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

Intended Use
Half Fraser (Demi-Fraser) Broth (ISO) is used with ferric ammonium citrate for the selective enrichment of *Listeria* species and is not intended for use in the diagnosis of disease or other conditions in humans.

Description
Primary enrichment broth for the isolation of *Listeria* spp. formulated according to ISO 11290. The selective components acriflavine and nalidixic acid are blended into the base powder and the ferric ammonium citrate is added to the tempered broth after sterilization.

Half Fraser (Demi-Fraser) Broth (ISO), NCM0001 was developed to give improved results for the isolation of *Listeria* spp. with both traditional and ELISA methods. As with the sister product, Fraser Broth, NCM0050 the selective components acriflavine and nalidixic acid are blended into the base powder and the Ferric Ammonium Citrate is added to the tempered broth after autoclaving. This format has been shown to give improved selectivity with pure cultures and food samples. Furthermore, more stable ELISA results are seen, resulting in fewer false positive results when the ferric ammonium citrate is omitted from the complete media.

Developed as a modification of UVM medium (NCM0012) and made according to ISO 11290, Fraser Broth is a secondary enrichment broth for the isolation of *Listeria* spp. and is similar to Palcam Broth (NCM0049) in that it contains esculin to indicate the presence of a potential *Listeria* isolate. It also contains lithium chloride in an attempt to suppress the growth of Enterococci in the medium (as does Palcam). *Listeria* spp. hydrolyze the esculin to form esculetin, which reacts with the ferric ammonium citrate in resulting in a black precipitate and a visible positive reaction. However, Enterococci can also perform this reaction so further plating is required onto an isolation medium such as Harlequin Listeria Chromogenic Agar according to Ottaviani & Agosti (NCM1004).

Note: Acriflavine and Nalidixic Acid in NCM0001 are half-strength of Fraser Broth NCM0050.

Typical Formulation

Peptone Mixture	15.0 g/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.
Ferric Ammonium Citrate Supplement (X211) or Fraser Broth Base Supplement (7984) may be used

Precaution
Refer to SDS

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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
4. Cool to 45-50°C.
5. Aseptically add 2 vials of Ferric Ammonium Citrate Supplement (X211) or 2 vials of Fraser Broth Base Supplement (7984)

Test Procedure

To isolate *Listeria monocytogenes* and other *Listeria* spp., refer to appropriate references.

Quality Control Specifications

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

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

Expected Cultural Response:
(Fertility and Selectivity Testing)

The medium was prepared according to label directions 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 of *E. faecalis* and *E. coli* cultures were sub-cultured onto Tryptone Soy Agar (NCM0020) at 30 ± 1°C and examined for growth at 22-26 hours.
*Incubation up to 48 hours may be required to observe blackening reaction.

MICROORGANISM	ATCC	APPROX. INOCULUM (CFU)	EXPECTED RESULTS		
			Growth in HFB	Reaction in HFB*	Recovery on TSA
Enterococcus faecalis	29212	> 10^4	Partial to Complete Inhibition	—	< 100 cfu
Enterococcus faecalis	19433	> 10^4	Partial to Complete Inhibition	—	< 100 cfu
Escherichia coli	25922	> 10^4	Inhibition	—	< 10 cfu
Escherichia coli	8739	> 10^4	Inhibition	—	—
Listeria innocua	33090	10-100	Growth	Blackening	NA
Listeria monocytogenes	35152	10-100	Growth	Blackening	NA
Listeria monocytogenes	13932	10-100	Growth	Blackening	NA

(Productivity Testing)

The medium was prepared according to label directions and 10mL volumes inoculated as a mixed culture using the organisms listed below. Cultures were incubated at 30 ± 1°C under aerobic atmosphere for 22-26 hours followed by sub-culture onto Harlequin® Listeria Chromogenic Agar according to Ottaviani and Agosti (NCM1004) at 37 ± 1°C and plates examined for growth at 40-48 hours.

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Mixed Culture Testing	ATCC	APPROX. INOCULUM (CFU)	EXPECTED RESULTS	
			Recovery on Harlequin® Listeria Chromogenic Agar**	Reaction on Harlequin® Listeria Chromogenic Agar**
Enterococcus faecalis + *Escherichia coli* + *Listeria monocytogenes*	29212 25922 35152	≥1000 ≥1000 10-100	Inhibited Inhibited >10 cfu	— — Blue colonies with opaque halo
Enterococcus faecalis + *Escherichia coli* + *Listeria monocytogenes*	19433 8739 13932	≥1000 ≥1000 10-100	Inhibited Inhibited >10 cfu	— — Blue colonies with opaque halo

The organisms listed are the minimum that should be used for quality control testing.
** according to Ottaviani and Agosti

Results
Listeria is presumptively indicated by the blackening of Half Fraser (Demi Fraser) Broth after 24 hours incubation at 30°C. For further identification and confirmation of *Listeria* species, consult appropriate references. Rapid slide and macroscopic tube tests can be used for definitive serological identification.

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.

Storage
Store dehydrated culture media 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.

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

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