One Broth One Plate for *Listeria* (OBOP-L)

INTENDED USE

One Broth One Plate for *Listeria* (OBOP-L) offers a rapid method for the enrichment and detection of *Listeria* spp. and *Listeria monocytogenes* using traditional culture methodology.

PRODUCT SUMMARY AND EXPLANATION

Listeria monocytogenes, described first in 1926 by Murray, Webb and Swann, is an extensive problem in public health and food industries. This organism has the ability to cause human illness and death, particularly in immunocompromised individuals and pregnant women. Epidemiological evidence from outbreaks of listeriosis indicates the principle route of transmission is via the consumption of foods contaminated with *Listeria monocytogenes*. Implicated vehicles of transmission include meat, eggs, chicken, vegetables, and dairy products.

Listeria spp. are ubiquitous in nature, present in a wide range of unprocessed foods and in soil, sewage, and river water. Certain strains of *Listeria* spp. are able to survive the manufacturing and ripening processes in dairy products. *Listeria* spp. grow over a pH range of 5.0 – 9.6 and survive in food products with pH level outside these parameters. *Listeria* spp. are microaerophilic, Gram-positive, asporogenous, non-encapsulated, non-branching, short, motile rods. Motility is pronounced at 20°C for *Listeria*.

One Broth One Plate for *Listeria* utilises Neogen's proprietary LESS Plus for the enrichment steps as this provides superior recovery of *Listeria* species in foods and environmental samples. This is followed by detection using *Listeria* Chromogenic Agar (according to the formulation of Ottaviani and Agosti), a selective medium for the isolation and presumptive identification of *Listeria monocytogenes* from foodstuffs and related materials as described in ISO 11290-1:2017 or Palcam for *Listeria* spp. only or RAPID'*L. mono* for *Listeria monocytogenes* only.

INTENDED USER

The method is designed for use by personnel with appropriate training.

PRODUCT CODES

Product Name	Format	Pack Size	Product Code
LESS Plus	DCM	500g	NCM0202A
		5kg	NCM0202B
		10kg	NCM0202C
	Bagged Media	3L x 3 bags	NCM3400
	Ready-to-reconstitute	20L x 5 bags	NCM3206
		1 x filter unit	NCM3200
LCA (O&A) DCM or RTU			
Listeria Chromogenic Agar according to Ottaviani & Agosti LCA (O&A)	DCM	500g	NCM1004A or equivalent
		5kg	NCM1004B or equivalent
		10kg	NCM1004C or equivalent
	Pre-poured plates	20 x 90mm plates	NCM3000 or equivalent
LCA (O&A) DCM Supplements			
Listeria Selective diagnostic supplement	LCA (O&A) DCM Supplements	10 vials (2 x Vials needed per 1L media)	NCM4001
Listeria Chromogenic Selective supplement		10 vials (2 x Vials needed per 1L media)	NCM4002
PALCAM DCM			
PALCAM	DCM	500g	NCM0111A or equivalent
		5kg	NCM0111B or equivalent
		10kg	NCM0111C or equivalent
Confirmation			
Blood Agar Base	DCM	500g	NCM0040A or equivalent
		5Kg	NCM0040B or equivalent
		10Kg	NCM0040C or equivalent
Listeria Carbohydrate Confirmation Broths	RTU	L-Rhamnose broth (200 tests) D-Xylose broth (200 tests)	NCM3800

*Pre-poured plates are only available in certain countries. Please ask your Account Manager for further details.

EQUIPMENT AND MATERIALS REQUIRED

- Stomacher or equivalent
- Stomacher-type bags for sample enrichment. Filter bags are recommended (Neogen item 6827 or equivalent)
- Graduated cylinder, 250 mL (Neogen item 9368 or equivalent)
- 1 L purified water

TYPICAL FORMULATIONS

Please refer to the specific product information sheets for formulation, reconstitution, QC organisms, interpretation and storage

LESS Plus	g/L
Peptone	15.0
Buffer	15.7
Growth enhancers	8.3
Selective mix	5.0
Final pH 7.0±0.2 at 25 °C	

Listeria Chromogenic Agar (LCA)	g/L
Enzymatic Digest of Animal Tissue	18.0
Enzymatic Digest of Casein	6.0
Yeast extract	10.0
Sodium Pyruvate	2.0
Glucose	2.0
Magnesium Glycerophosphate	1.0
Magnesium Sulfate (anhydrous)	0.5
Sodium Chloride	5.0
Lithium Chloride	10.0
Disodium Hydrogen Phosphate (anhydrous)	2.5
5-bromo-4-chloro-3-indolyl-β-D-Glucopyranoside	0.05
Agar	12.5
Final pH 7.2 ± 0.2 at 25°C	

Method for reconstitution of LESS Plus from DCM

1. Dissolve 44 g of NCM0202 in one litre of purified water.
2. Heat with frequent agitation to completely dissolve the medium, if necessary.
3. Autoclave at 121°C for 15 minutes.

Method for reconstitution of LCA (O&A) from DCM

1. Suspend 69.5 grams of the medium in 950mL of purified water. Mix thoroughly
2. Heat with frequent agitation and boil for one minute to completely dissolve the medium.
3. Autoclave at 121°C for 15 minutes.
4. Cool to 48-50°C and add 2 vials of reconstituted NCM4002 supplement.
5. Add 2 vials of NCM4001 supplement (pre-heated to 48-50°C).
6. Mix well with gentle end-over-end mixing and pour into Petri dishes.

Expected Cultural Response:

Cultural response on *Listeria* Chromogenic Agar (supplemented with NCM4001 & NCM4002), incubated aerobically at 37±2°C and examined for growth after 44 – 52 hours incubation.

Microorganism	WDCM	Expected results
Listeria monocytogenes	00021	Good Growth, Blue to blue/green, surrounded by opaque halo
Listeria monocytogenes	00109	Good Growth, Blue to blue/green, surrounded by opaque halo
Escherichia coli	00012 or 00013	Inhibited
Enterococcus faecalis	00009 or 00087	Inhibited
Listeria innocua	00017	Blue/green colonies without an opaque halo

INTERPRETATION

Growth characteristics on LCA (O&A)		
Microorganism	Growth	Colour
Listeria monocytogenes	Good Growth	Blue to blue-green, surrounded by opaque halo
Listeria spp.	Good Growth	Blue to blue-green, without opaque halo

PRECAUTION:

Refer to SDS.

https:/www.neogen.com/solutions/microbiology/harlequin-chromogenic-agar-salmonella-esterase/

STORAGE

Product Name	Format	Storage conditions
LESS Plus	DCM (as supplied)	Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping the container tightly closed.
	DCM (reconstituted)	Store in the dark at 2-8°C, use within the same day as prepared.
	Bagged Media	Store in the dark at 2-8°C. Use within stated shelf life.
	Ready-to-reconstitute (as supplied)	Store in the dark at 2-30°C. Use within stated shelf life.
	Ready-to-reconstitute (reconstituted)	Store in the dark at 2-30°C for up to 5 days (providing asepsis is maintained)
Agars	DCM (as supplied)	Store sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping the container tightly closed.
	DCM (reconstituted)	Store in the dark at 2-8°C, use within 7 days of preparation.
	Pre-poured plates	Store in the dark at 2-30°C. Use within stated shelf life.
Listeria Carbohydrates Confirmation	RTU (as supplied)	Store in the dark at 2-8 °C until the expiry date. Once opened, ensure that partially used packs are properly sealed prior to continued storage at 2-8 °C.

TECHNICAL PRODUCT INFORMATION

FLOW DIAGRAM

x g or *x* mL of sample in 9 x *x* mL of LESS Plus broth incubate for 25±3h for *Listeria* spp. or 27±3h for *Listeria monocytogenes* at 30 ±1°C

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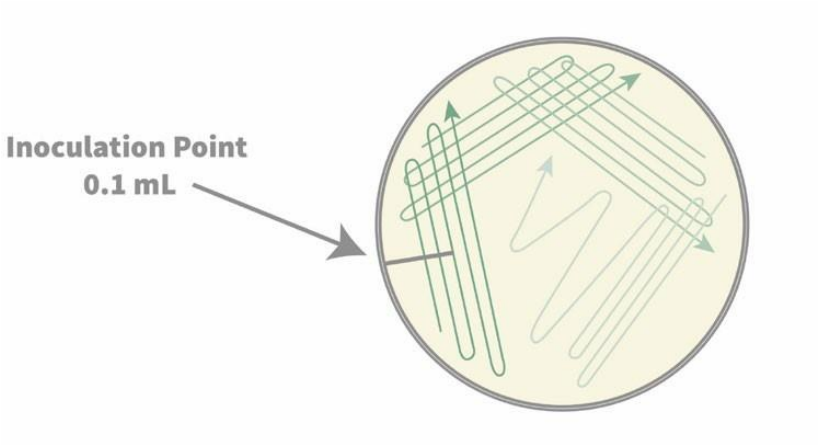
Detection and isolating 0.1mL on 1 plate and incubate 24h (up to 48h) at 37±2°C



Listeria spp.	*Listeria monocytogenes*
• *Listeria* Chromogenic Aga according to Ottaviani & Agosti • PALCAM	• *Listeria* Chromogenic Agar according to Ottaviani & Agosti • RAPID'L.mono

↓

Confirmation of typical *Listeria monocytogenes* (blue/green colonies with opaque halo) using standard tests described in the standardized CEN or ISO methods (NCM0040 and NCM3800) or ANSR methods

TEST PROCEDURE

1. Weigh ***x*** g sample in Stomacher-type bag.
2. Dilute 1:10 ***x*** g or ***x*** mL of sample in 9 x ***x*** mL of LESS Plus broth, i.e add 25 g or 25 mL of sample in 225mL of LESS Plus broth.
   NOTE: For swab testing, the volume of broth should cover the swab sample
3. Homogenize in a Stomacher blender
4. Incubate the enrichment broth and samples at 30 ± 1°C for 25 ± 3 hours (for *Listeria* spp.) or 27 ± 3hours (for *Listeria monocytogenes*).
   NOTE: Samples can be maintained at room temperature for two hours when carrying out the analysis.
   NOTE: It is possible to store the enriched LESS Plus broth between 2-8°C for 72 hours maximum, following the last incubation at 30°C.
   NOTE: In the context of NF VALIDATION, test portions weighing more than 25g have not been tested.
5. Using a sterile inoculating loop, remove 0.1 mL from the LESS Plus enrichment broth and isolate onto the surface of a selective agar plate Agar *Listeria* according Ottaviani and Agosti or Palcam (*Listeria* spp. only) or RAPID'*L. mono* (*Listeria monocytogenes* only). Streak this inoculum with a loop on half of the plate, then streak on the other half of the plate coming back onto the first half as described in the scheme above (4 quadrant streak method).
6. Incubate the plate at 37±2°C. Read plates after 24 to 48 hours. It is not necessary to prolong incubation to 48 hours for the plates screened at 24 hours whatever the result of the screening, except for PALCAM necessitating 48h for a final reading.
   NOTE: Please refer to the specific product information sheets for information on how to read the plates.

INTEPRETATION AND CONFIRMATION

Take a reading after 27 ± 3 hours, *Listeria* spp. colonies are blue/green.

NOTE: After incubation, the LCA (O&A) plates can be stored in a refrigerator (2-8 °C) for 72 hours, before reading and confirmation.

In the context of standard method confirmation, one isolated colony can be confirmed from the LCA (O&A) plate in one of two ways:

1. Using standard tests described in the standardized CEN or ISO methods (e.g. using a carbohydrate utilization test (NCM3800) after a subculture on Blood agar (NCM0040) or TSYEA/TSYEB followed by a haemolytic test).
2. Using ISO 16140-6 validated methods and certified, starting from a colony.

In the context of the NF VALIDATION certified method, all positive culture media screening results need to be confirmed by:

1. Using the conventional tests described in the methods standardized by CEN or ISO methods (e.g. using a carbohydrate utilization test (NCM3800) after a subculture on Blood agar (NCM0040) or TSYEA/TSYEB followed by a haemolytic test).
2. Using ANSR *Listeria* or ANSR *Listeria monocytogenes*. For the confirmation test, it is necessary to start from the LESS Plus enrichment broth after the full enrichment at 30°C.
3. Using any ISO 16140-6 validated method.

NOTE: In the event of results that are not in agreement, between the detection method and one of the confirmation options listed above, the laboratory should follow the necessary steps to ensure the validity of their results.

PRECAUTIONS & LIMITATION OF THE METHOD

1. Use good microbiology laboratory practices, such as ISO 7218.
2. Please note that colonies of *L. ivanovii* on LCA can give blue colonies and a small zone of precipitation and colonies of *B. cereus* on LCA can give blue colonies, confirmation is mandatory in the context of the NF Validation.
3. Please note that some organisms such as *Bacillus cereus*, Enterococci and *Staphylococcus* can present as a target grey/green colour on PALCAM agar, confirmation is mandatory in the context of the NF Validation.
4. For precautions and limitations on Rapid'*L. mono* and PALCAM please refer to the respective supplier technical sheet.

DISPOSAL

Enrichment cultures should be disposed of as biohazard waste. The preferred method of treatment for biohazard waste is autoclaving. Items that cannot be autoclaved may be disinfected with bleach solution. Consult with the safety advisor for your facility for detailed instructions.

CUSTOMER SERVICE

NEOGEN Customer Services and Technical Services can be reached by using the contact information below. Training on this product, and all NEOGEN test kits, is available upon request to your Account Manager.

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WARRANTY

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VALIDATIONS

1. One Broth One Plate for *Listeria* (OBOP-L) has been certified by NF VALIDATION as an alternative to the reference standard ISO 11290-1, according to the ISO 16140 part 2 protocol, for the detection of *Listeria* spp. in all food products for human consumption and in environmental samples.

   End of NF VALIDATION: please see the certificate

   NEO: 35/05-07/16

2. One Broth One Plate for *Listeria monocytogenes* (OBOP-LMO) has beencertified by NF VALIDATION as an alternative to the reference standard ISO 11290-1, according to the ISO 16140 part 2 protocol, for the detection of *Listeria monocytogenes* in all food products for human consumption and in environmental samples.

   End of NF VALIDATION: please see the certificate

   NEO: 35/06-07/16

REFERENCES

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