Carbohydrate Utilization Broths *Listeria monocytogenes* Confirmation 200 tests (ISO) (NCM3800)

NCM3801	Carbohydrate utilization Broth with L-Rhamnose, 200 reactions
NCM3802	Carbohydrate utilization Broth with D-Xylose, 200 reactions

Revision: 2 Effective Date: 6/11/2021

Intended Use

Carbohydrate utilization broths are used for the confirmation of *Listeria monocytogenes* from non-selective agar according to ISO 11290-1:2017, and is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Formulated as described by ISO 11290-1:2017, carbohydrate ultilization broths are simple formulations that allows the visualization of carbohydrate fermentation. D-Xylose is used for the confirmation of *L. monocytogenes*, which is L-Rhamnose positive, and D-Xylose negative. Enzymatic digest of animal tissues and meat extract provide all the necessary amino acid, vitamins and minerals for growth. Sodium chloride maintains osmotic balance and bromocresol purple changes the broth from purple to yellow, when acid is produced from carbohydrate fermentation

The product can be used as part of the ISO 11290 workflow or as part of the Neogen *Listeria* One Broth One Plate (OBOP) alterative workflow.

Each box contains a universal tube for each test, L-Rhamnose and D-Xylose, with 20mL of the ready to use test reagent.

Principle of the procedure

Fermentation of the carbohydrate produces acid which decreases the pH of the medium. Bromocresol purple is a pH indicator dye which is purple when above pH 6.8 and yellow when below pH 5.2. *L. monocytogenes* can ferment L-Rhamnose but not D-Xylose. This profile aids the differentiation of *L. monocytogenes* from organisms that may present a false positive on Listeria Chromogenic Agar (Ottaviani & Agosti).

Typical Formulation

Enzymatic Digest of Animal Tissues	10.0 g/L
Meat Extract	1.0 g/L
Sodium Chloride	5.0 g/L
Bromocresol Purple	0.02 g/L
Carbohydrate*	5.00 g/L

*L-Rhamnose or D-Xylose

Final pH: 6.8 ± 0.2 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

Precaution

Refer to SDS

Recommended Test Procedure

Typical *Listeria monocytogenes* obtained from LCA (NCM1004/NCM3000) (blue/green colonies with an opaque halo), should be sub-cultured onto non-selective agar e.g. Blood agar and incubated for at 37°C for 18-26 hours. Isolated colonies exhibiting hemolysis should be tested for the ability to ferment both L-Rhamnose and D-Xylose.

NEOGEN
620 Lesher Place • Lansing, MI 48912
800-234-5333 (USA/Canada) • 517-372-9200
foodsafety@neogen.com • foodsafety.neogen.com

As referenced in ISO 11290-1:2017, reactions tested in micro-volumes provide more rapid results. As such, 0.1 mL should be aseptically dispensed into suitable sterile containers i.e. microcentrifuge tube. Using a sterile loop inoculate the dispensed broth with one isolated colony. The inoculated broths should be incubated at **37°C for 1 - 24 hours**. A positive fermentation reaction is indicated by a color change from purple to yellow. The rate of the potential color change is dependent on the inoculation level and the rate of growth of the isolate. Typically, most reactions are complete with 4-6 hours. If the reaction is unclear it should be left for the full incubation time.

Quality Control Specifications
Prepared Appearance: Clear purple solution

Minimum QC:
Listeria monocytogenes WDCM 00021

Results
Listeria monocytogenes should produce a positive reaction for L-Rhamnose but a negative reaction for D-Xylose.

Picture 1. Typical appearance of a positive fermentation reaction (left) and a negative reaction (right)

Expiration
Refer to expiration date stamped on the container. Expiry applies to medium in its intact container when stored as directed. Do not use if the broth has changed in color or appearance.

Limitations of the Procedures
Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium. There are rare strains of *L. monocytogenes* which do not ferment L-Rhamnose. If the broth is overloaded with biomass the color may appear to change, a single, well isolated colony should be used for testing. Any uncertain results should be enriched for the full 24-hour incubation and the test repeated where necessary. The product should be used alongside further confirmation test as specified by the relevant standard.

Storage
Store unopened media upright at 2-8 °C and away from direct sunlight until the expiry date. Once opened, ensure that partially used packs are properly sealed prior to continued storage at 2-8 °C and in the dark. Asepsis should be maintained throughout the use of the pack to ensure accurate results.

Note:

Semi-regular (i.e. once a month) quality control of the media is recommended by testing for a positive reaction (yellow colour change) with *L. monocytogenes* WDCM 00021 for L-Rhamnose (NCM3801) and *L. ivanovii* WDCM 00018 for D-Xylose (NCM3802).

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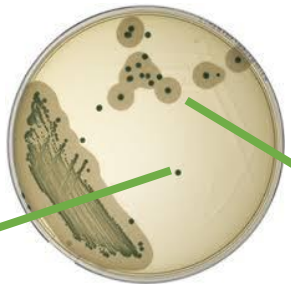
OBOP-L confirmation workflow according to Retailer Supplementary Audit (RSA) and ISO 11290



Listeria spp.

***Listeria* spp. confirmation according to ISO 11290**

This confirmation is accepted as a retailer approved method, meaning the workflow can be used by manufacturers supplying products to some of the UK's leading supermarkets.

Take 5 blue/green colonies without halo and isolate them on 2 TSYEA plates and incubate at 37°C for 18-24h

From isolated colonies suspended in a drop of peroxide solution, perform catalase test. This is an immediate reaction

If positive, carry out gram stain test

If negative, stop - not *Listeria* spp.

If gram positive, stop - confirmed *Listeria* spp.

If negative, stop - not *Listeria* spp.

Listeria monocytogenes

***Listeria monocytogenes* confirmation according to ISO 11290**

This confirmation is accepted as a retailer approved method, meaning the workflow can be used by manufacturers supplying products to some of the UK's leading supermarkets.

Take 5 blue/green colonies with a halo and streak them on 5 different lines on 1 blood agar plate. Incubate at 37°C for 20-24h

If less than 5 colonies with halos are available on the plate confirm all of them

Check for haemolysis

If haemolysis positive, carry out D-Xylose and L-Rhamnose tests for one colony at 37°C for 1-4h

If haemolysis negative, test haemolysis reaction using red blood corpuscles

If L-Rhamnose positive & D-Xylose negative, stop - confirmed *Listeria monocytogenes*

If L-Rhamnose negative, repeat up to 4 times

If negative, stop - not *Listeria monocytogenes*

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References

1. ISO 11290-1:2017 Microbiology of the food chain– Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp.
2. ISO 11290-2:2017 Microbiology of the food chain- Horizontal method for the detection and enumeration of Listeria monocytogenes and Listeria spp.- Part 2: Enumeration method
3. ISO 11133-1:2014 + A2:2020 Microbiology of food animal feed and water – Preparation, production, storage and performance testing of culture media – Amendment 2
4. Liu D., Lawrence M.L., Wiedmann M., Gorski L., Mandrell R.E., Ainsworth A.J. *et al*. *Listeria monocytogenes* subgroups IIIA, IIIB, and IIIC delineate genetically distinct populations with varied pathogenic potential. J. Clin. Microbiol. 2006, 44 pp. 4229–4233
5. Roberts A., Nightingale K., Jeffers G., Fortes E., Kongo J.M., Wiedmann M. Genetic and phenotypic characterization of *Listeria monocytogenes* lineage III. Microbiology. 2006, 152 pp. 85–693

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