN ISO 16140-2 protocol) as alternative methods to reference method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* genus.

NF VALIDATION by AFNOR Certification:

Target	Reading of typical colonies	Protocol	Certificate references	Scope	Reference method	Protocol
Listeria monocytogenes	Blue to blue-green with opaque halo	Detection	BRD 07/16-01/09 (AL Detection) BRD 07/17-01/09 (AL Enumeration) ALTERNATIVE ANALYTICAL METHODS FOR AGRIBUSINESS http://nf-validation.afnor.org/en	Broad range of food and environmental samples	EN ISO 11290-1	EN ISO 16140-2
		Enumeration			EN ISO 11290-2	
Listeria genus	Blue to blue-green	Detection			EN ISO 11290-1	

STORAGE / SHELF-LIFE / BATCH

- Dehydrated: + 15° to 25°C, in carefully sealed package, in a cool, dry and dark place
- Agar base in bottle, Supplement 1 & 2 and Pre-poured: + 2° to 8°C in a dark place
- Expiration date and batch number are shown on the package

THEORETICAL FORMULA

Meat peptone	18 g	Chromogenic substrate (5-bromo-4-chloro-3-indolyl-β-D-glucopyranoside)	0.05 g
Tryptone	6 g		
Yeast extract	10 g	Nalidixic acid	0.02 g
Sodium pyruvate	2 g	Ceftazidime	0.02 g
Glucose	2 g	Polymyxin B sulphate	76700 U
Anhydrous magnesium glycerophosphate	1 g	Cycloheximide	0.05 g
Anhydrous magnesium sulphate	0.5 g	Phosphatidylinositol	2 g
Sodium chloride (NaCl)	5 g	Agar	12 g
Lithium chloride (LiCl)	10 g	Distilled water	1,000 ml
Anhydrous Na2HPO4	2.5 g		

Final pH (25°C) = 7.2 ± 0.2

PREPARATION COMPLETE MEDIUM

AL supplement 1, code 3564041 (Box of 10 vials, 1 Vial for 500 ml of complete medium)

- Wearing latex gloves, aseptically reconstitute the contents of the vial with 5 ml of sterile distilled water using a sterile pipette. Reconstituted supplement can be stored for 1 week at 4°C.

AL supplement 2, code 3564042 (Box of 10 vials, 1 Vial for 500 ml of complete medium)

- Pre-heat the supplement to 44-47°C for at least 5 minutes (up to 6 hours) in a water bath.

Dehydrated base, code 3564043 (500 g of powders makes 7.2 liters of complete medium)
Always shake well before use

- Dissolve 34.55 g of powder in 470 ml of liters of distilled water
- Heat gently, swirling frequently, then bring to boiling point until completely dissolved
- Autoclave at 121 °C (± 1°C) for 15 minutes

Complete medium (250 ml)

- Melt the AL base medium (bottle code 3555200 or base prepared from the dehydrated base) and cool to 44-47°C
- Add 2.5 ml of the reconstituted AL supplement 1 in 237.5 ml of AL base medium and mix
- Add 12.5 ml of AL supplement 2 pre-heated at 44-47°C and mix
- Pour into Petri dishes

Note 1:

- The temperature of the base should not exceed 50°c when adding the supplement 2
- Stack Petri dishes together (e.g. 5) to allow slow cooling and then dry the agar surface well
- The reconstituted plates can be stored for 2 weeks at 4°C

PRODUCTS AND MATERIALS REQUIRED (Not supplied and non-exhaustive list)

Sample preparation:

Tryptone Salt diluent:

3555754	9 ml x 25 tubes
3555756	90 ml x 6 bottles
3564544	500 g
3555796	4 x 3 L bags

Fraser ½ broth:

3555797	6 x 225 ml bottles
3555794	4 x 3 L bags
3564604	Dehydrated base 500 g
3564616	Supplement

Buffered peptone water:

3554179	6 x 225 ml bottles
3564684	Dehydrated 500 g
3555795	4 x 3 L bags
3555790	2 x 5 L bags

Confirmation:

RAPID'*L.mono* agar:

3563694	20 dishes x 90 mm
3563964	120 dishes x 90 mm
3555294	Ready-to-use pack
3564293	Dehydrated base 500 g
3564294	Supplement 1
3564746	Supplement 2

PALCAM:

3564754	Dehydrated 500g
3564752	Supplement

iQ-Check® Kits:

3578124	for *Listeria monocytogenes*
3578113	for *Listeria* spp.

Equipment and material:

- Scales
- Sterile weighing bags
- Mill
- Stirrer-homogenizer
- Sterile Petri dishes (Ø 90 mm)
- Sterile pipettes
- Sterile spreaders
- Sterile Pasteur pipette
- Water-bath
- Incubators or incubation room
- All usual laboratory equipment

ALTERNATIVE PROTOCOLS

• *Listeria monocytogenes* and *Listeria* genus detection

- **Preparation of sample**

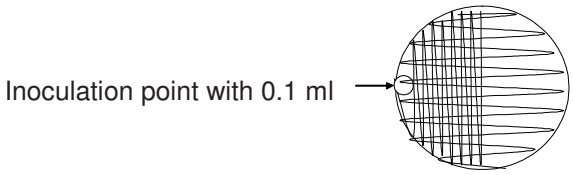
- Dilute n g or n ml of sample in 9 x n ml of Fraser ½ broth
- Incubate at 30°C (± 1°C) for 24 h (± 2 h)

Note 2:

- After incubation, the enrichment broth may be stored at 2-8°C for 72h, before the inoculation
- In the context of NF VALIDATION mark, no samples of over 25 g were tested
- Using a stick swab as sample: ensure that the swab is completely immersed in the broth (at least 9 ml)
- Using a sponge, cloth or gauze pad as sample: Add 9 times the weight of the moistened wiping device, of broth in the plastic bag containing the wiping device which must be completely soaked in the broth

- **Inoculation**

- Pipette 0.1 ml from the enriched Fraser ½ broth and lay it as a drop onto the surface of an AL agar plate close to the side of the plate
- Streak this inoculum with a loop on half of the plate, than streak on the other half of the plate coming back onto the first half as described in the scheme below:



- **Incubation**

- 37°C (± 1°C) for 24 h (±2 h)

Note 3:

- Incubation can be extended to 48h (±2 h)
- After incubation, the AL agar plates may be stored at 2-8°C for 72h

- **Interpretation**

- Typical colonies of *Listeria monocytogenes* are blue / blue-green colonies with halo
- Typical colonies of *Listeria* genus are blue / blue-green colonies with or without halo

- **Confirmation**

- See “Confirmation” section (page 4)

Note 4:

- In case of characteristic *Listeria monocytogenes* colonies using the AL detection protocol, it is not necessary to perform a confirmation if the sample has been already confirmed as positive in the enumeration protocol

• *Listeria monocytogenes* enumeration

- **Preparation of sample**

- Dilute n g or n ml of sample in 9 x n ml of Fraser ½ broth or Buffered Peptone Water
- Incubate at 20°C (± 2°C) for 1h (± 5 min). This step is optional using the Buffered Peptone Water

- **Inoculation**

- Spread 0.1 ml onto the surface of an AL agar plate or transfer 1 ml into an empty Petri dish and pour 15 ml of melted (44-47°C) AL agar

Note 5:

- If for some products it is necessary to estimate small numbers, spread 1 ml over 3 Ø 90 mm dishes or 1 Ø 140 mm dish of AL agar or 1 ml in inclusion in one dish of AL agar (pour plate inoculation)

- **Incubation**

- 37°C (± 1°C) for 48 h (±3 h)

Note 6:

- After incubation, the AL agar plates may be stored at 2-8°C for 72h before reading and confirmation
- A first reading at 24h allows a more rapid detection of heavily contaminated samples. However the final result count is reached after 48 h (±3 h)

- **Interpretation**

- Typical colonies of *Listeria monocytogenes* are blue / blue-green colonies with halo

- **Confirmation**

- See "Confirmation" section

Note 7:

- In case of characteristic *Listeria monocytogenes* colonies using the AL enumeration protocol, it is not necessary to perform a confirmation if the sample has been already confirmed as positive in the detection protocol

Confirmation of characteristic colonies

- Within the context of NF VALIDATION mark, all the positive results have to be confirmed
- In the event of discrepant results (positive with AL protocols, negative with the confirmation option), the laboratory should take sufficient measures to ensure the validity of its findings

Listeria monocytogenes:

- Using the conventional tests described in the standardised ISO reference methods (with purification step)

or

- 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 3578124)

or

- A colony isolated on AL agar may be confirmed by means of spot sub-culture on RAPID'*L.mono* agar. Up to 12 colonies can be confirmed on one dish of RAPID'*L.mono* agar

or

- Using any NF VALIDATION certified method based on a different principle from that of AL agar. The detection protocol of the validated second alternative method shall 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

Note 8:

- In the context of enumeration protocol and NF VALIDATION mark, if the first colony confirmed gives a negative result, continue the confirmations on other colonies (up to five)
- In the context of enumeration protocol, 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*

Listeria genus:

- Using the conventional tests described in the standardised ISO reference methods (with purification step)

or

- 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 3578113)

or

- A colony isolated on AL agar may be confirmed by streak sub-culture on PALCAM. Up to 6 colonies can be confirmed on one dish of PALCAM agar

or

- Using any NF VALIDATION certified method based on a different principle from that of AL agar. The detection protocol of the validated second alternative method shall 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

STANDARD PROTOCOLS

The AL agar formula is compliant to the agar *Listeria* according to Ottaviani and Agosti described into standard protocols (i.e. FDA Bacteriological Analytical Manual Chapter 10 Detection and Enumeration of *Listeria monocytogenes* in Foods, MFHPB-30: Isolation of *Listeria monocytogenes* and Other *Listeria* spp. from Foods and Environmental Samples, or ISO 11290-1 & 2 -- Microbiology of the food chain -- Horizontal method for the detection and enumeration of *Listeria monocytogenes* and other *Listeria* spp.). As a result, it is possible to use AL agar by following the instructions given in these standards.

PRECAUTIONS

- Standard precautions concerning the handling of potentially contaminated products in a microbiology laboratory should be observed. Media that have come in contact with food samples should be considered contaminated and should be autoclaved prior to disposal.
- Comply with Good Laboratory Practice. (EN ISO 7218).
- End of NF VALIDATION: please see the certificate BRD 07/16 - 01/09 BRD (AL Detection) & 07/17 - 01/09 (AL Enumeration). These certificates are available from Bio-Rad representative or AFNOR Certification.
- See SDS for Product Safety Information, www.bio-rad.com
- In the event of discrepant results (positive with AL protocols, negative with the confirmation option), the laboratory should take sufficient measures to ensure the validity of its findings.
- For heavily-loaded dishes with intensely opaque agar, reading can be facilitated by comparing opacity of the agar with a non-inoculated AL dish.
- Other Gram positive β-D-glucosidase positive bacteria exist without halos (e.g. *Enterococcus* spp.) and with halos (e.g. *Bacillus circulans*)
- Before using the AL agar dishes, leave them to dry, 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.

TECHNICAL SUPPORT IN THE UNITED STATES

In the United States, for technical assistance please call (800) 4BIORAD. Select option 2 for technical support and option 2 again for the food science division. To place an order, please call (800) 4BIORAD and press option 1 for customer care.

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 according to EN ISO 11133 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.

QUALITY AND PERFORMANCE OF THE TEST

See quality certificate available on www.bio-rad.com/certificate (Catalog#/ref# and Lot# number are required)

KEY WORDS

Agar *Listeria* according to Ottaviani and Agosti (AL) / *Listeria monocytogenes* / Detection / Enumeration / Food products / Fraser / Glucosidase / Phospholipase / Chromogenic / Medium

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