Half Fraser (Demi-Fraser) Broth (ISO) (NCM0001)

NCM0001 500G, 5KG & 10KG DCM Packs
NCM3205 20L Ready-to-Reconstitute Media Bags (pre-supplemented with FAC at 0.5g/L)

Intended Use

Half Fraser (Demi-Fraser) Broth (ISO) is a selective medium used for the primary enrichment of *Listeria monocytogenes* and *Listeria spp.*, as described in ISO 11290-1:2017. Half Fraser (Demi-Fraser) Broth (ISO) is not intended for use in the diagnosis of disease or other conditions in humans.

Description

Half Fraser (Demi-Fraser) Broth (ISO) was developed as a modification of UVM medium (NCM0012) and is based on the formula as described by Fraser and Sperber. *Listeria spp.* hydrolyze the esculin to esculetin which reacts with the ferric ammonium citrate (NCM4009) resulting in a black precipitate and a visible positive reaction. Lithium chloride suppresses the growth of *Enterococci* in the medium, as *Enterococci* can also hydrolyze esculin. Enzymatic digest of animal tissues, casein, meat extract and yeast extract provide the essential vitamins, minerals, amino acids, nitrogen and carbon, while sodium chloride maintains the osmotic balance. The selective components acriflavine and nalidixic acid are blended into the base powder and inhibit the growth of *Escherichia coli*. Ferric ammonium citrate is added to the tempered broth after sterilization. According to ISO 11290-1:2017, the test portion is inoculated into the primary enrichment medium Half Fraser (Demi-Fraser) Broth (ISO), followed by secondary enrichment in Fraser Broth (ISO) and then sub-cultured onto Harlequin Listeria Chromogenic Agar according to Ottaviani & Agosti (NCM1004). The medium conforms to the performance and formulation requirements of ISO 11290-1:2017.

Typical Formulation

Enzymatic Digest of Animal Tissues	5.0g/L
Enzymatic Digest of Casein	5.0g/L
Meat Extract	5.0g/L
Yeast Extract	5.0 g/L
Esculin	1.0 g/L
Disodium Hydrogen Phosphate	9.6 g/L*
Potassium Dihydrogen Phosphate	1.35 g/L
Sodium Chloride	20.0 g/L
Lithium Chloride	3.0 g/L
Acriflavine	0.0125 g/L
Nalidixic Acid	0.01 g/L

pH: 7.2 ± 0.2 at 25°C
*equivalent to Disodium Hydrogen Phosphate Dihydrate 12.0 g/L
Formula may be adjusted and/or supplemented as required to meet performance specifications.

Supplement

NCM4009 Ferric Ammonium Citrate

Precaution

Refer to SDS

Dehydrated Culture Medium Preparation

1. Dissolve 55 grams of the medium in one liter of purified water.
2. Mix thoroughly.
3. Autoclave at 121°C for 15 minutes

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4. Cool to 45-50°C.
5. Aseptically add 2 vials of NCM4009-0.5* Ferric Ammonium Citrate Supplement.

*Larger vials may be available. Please see appropriate supplement data sheet for availability and preparation instructions.

Ready-to-Reconstitute Media Bag Preparation

Please refer to the Instructions for Use for the range.

Test Procedure

To isolate *Listeria monocytogenes* and other *Listeria* spp., refer to ISO 11290-1:2017.

Quality Control Specifications

Dehydrated Appearance: Powder is homogeneous, free flowing, and beige.

Prepared Appearance: Prepared medium is yellow to amber and clear with none to slight precipitate.

Expected Cultural Response: The medium was prepared according to label directions, incorporating NCM4009 Ferric Ammonium Citrate and 10mL volumes inoculated with the organisms listed below. Cultures were incubated at 30 ± 1°C under aerobic atmosphere and examined for growth at 22-26 hours. Following incubation, 10µL was sub-cultured onto TSA at 37 ± 1°C and examined for growth at 22-26 hours or onto Harlequin® Listeria Chromogenic Agar (HLCA, NCM1004, PIN001)* at 37 ± 1°C and plates examined for growth at 40-48 hours.

MICROORGANISM	WDCM	APPROX. INOCULUM (CFU)	EXPECTED RESULTS				ACTUAL RESULTS
			Growth in HF	Reaction in HF*	Recovery on HLCA**	Recovery on TSA	
Listeria monocytogenes + *Escherichia coli* + *Enterococcus faecalis*	00021 00013 00087	10 – 100 $> 10^4$ $> 10^4$	Growth	Black coloration	>10 cfu Blue colonies with opaque halo	Not applicable	Meets Expected Result
Listeria monocytogenes + *Escherichia coli* + *Enterococcus faecalis*	00109 00012 00009	10 – 100 $> 10^4$ $> 10^4$	Growth	Black coloration	>10 cfu Blue colonies with opaque halo	Not applicable	Meets Expected Result
Escherichia coli	00012	$> 10^4$	Inhibition	None	Not applicable	Total inhibition	Meets Expected Result
Escherichia coli	00013	$> 10^4$	Inhibition	None	Not applicable	Total inhibition	Meets Expected Result
Enterococcus faecalis	00009	$> 10^4$	Partial to Complete Inhibition	None	Not applicable	< 100 cfu	Meets Expected Result
Enterococcus faecalis	00087	$> 10^4$	Partial to Complete Inhibition	None	Not applicable	< 100 cfu	Meets Expected Result

The organisms listed are the minimum that should be used for quality control testing.

Results

Listeria is presumptively indicated by the blackening of Half Fraser (Demi-Fraser) Broth (ISO) after 25 ± 1 hours incubation at 30°C ± 1°C.

*Sub-culture onto LCA provides presumptive *L. monocytogenes* or *L. ivanovii,* if colonies are blue-green surrounded by an opaque halo. Consider presumptive *Listeria spp.* if blue-green colonies with or without opaque halo.

Expiration

Refer to expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if appearance has changed from the original color. Expiry applies to medium in its intact container.

Limitations to the procedure

1. Some strains of *L. monocytogenes* exposed to stress conditions, particularly acid stress, can show a very weak halo
2. Some rare *L. monocytogenes* are characterized by a slow PIPLC activity. Such organisms will take longer to develop a halo.

Storage

Store dehydrated culture media (NCM0001) at 2 – 30°C away from direct sunlight. Once opened and recapped, place the container in a low humidity environment at the same storage temperature. Protect from moisture and light by keeping container tightly closed.
Store ready-to-reconstitute media bags (NCM3205) at 10-25°C away from direct sunlight. Once reconstituted store at 10-25°C away from direct sunlight for up to 5 days (providing asepsis is maintained).

References

1. Fraser, J.A. and Sperber, W.H. (1988). Rapid detection of Listeria spp in food and environmental samples by esculin hydrolysis. *J. Food Protect*. 51, No.10, 762-765.
2. McClain, D. and Lee, W.H. (1989). FSIS method for isolation of L. monocytogenes from processed meat and poultry products. Lab.Comm.No.57, Revised May 24, (1989). US Dept of Agric. FSIS, Microbiol. Div.
3. ISO 11290-1:2017 Microbiology of the food chain- Horizontal method for the detection and enumeration of *Listeria monocytogenes* and *Listeria* spp.- Part 1: Detection method
4. 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
5. ISO 11133:2014+A1:2018 Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media.