CULTURE MEDIA

This document presents formulae and a brief instruction of preparation of culture media required for some of the methods (approved by the Department of Agriculture). This is not meant to be a complete list, nor is it meant to provide all the information required to prepare specific media. For detail procedures of media preparation and quality control, laboratories are required to consult with the relevant standards and manufacturer's instructions.

To achieve optimum performance dehydrated and prepared culture media must be stored under specified conditions and must be used by their expiry dates. Expiry date of dehydrated media as well as prepared media including storage conditions can be found on the product label or insert of each medium.

Shelf-life and storage conditions of some prepared media are provided below:

Media	Shelf-life*
Buffered Peptone Water (BPW)	Sealed, 2 weeks at 2-8°C
Demi-Fraser Broth/Half Fraser Broth	Sealed, 2 weeks at 2-8°C
Double Modified Lysine Iron Agar (DMLIA)	Sealed, 3 weeks at 2-8°C
Horse Blood Overlay Medium (HBO or HL)	Sealed, 2 weeks at 2-8°C
Modified Rainbow Agar (mRBA)	Sealed, 3 weeks at 2-8°C
XLT4	Sealed, 3 months at 4°C

*FSIS Laboratory Manual and Oxoid

This table is intended as a guide only. Laboratories may use this information in their quality program or may choose to validate alternate shelf-life data.

Agar Listeria according to Ottaviana & Agosti (ALOA)

AS 5013.24.1

Formula Base Media in g

Enzymatic digest of animal tissue	18.0 g
Enzymatic digest of casein	6.0 g
Yeast extract	10.0 g
Sodium pyruvate	2.0 g
Glucose	2.0 g
Magnesium glycerolphosphate	1.0 g
Magnesium sulfate (anhydrous)	0.5 g
NaCl	5.0 g
LiCl	10.0 g
Na2HPO4	2.5 g
5-Bromo-4-chhloro-3-indolyl-b-D-glucopyranoside.	0.05 g
Agar	12-18 g
Water	930 mL

Dissolve ingredients in water by boiling, sterilise 121°C for 15 minutes. Adjust pH to 7.2±0.2.

Complete media

Base media	930 mL*
Nalidixic acid	5 mL
Ceftazidime	5 mL
Polymyxin B	5 mL
Cycloheximide	5 mL
or Amphotericin B	10 mL
Supplement	50 mL

*925 mL if Amphotericin B is used

Buffered peptone water

AS 5013.10/AS 5013.15

Recommended as a diluent for the homogenization of samples and also use for pre-enrichment media for *Salmonella* (AS 5013.10).

Formula

Enzymatic digest of casein	10.0 g
Sodium chloride	5.0 g
Disodium hydrogen phosphate dodecahydrate	9.0 g
Potassium dihydrogen phosphate	1.5 g
Water	1.0 L

Rehydrate ingredients in the water, apply heat to dissolve if necessary. Sterilize for 15 min at 121°C. Adjust pH 7.0 ± 0.2 at 25°C if necessary.

Butterfield's diluent / Butterfield's phosphate buffer (BPB) diluent

AOAC 990.12 / AOAC 2000.14

Used as a diluent and for moistening sponges for use with Petrifilm. Butterfield's Diluent can be used for both *E. coli* and TVC Petrifilm.

Stock Solution

KH_2PO_4	34.0 g
Purified water	1.0 L

Dissolve 34g KH_2PO_4 in 500mL of boiled and cooled purified water, adjust the pH to 7.2 using 1M NaOH and dilute to 1 L with water. Store refrigeration.

Working Solution

Dilute 1.25 mL of stock in one litre of boiled and cooled purified water (final concentration of KH_2PO_4 0.0425 g/L). Autoclave 15 min at 121°C.

Buffered *Listeria* Enrichment Broth (BLEB)

FDA BAM Chapter 10

Formula (per litre of purified water)

Trypticase soy broth	30.0g
Yeast extract	6.0 g
Monopotassium phosphate (anhydrous)	1.35 g
Disodium phosphate (anhydrous)	9.60 g
Sodium Pyruvate (sodium salt)	1.11 g

Weigh ingredients and dissolve in water. Autoclave 15 min at 121°C. Final pH 7.3 ± 0.1.

Selective Supplements

The following filter sterilised supplements are added to enrichment after 4 hours incubation: 10 mg/L acriflavin HCL (0.5% w/v aqueous solution)
40 mg/L nalidixic acid (0.5% w/v aqueous solution)
50 mg/L cycloheximide (1% solution in 40% ethanol)

Demi-Fraser broth (Half Fraser Broth)

AS 5013.24.1

Demi-Fraser broth is a modification of Fraser broth for the primary enrichment of *Listeria monocytogenes* in meat and poultry samples.

Formula of base medium (per litre of purified water)

Proteose peptone	5.0	g
Tryptone	5.0	g
Lab Lemco powder (Oxoid)	5.0	g
Yeast extract	5.0	g
NaCl	20.0	g
KH_2PO_4	1.35	g
Na_2HPO_4	12.0	g
Esculin	1.0	g
Nalidixic acid (1% in 0.1M NaOH)	1.0	mL
LiCl	3.0	g

Rehydrate ingredients in 1 L distilled water, apply heat to dissolve. Autoclave 121°C for 15 minutes. Final pH 7.2 ±0.2. The following filter sterilised supplements are added to the base media immediately prior to use:
12.5 mg/L acriflavin HCL (10 mL of 1.25 mg/mL aqueous solution)
0.5g/L ferric ammonium citrate (10 mL of 5% aqueous solution)

Double Modified Lysine Iron Agar (DMLIA)

FSIS MLG Appendix 1.08

Formula

Lysine Iron Agar	34.0	g
Bile Salts No. 3	1.5	g
Lactose	10.0	g
Sucrose	10.0	g
Sodium Thiosulfate	6.76	g
Ferric Ammonium Citrate	0.3	g
Distilled water	1.0	L
Sodium Novobiocin	0.015	g

Dehydrate ingredients except Sodium Novobiocin in 1 L distilled water, heat to 100°C for 10 minutes. Cool to approximately 50°C. Add Sodium Novobiocin from a filter-sterilized stock solution mix and pour 15 – 20 mL/plate. Store refrigerated for up to 3 weeks. Final pH 6.7 ± 0.2 at 25°C.

EHEC Enrichment Broth (EEB)

FDA BAM Chapter 4A(K) (previous version)

Note: FDA introduced modified Buffered Peptone Water with pyruvate (mBPWp) as enrichment medium for analysis of *E. coli* O157:H7 using FDA BAM Chapter 4A(K)

Reagents (per litre of purified water)

Trypticase soy broth	30.0	g
Bile salts No. 3	1.5	g
K_2HPO_4	1.5	g

Media is autoclaved at 121 °C for 15 min. The final pH of the medium should be 7.4 ± 0.2. The following filter sterilised antibiotics are added after autoclaving and tempering just prior to use.

Cefixime 0.0125 mg/L
Cefsulodin 10 mg/L
Vancomycin 8 mg/L

Fraser Broth

FSIS MLG Appendix 1.08/ISO 5013.24.1

Formula

Proteose Peptone	5.0 g
Tryptone	5.0 g
Beef Extract (Oxoid LabLemco)	5.0 g
Yeast Extract	5.0 g
NaCl	20.0 g
KH_2PO_4	1.35 g
Na_2HPO_4	12.0 g
Esculin	1.0 g
Naladixic Acid † (2% in 0.1 M NaOH)	1.0 mL
Acriflavin	25.0 mg
Lithium Chloride	3.0 g
Distilled water	1.0 L

Dissolve ingredients and dispense into test tubes. Sterilize at 121°C for 15 minutes. Store at 2-8°C. Add 0.1 mL of ammonium iron (III) citrate stock solution to each 10 mL tube just before use.
Final pH 7.2 ± 0.2 at 25ºC.

Ammonium iron (III) citrate (Ferric Ammonium Citrate)

Ammonium iron (III) citrate (Sigma)	5.0 g
Distilled water	100 mL

Dissolve 5 g of ammonium iron (III) citrate (Sigma) in water. Bring to volume and filter sterilize. Store at 2-8°C.

Frazier Broth may be prepared from Modified UVM by adding the appropriate amounts of lithium chloride and acriflavin and ammonium iron (III) citrate after sterilization.

Horse Blood Overlay Medium (HBO or HL)

FSIS MLG Appendix 1.08

Base Layer

Columbia Blood Agar Base	1.0 L

Prepare as per manufacturer's specifications. Autoclave at 121°C for 15 minutes. Pour 10 mL per 100 mm diameter Petri dish and allow to solidify. Overlay with blood agar as described below.

Top Layer

Add 4 mL of sterile horse blood to each 100 mL of autoclaved but cooled to 46°C Columbia Blood Agar Base. Swirl to mix evenly and add 5 to 6 mL on top of the base layer and tilt the plates to spread top layer evenly. Final pH 7.2 ± 0.2 at 25ºC. Store at 2-8°C and used by 2 weeks.

Lithium Chloride-Phenylethanol-Moxalactam (LPM) Medium

FDA BAM Chapter 10

Formula

Phenylethanol agar (Difco)	35.5 g
Glycine anhydride	10.0 g
Lithium chloride	5.0 g
Moxalactam stock solution,	
1% in phosphate buffer, pH 6.0	2 mL
Distilled water	1.0 L

Dissolve and sterilize at 121°C for 15 min. Cool to 48-50°C and add filter-sterilized moxalactam solution.

Moxalactam stock solution

Dissolve 1 g moxalactam, Eli Lilly Co, (ammonium or sodium salt) in 100 mL 0.1 M potassium phosphate buffer, pH 6.0. Filter-sterilized and dispense 2 mL aliquots, store frozen. Prepare plates by pouring 12-15 mL per standard petri dish cool and refrigerate.

LPM Plus Esculin and Ferric Iron

FDA BAM Chapter 10

Esculin	1.0 g
Ferric ammonium citrate	0.5 g

Add ingredients to those for LPM medium. Sterilize and add filter-sterilized moxalactam as described for LPM medium.

Modified Buffered Peptone water with pyruvate (mBPWp)

FDA BAM Chapter 4A(K)

Formula

Peptone	10.0 g
NaCl	5.0 g
Na_2HPO_4	3.6 g
KH_2PO_4	1.5 g
Casamino acids	5.0 g
Yeast extract	6.0 g
Lactose	10.0 g
Sodium Pyruvate	1.0 g
Distilled water	1.0 L
	(use 500 mL for 2X strength)

Sterilize by autoclaving. Final pH to 7.2 ± 0.2.

Acriflavin-Cefsulodin-Vancomycin (ACV) Supplement for mBPWp

Supplement	Conc. in mBPWp	Conc. in stock	Amount of stock/225 mL
Acriflavin	10 mg/L	1.125 g/ 500 mL	1 mL
Cefsulodin	10 mg/L	1.125 g/ 500 mL	1 mL
Vancomycin	8 mg/L	0.90 g/ 500 mL	1 mL

Add 1mL of each filter sterilized supplements to 225 mL mBPWp for selective overnight enrichment.

Modified EHEC media (mEHEC), BioControl

AOAC 2005.04

Formula

BioControl mEHEC base	30.0 g
Sterile deionized water	1.0 L

Dissolve 31.6 g medium in 1.0 L pre-warm (42 ± 0.5°C) sterile deionized water, gently mix to dissolve. Use within 6 hours of preparation. mEHEC media can be autoclave at 121°C for 15 min. Store at refrigeration temperature. Must be pre-warmed at 42 ± 0.5°C overnight before use.

Modified Oxford Medium (MOX)

FSIS MLG Appendix 1.08

Formula

Columbia Blood Agar Base (depending on brand) ..	38-44.0 g
Esculin	1.0 g
Ferric Ammonium Citrate	0.5 g
Lithium Chloride (Sigma L0505)	15.0 g
Colistin	0.01 g
Distilled water	1.0 L

Dissolve medium base with constant stirring using a magnetic mixer. Autoclave at 121°C for 15 minutes, cool to 45° to 50°C. Add 2 mL of 1% filter sterilized Moxalactam solution mix well & pour 12 mL per plate. Final pH 7.0 ± 0.2 at 25°C.

Modified Rainbow Agar O157 (mRBA)

FSIS MLG Appendix 1.08

Formula

Rainbow agar base	60.0 g
Potassium Tellurite solution	0.15 mL
Sodium Novobiocin solution	1.25 mL
Cefixime solution (concentration of 0.5mg/mL)	0.1 mL
Distilled water	1.0 L

Rehydrate 60 g of Rainbow agar base (Biolog Inc., Hayward California) to 1 L of distilled water and boil gently until dissolved. Autoclave at 121°C for 10 min. Cool to 50°C and add 1.25 mL of novobiocin solution, 0.15 mL of potassium tellurite solution, and 0.1mL of cefixime solution. Mix well and dispense approx 20 mL per plate into petri plates. Store dark in a closed container. Shelf life is 21 days if stored under refrigeration in sealed container.
Final pH 8.1 ± 0.2 at 25°C

Potassium Tellurite Solution

Potassium tellurite	0.010 g
Distilled water	10.0 mL

Dissolve the potassium tellurite in the distilled water. Filter sterilize and store in the dark at 2-8°C for up to 8 days.

Sodium Novobiocin Solution

Sodium novobiocin	0.4 g
Distilled water	100 mL

Dissolve the sodium novobiocin in the distilled water. Filter sterilize and store in the dark up to one year at 2 – 8°C.

Cefixime Solution

Cefixime Trihydrate	0.050 g
Methanol	10 mL

Dissolve the cefixime trihydrate in methanol. Dilute solution by adding 1 mL to 9 mL of water (to get a working concentration of 0.5 mg/mL). The methanol solution can be stored at -20°C for six months. Filters sterilize the 1:10 dilution and use on the day of making.

Modified Tryptone Soya Broth (mTSB)

FSIS MLG Appendix 1.08

The novobiocin supplement of mTSB+n with 8 mg/L of sodium novobiocin was removed to allow the same sample enrichment, Modified Tryptone Soya Broth (mTSB), to be used to analyze raw beef product and environmental and carcass sponges for *E. coli* O157, non O157 Shiga toxin-producing *E. coli, and Salmonella.*

Formula of basal medium

Modified Tryptone Soya Broth*	33.0 g
Casaminoacids (casein acid hydrolysate)	10.0 g
Distilled water	1.0 L

* Oxoid product # CM0989B or current (other brands are permitted if the formula is equivalent).

Dissolve the basal ingredients by stirring then autoclave for 20 minutes @ 121°C. Cool to 50°C and then add 2 mL of filter sterilized aqueous sodium novobiocin solution (4 mg/mL adjusted for potency; Sigma N1628) for each litre of medium. Final pH 7.4 ± 0.2 at 25°C.

Modified Tryptone Soya Broth plus Novobiocin (mTSB+N)

FDA BAM Chapter 4A

Formula (per litre of purified water)

Trypticase soy broth	30.0	g
Bile salts No. 3	1.5	g
Dipotassium phosphate	1.5	g

Media is autoclaved at 121°C for 15 min. The final pH of the medium should be 7.4 ± 0.2. Novobiocin solution is added just prior to use at a concentration of 20 mg per litre of final media.

Modified Tryptone Soya Broth plus Novobiocin (mTSB+N)

ISO 16654:2001

Modified Tryptone Soya Broth is a base medium used for the selective enrichment of *E. coli* O157:H7. Novobiocin is added to increase specificity.

Reagents (per litre of purified water)

Enzymatic digest of casein	17.0	g
Enzymatic digest of soya	3.0	g
D(+)-Glucose	2.5	g
Bile salts No. 3	1.5	g
Sodium Chloride	5.0	g
K2HPO4	4.0	g

Media is autoclaved at 121 °C for 15 min. The final pH of the medium should be 7.4 ± 0.2. One mL of filter sterilised novobiocin (0.45%) is added to 225 mL of cooled mTSB on the day of use. The final concentration of novobiocin is 20 mg per litre of mTSB.

Modified Tryptone Soya Broth with Novobiocin (mTSB+N, with 8 mg/L of sodium novobiocin)

FSIS MLG Appendix 1.08

Basal Ingredients

Modified Tryptone Soya Broth*	33.0 g
Casaminoacids (casein acid hydrolysate)	10.0 g
Distilled water	1.0 L

Supplement

Sodium novobiocin solution	2 mL

* Oxoid product # *CM0989B* or current (other brands are permitted if the formula is equivalent).
Dissolve the basal ingredients by stirring then autoclave for 20 minutes @ 121°C. Cool to 50°C and then add 2 mL of filter sterilized aqueous sodium novobiocin solution (4 mg/mL adjusted for potency; Sigma N1628) for each litre of medium. Final pH 7.4 ± 0.2 at 25°C.

Modified UVM Both

FSIS MLG Appendix 1.08

Formula

Proteose Peptone	5.0 g
Tryptone	5.0 g
Lab Lemco Powder (Oxoid)	5.0 g
Yeast Extract	5.0 g
NaCl	20.0 g
KH_2PO_4	1.35 g
Na_2HPO_4	12.0 g
Esculin	1.0 g
Nalidixic Acid (2% in 0.1 M NaOH)	1.0 mL
Acriflavin	12.0 mg
Distilled water	1.0 L

Sterilize at 121ºC for 15 minutes. Store at 2-8°C. Final pH 7.2 ± 0.2 at 25ºC.

Morpholine Propanesulfonic Acid-Buffered Listeria Enrichment Broth (MOPS-BLEB)

FSIS MLG Appendix 1.08

Formula

Powdered Listeria Enrichment Broth	36.1 g
MOPS free acid (3-[N-Morpholino] propanesulfonic acid)	6.7 g
MOPS sodium salt (3-[N-Morpholino] propanesulfonic acid sodium salt)	10.5 g
Distilled water	1.0 L

Dissolve ingredients, dispense and autoclave at 121°C for 15 min. Final pH 7.3 ± 0.2 at 25°C.

Oxford Medium

FDA BAM Chapter 10/AS 5013.24.1

Formula

Columbia blood agar base	39.0 g
Esculin	1.0 g
Ferric ammonium citrate	0.5 g
Lithium chloride	15.0 g
Cycloheximide	0.4 g
Colistin sulphate	0.02 g
Acriflavin	0.005 g
Cefotetan	0.002 g
Fosfomycin	0.010 g
Distilled water	1.0 L

Rehydrate the 55.5 g of the first 4 components to 1 L distilled water. Heat gently to dissolve completely. Autoclaving at 121°C for 15 min. Cool to 50°C and aseptically add supplement. Mix and pour into petri dishes.
Dissolve supplement, cycloheximide, colistin sulfate, acriflavin, cefotetan, and fosfomycin in 10 mL of 1:1 mixture of ethanol and distilled water. Filter-sterilize supplement before use.

PALCAM Listeria Selective Agar

FDA BAM Chapter 10/AS 5013.24.1

Basal medium

Peptone	23.0 g
Starch	1.0 g
NaCl	5.0 g
Columbia agar	13.0 g
Mannitol	10.0 g
Ferric ammonium citrate	0.50 g
Esculin (aesculin)	0.80 g
Dextrose (glucose)	0.50 g
Lithium chloride	15.0 g
Phenol red	0.08 g
Distilled water	1.0 L

Selective agents

Polymyxin B sulfate	10 mg
Acriflavin	5 mg
Ceftazidine	20 mg
Distilled water	2 mL

Dissolve 34.4 g of basal medium to 500 mL water. Sterilize at 121°C for 15 min. Cool to 50°C. Dissolve selective agents in 2 mL sterile distilled water and filter sterilize. Add 1 mL selective agent to 500 mL autoclaved medium, mix gently and pour plates. Final pH 7.2 ±0.1.

Peptone Salt Solution 0.1% / Saline Peptone Water / Peptone Saline Diluent

ISO 6887

This medium is recommended by ISO 6887 to use as a diluent for making initial suspension of microbiological samples.

Formula in g/L

Casein Peptone	1.0 g
Sodium Chloride	8.5 g

Rehydrate 9.5 g of the medium in 1 L of purified water, mix and heat until dissolve. Dispense into appropriate container and autoclave at 121°C for 15 min. Store at 2-8°C.

Rappaport-Vassiliadis Broth, modified (Fluka)

FSIS MLG Appendix 1.08

Formula

Papaic digest of soybean meal	5.0 g
Sodium Chloride	8.0 g
Monopotassium Phosphate	1.6 g
Magnesium Chloride hexahydrate	18.7 g
Malachite Green	0.04 g
Purified water	1.1 L

Dissolve ingredients to distilled water. Heat gently if necessary to dissolve completely. Autoclave at 115°C for 15 minutes. Final pH 5.2 ± 0.2 at 25°C.

Sheep Blood Agar or SBA (Trypticase Soy Agar with 5% Sheep Blood OR TSA-SB)

MLG Appendix 1.08

Trypticase® (Tryptic)	15.0 g
Phytone	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g
Distilled water	1.0 L

Rehydrate ingredients in distilled water. Gently heat until dissolved. Sterilize at 121°C for 15 min. Cool to approximately 50°C and add 5% sterile defibrinated sheep blood and swirl. Pour 15 mL standard petri dishes. Pour 9 ±1 mL on standard petri dish for *Listeria monocytogenes* CAMP test. Final pH 7.3 ± 0.2 at 25°C.

Tryptone Soya Yeast Extract Agar (TSYEA)

AS 5013.24.1

Formula

Tryptone	17.0 g
Soya peptone	3.0 g
Sodium chloride	5.0 g
Dipotassium phosphate	2.5 g
Glucose	2.5 g
Yeast extract	6.0 g
Agar	9 to 18 g
Water	1.0 L

Dissolve components in distilled water by heating if necessary. Autoclave at 121°C for 15 min. Cool to approx 50°C and dispense into petri dishes. pH after autoclave should be 7.3 ± 0.2.

TT Broth (Hajna and Damon, 1956)

FSIS MLG Appendix 1.08

Broth Base

Yeast Extract	2.0 g
Tryptose	18.0 g
Dextrose	0.5 g
d-Mannitol	2.5 g
Sodium Desoxycholate	0.5 g
Sodium Chloride	5.0 g
Sodium Thiosulfate	38.0 g
Calcium Carbonate	25.0 g
Brilliant Green	0.01 g
Distilled water	1.0 L

Rehydrate and heat to boiling to dissolve. DO NOT AUTOCLAVE. Cool below 50°C and add 40 mL iodine solution. Use the day it is prepared. Final pH 7.6 ± 0.2 at 25°C after addition of iodine.
Base medium may be stored at 2-8°C for up to six months prior to the addition of iodine.

Iodine Solution

Potassium Iodide	8.0 g
Iodine crystals	5.0 g
Distilled water	20 mL

Add potassium iodide in 20 mL distilled water. Add iodine crystals and stir until completely dissolved. Bring volume to 40 mL and mix thoroughly. Store dark at either 2-8°C or at room temperature. Final pH 7.6 ± 0.2 at 25°C after addition of iodine.

XLT4 Agar

FSIS MLG Appendix 1.08

Formula

XL Agar Base (Difco #0555-01-8)	47.0 g
Bacto Agar (Difco #0140-01-0)	3.0 g
Ferric Ammonium Citrate	0.8 g
Sodium Thiosulfate (Anhydrous)	6.8 g
Proteose Peptone #3 (Difco #0122-01-2)	1.2 g
Niaproof 4 (Sigma, formally Tergitol 4)	4.6 mL
Distilled water	1.0 L

Dissolve Niaproof 4 in distilled water in a large Erlenmeyer flask and mix with a magnetic stir-bar. Add other ingredients, mix and apply heat until dissolved. Cool to 45 - 50°C. Pour plates about 5 mm deep. pH of XLT4 plates = 7.5 ± 0.2. Self life 3 months when stored refrigerated in closed container.