TECHNICAL DATA SHEET

HALF-FRASER BROTH

SELECTIVE PRIMARY ENRICHMENT FOR *LISTERIA*

1 INTENDED USE

Half-Fraser broth is used for the selective and differential (primary enrichment broth) enrichment of *Listeria monocytogenes* and of *Listeria* spp. in food products, according to the Standard NF EN ISO 11290-1.
The media is also used in the context of alternative rapid methods for the detection and enumeration of *Listeria monocytogenes* or *Listeria* spp.

2 HISTORY

The medium studied by Fraser *et al.* in 1988 is a modification of the formulation of Donnelly and Baigent. The composition of the base is identical to that of UVM Broth and was modified by the addition of lithium chloride as selective agent and of ferric ammonium citrate to visualize cultures that hydrolyze esculin, by the resultant blackening of the medium.

3 PRINCIPLES

The very good recovery of *Listeria monocytogenes* is assured by the concentration differences in nalidixic acid and acriflavine between Half-Fraser and Fraser, as well as the two enrichment steps themselves. Half-Fraser Broth allows the primary enrichment step, with secondary enrichment being performed in Fraser Broth.

Polypeptone, yeast extract and meat extract furnish the nutrients required for the growth of *Listeria*.

The high sodium chloride content increases the selectivity of the medium.

Phosphates act as buffers and maintain the pH of the medium.

Esculin is hydrolyzed by *Listeria* to glucose and esculetin, the latter compound forming a black complex with ferric ions supplied by ferric citrate, added just before use, which also favors the growth of *Listeria*.

Lithium chloride inhibits the growth of most enterococci which can also hydrolyze esculin.

Nalidixic acid blocks the DNA replication of bacteria sensitive to this antibacterial agent.

The growth of secondary Gram-positive microflora is inhibited by acriflavine.

4 TYPICAL COMPOSITION

The composition can be adjusted in order to obtain optimal performance.

For 1 liter of media :

- Enzymatic digest of animal tissues ..... 5,0 g
- Enzymatic digest of casein ..... 5,0 g
- Yeast extract ..... 5,0 g
- Meat extract ..... 5,0 g
- Sodium chloride ..... 20,0 g
- Disodium phosphate, anhydrous* ..... 9,6 g
- Monopotassium phosphate ..... 1,35 g
- Esculin ..... 1,0 g
- Lithium chloride ..... 3,0 g
- Nalidixic acid ..... 10,0 mg
- Acriflavine hydrochloride ..... 12,5 mg
- Ferric ammonium citrate ..... 0,5 g

pH of the ready-to-use media at 25 °C : 7,2 ± 0,2.

For 55 g of dehydrated base media BK133

- Enzymatic digest of animal tissues.....5,0 g
- Enzymatic digest of casein .....5,0 g
- Yeast extract .....5,0 g
- Meat extract.....5,0 g
- Sodium chloride.....20,0 g
- Disodium phosphate, anhydrous*.....9,6 g
- Monopotassium phosphate .....1,35 g
- Esculin.....1,0 g
- Lithium chloride .....3,0 g

For one vial of supplement BS030

- Nalidixic acid .....5,00 mg
- Acriflavine hydrochloride .....6,25 mg
- Ferric ammonium citrate.....0,25 g

For one vial of supplement BS032

- Nalidixic acid .....22,5 mg
- Acriflavine hydrochloride .....28,125 mg
- Ferric ammonium citrate.....1,125 g

For 55 g of dehydrated base media BK173

- Enzymatic digest of animal tissues.....5,0 g
- Enzymatic digest of casein .....5,0 g
- Yeast extract .....5,0 g
- Meat extract.....5,0 g
- Sodium chloride.....20,0 g
- Disodium phosphate, anhydrous*.....9,6 g
- Monopotassium phosphate .....1,35 g
- Esculin.....1,0 g
- Lithium chloride .....3,0 g
- Nalidixic acid .....10,0 mg
- Acriflavine hydrochloride .....12,5 mg

For one tube of liquid supplement BS062 (10 mL)

- Ferric ammonium citrate.....0,5 g

For one vial of liquid supplement BS059 (90 mL)

- Ferric ammonium citrate.....4,5 g

* NOTE : Equates to 12 g of Disodium hydrogen phosphate dehydrate.

5 PREPARATION

- Dissolve 55,0 g of dehydrated Fraser broth (BK133 or BK173) in 1 liter of distilled or demineralized water.
- Stir slowly until complete dissolution.
- Dispense in vials, at 225 mL per vial.
- Sterilize in an autoclave at 121 °C for 15 minutes.
- Cool to room temperature.

✓ **Reconstitution : 55,0 g/L**

✓ **Sterilization : 15 min at 121 °C**

Use of dehydrated base media BK133

- Reconstitute the freeze-dried supplement for Half-Fraser qsp 500 mL (BS030) with 5 mL of a 1:1 ethanol / sterile distilled water or with supplement qsp 2,25 L (BS032), with 20 mL of the same 1:1 solution.
- Mix well to insure a complete dissolution, taking care to avoid overdue foaming.
- Add 2,25 mL of reconstituted supplement BS030 or 2 mL of reconstituted BS032 supplement into each vial of 225 mL of broth.
- Mix well.

Use of dehydrated base media BK173

- Aseptically add to each 225 mL vial of broth, 2,25 mL of a sterile solution of 5% ferric ammonium citrate (BS059 or BS062).
- Mix well.

6 INSTRUCTIONS FOR USE

- From vials prepared as above or by using complete ready-to-use media in vials or flexible bags (BM016, BM133, BM134), aseptically add 25 g of the product to test.
- Mix well.
- Incubate at 30 ± 1 °C for 24 to 26 hours.

✓ **Inoculation :**
25 g in 225 mL

✓ **Incubation :**
24 to 26 h at 30 °C

NOTE
Half-Fraser broth can also be used as a diluent in the context of the enumeration of *Listeria monocytogenes* (NF VALIDATION, BKR 23/05-12/07).

7 RESULTS

All tubes, presenting blackening or not, must be re-inoculated into secondary enrichment media or onto selective isolation media. A minimum incubation of 24 hours is necessary to visualize the blackening reaction.

Re-inoculate 0,1 mL from each tube into a tube of Fraser broth for normalized (standardized) methods.
Re-inoculate onto COMPASS® *Listeria* Agar for the rapid detection of *Listeria monocytogenes* and *Listeria* spp (NF VALIDATION, BKR 23/02-11/02).

NOTE :
For all other applications, use the reference method in vigor.

8 QUALITY CONTROL

Dehydrated media : yellowish powder, free-flowing and homogeneous.
Freeze-dried selective supplements : brown pellets, giving after reconstitution a brown solution, with possible precipitates.
Ferric ammonium citrate 5% solution : brown liquid, may present a slight precipitate.
Prepared (complete) media : brownish solution with bluish reflections, may contain a slight precipitate.

Typical culture response after 24 hours of incubation at 30 °C, followed by subculture (NF EN ISO 11133) :

Microorganisms		Growth
Listeria monocytogenes 4b + *Enterococcus faecalis* + *Escherichia coli*	WDCM 00021 WDCM 00087 WDCM 00013	> 10 characteristic colonies
Listeria monocytogenes 1/2a + *Enterococcus faecalis* + *Escherichia coli*	WDCM 00109 WDCM 00087 WDCM 00012	> 10 characteristic colonies
Enterococccus faecalis *Escherichia coli*	WDCM 00087 WDCM 00013	< 100 colonies Inhibited

9 STORAGE / SHELF LIFE

Dehydrated base media : 2-30 °C.
Half-Fraser broth selective supplements : 2-8 °C.
Sterile 5% solution of ferric ammonium citrate : 2-25 °C.
Complete media in vials or flexible bags : 2-8 °C, shielded from light.
The expiration dates are indicated on the labels.

NOTE :
Complete ready-to-use media in vials or in flexible bags can be kept 2 months at 15 - 25 °C, shielded from light, without any impact on microbiological performance.

Prepared base media in vials BK133 (*) : 180 days at 2-8 °C.
Prepared based media in vials BK173 (*) : 180 days at 2-8 °C, shielded from light.
Prepared complete media in vials (*) : 180 days at 2-8 °C, shielded from light.
Rehydrated freeze-dried supplements (*) : 30 days at 2-8 °C, shielded from light.
(*) Benchmark value determined under standard preparation conditions, following manufacturer's instructions.

10 PACKAGING

Dehydrated media FRASER base II :
500 g bottle BK133HA
5 kg drum BK133GC

Half-FRASER selective supplement :
10 vials qsp 500 mL BS03008
8 vials qsp 2,25 L BS03208

Half-FRASER dehydrated media base (without ferric ammonium citrate) :
500 g bottle BK173HA
5 kg drum BK173GC

Sterile 5% solution Ferric ammonium citrate :
10 x 90 mL vials BS05908
7 x 10 mL tubes BS06208

Ready-to-use media in vials :
10 x 225 mL vials BM01608

Ready-to-use media in flexible bags :
3 x 3 L bags BM13308
2 x 5 L bags BM13408
40 x 5 L bags (carton) BM18808

11 BIBLIOGRAPHY

Donnelly, C.W., and Baigent, G.J.. 1986. Method for flow cytometric detection of *Listeria monocytogenes* in milk. Applied and Environmental Microbiology, **52** : 689-695.

Fraser, J.A., and Sperber, W.H.. 1988. Rapid detection of *Listeria* spp. in food and environmental samples by esculin hydrolysis. Journal of Food Protection, **51** : 762-765.

NF EN ISO 11133. Juillet 2014. Microbiologie des aliments, des aliments pour animaux et de l'eau - Préparation, production, stockage et essais de performance des milieux de culture (Tirage 2 (2016-01-01)).

NF EN ISO 11290-1. Juillet 2017. Microbiologie de la chaîne alimentaire - Méthode horizontale pour la recherche et le dénombrement de Listeria monocytogenes et Listeria spp. - Partie 1 : méthode de recherche.

12 ADDITIONAL INFORMATION

COMPASS® is a registered trademark of SOLABIA S.A.S.

The information provided on the labels take precedence over the formulations or instructions described in this document and are susceptible to modification at any time, without warning.

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