



## Laboratory Guidebook Notice of Change

Chapter new, **revised**, or archived: MLG Appendix 1.06

Title: Media and Reagents

Effective Date: 11/4/11

Description and purpose of change(s):

Preparation instructions for Modified Rainbow Agar O157 (mRBA) and Modified Tryptone Soya Broth with Novobiocin (mTSB+n) with 8 mg/L of Sodium Novobiocin were added.

The title for the preparation instructions of mTSB+n was revised to differentiate mTSB+n with 20 mg/L of Sodium Novobiocin from mTSB+n with 8 mg/L of Sodium Novobiocin.

The agar ingredient was added to the Campy-cefex agar preparation.

**The methods described in this guidebook are for use by the FSIS laboratories. FSIS does not specifically endorse any of the mentioned test products and acknowledges that equivalent products may be available for laboratory use.**

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**APP 1 Specific Procedure(s)**

**APP 1.1 Introduction**

- All media and reagents necessary for each analysis are listed in each chapter. The formulations and procedures for preparing the special media and reagents used throughout this Guidebook are presented in alphabetical order in this appendix.
- Formulations and preparations for basic media that may be used for general microbiological procedures, which are not listed in this appendix, may be obtained by consulting readily available reference materials such as general microbiology textbooks, commercially available media formulation handbooks, FDA's Bacteriological Analytical Manual, and APHA's Compendium of Methods for the Microbiological Examination of Foods.
- The carbohydrates (sugars) should be chemically pure and suitable for biological use; inorganic chemicals used as reagents should be American Chemical Society (ACS) grade; dyes must be certified by the "Biological Stain Commission" for use in media.
- The ingredients and the chemicals used for preparing media and reagents may be the product of any manufacturer if comparative tests show satisfactory results. For convenience, dehydrated media of any brand equivalent to the formulation may be used unless instructions indicate otherwise. Pre-mixed, dehydrated media should be examined before use for indications of separation or deterioration. Each batch of medium should be tested for sterility and growth promotion/inhibition characteristics, as appropriate following the QC procedures described by the manufacturer.
- Hydrogen ion concentration (pH) of media should be determined using an electronic pH meter which is standardized against known buffers, prepared according to the Official Methods of Analysis of the Association of Official Analytical Chemists (16th Edition). If necessary the pH of a medium should be adjusted by adding sufficient 1 N sodium hydroxide or 1 N hydrochloric acid. For testing the pH of agar media, the use of an automatic temperature adjusting pH meter/probe and/or a surface-testing probe are recommended. If a recipe is made from individual components instead of a commercially available dehydrated media, it is recommended that the pH be checked prior to sterilization.
- Precautions: All manufacturers' precautions should be followed. The personnel who handle the material should read the product's Material Safety Data Sheets. Chemicals with '†' are of particular concern.

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- Unless otherwise indicated, up to 1 liter of a medium should be sterilized by steam under pressure at 121°C (15-16 psi) for 15 minutes. Alternatively, media may be filter-sterilized.
- Any departures from standard media preparation practices/techniques (i.e. preparation volumes, sterilization/heating requirements, formulations, etc.) will require equivalency data to support the change(s). The laboratory will retain all records. Where the instructions say to dissolve by gently heating, the media shall be checked visually to determine that it is well dissolved. Do not over heat. This can be an essential step in obtaining the correct pH for the final medium.
- Depending on the type and quantity of media needed, tubed media may be either dispensed directly into tubes and sterilized by autoclaving or may be autoclaved in bulk and then aseptically dispensed into pre-sterilized tubes. Dilution tubes, or any tubes where the exact volume is critical, should only be dispensed after autoclaving.
- If commercial dehydrated medium is used, follow the manufacturer's instructions for specified pH, time and temperature of sterilization, etc.
- Microbiology Suitable (MS) water requirements.

Only water that has been treated to be free from traces of dissolved metal, bactericidal, and inhibitory compounds shall be used to prepare culture media, reagents, and dilution blanks. Inhibitor free water is referred to as microbiologically suitable (MS) water. The following tests are performed on the water source to ensure that the water is inhibitor free. Records of the following parameters shall be kept.

Weekly testing (or prior to use):

- >1.0 megohms-cm resistance at 25°C or
- <1.0 microSiemens-cm conductivity at 25°C.

Monthly testing:

- Total Residual Chlorine shall be < 0.1 mg/l
- Aerobic Plate Count shall be < 1,000 colony forming unit (cfu) ml

Annual testing:

- Heavy Metals (Cd, Cr, Cu, Ni, Pb, and Zn-single) shall be < 0.05 mg/L
- Heavy Metals (total) shall be < 0.10 mg/L

The suitability of water for microbiological analyses shall pass the test for toxicity annually.

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**APP 1.2 MEDIA PREPARATION INSTRUCTIONS**

**A-K AGAR #2 (SPORULATING AGAR)**

Pancreatic Digest of Gelatin	6.0 g
Pancreatic Digest of Casein	4.0 g
Yeast Extract	3.0 g
Beef Extract	1.5 g
Dextrose	1.0 g
Agar	15.0 g
Manganous Sulfate (MnSO <sub>4</sub> .7H <sub>2</sub> O)	0.3 g
MS water	1.0 L

Suspend above ingredients. Heat and check visually to ensure that it is well dissolved.  
Dispense and autoclave at 121°C for 15 minutes.

Final pH 6.6 ± 0.2 at 25°C.

**ANTIBIOTIC MEDIUM #2 with Dextrose**

**Antibiotic Medium #4 may be used instead of adding dextrose to Medium #2.**

Bacto Peptone	6.0 g
Beef Extract	1.5 g
Yeast Extract	3.0 g
Agar	15.0 g
Dextrose solution, sterile (10g/100 ml)	10.0 ml
MS water	1.0 L

Combine all ingredients except the dextrose solution. Heat the mixture until it is well dissolved. Dispense and autoclave at 121°C for 15 minutes. When cooled but still liquid (60-65°C), add sterile dextrose solution to a final concentration of 1 g/L. Commercially available powdered media may be used with the addition of the dextrose solution after autoclaving.

Final pH 6.5 to 6.6 at 25°C.

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**ANTIBIOTIC MEDIUM #4**

Bacto Peptone	6.0 g
Beef Extract	1.5 g
Yeast Extract	3.0 g
Dextrose	1.0 g
Agar	15.0 g
MS water	1.0 L

Heat the mixture until visual examination shows that it is well dissolved. Dispense and autoclave at 121°C for 15 minutes.

Final pH 6.5 – 6.6 at 25°C

**ANTIBIOTIC MEDIUM #5**

Bacto Peptone	6.0 g
Beef Extract	1.5 g
Yeast Extract	3.0 g
Agar	15.0 g
MS water	1.0 L

Heat the mixture until visual examination shows that it is well dissolved. Dispense and autoclave at 121°C for 15 minutes.

Final pH 7.8 – 8.0 at 25°C or as specified by the manufacturer if using commercial dehydrated medium.

**ANTIBIOTIC MEDIUM #8**

Bacto Peptone	6.0 g
Beef Extract	1.5 g
Yeast Extract	3.0 g
Agar	15.0g
MS water	1.0 L

Heat the mixture until visual examination shows that it is well dissolved. Dispense and autoclave at 121°C for 15 minutes. Final pH 5.8 – 5.9 at 25°C or as specified by the manufacturer if using commercial dehydrated medium.

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**ANTIBIOTIC MEDIUM #11 (NEOMYCIN ASSAY AGAR)**

Gelsate™ Peptone or Bacto Peptone	6.0 g
Trypticase Peptone or Bacto Casitone*	4.0 g
Yeast Extract	3.0 g
Beef Extract	1.5 g
Dextrose	1.0 g
Agar	15.0 g
MS water	1.0 L

\*Pancreatic digest of casein

Heat the mixture until visual examination shows that it is well dissolved. Dispense and autoclave at 121°C for 15 minutes. Refrigerate.

Final pH 7.95 ± 0.05 at 25°C or as specified by the manufacturer if using commercial dehydrated medium. Adjust pH if necessary.

**APT AGAR**

Pancreatic digest of casein	12.5 g
Dextrose	10.0 g
Yeast Extract	7.5 g
Sodium Chloride	5.0 g
K <sub>2</sub> HPO <sub>4</sub>	5.0 g
Sodium Citrate	5.0 g
Na <sub>2</sub> CO <sub>3</sub>	1.25 g
MnCl <sub>2</sub> .4H <sub>2</sub> O	0.14 g
MgSO <sub>4</sub> .7H <sub>2</sub> O	0.8 g
Polysorbate 80	0.2 g
FeSO <sub>4</sub> .7H <sub>2</sub> O	0.04 g
Thiamine Hydrochloride	1.0 mg
Agar	15.0 g
MS water	1.0 L

Add components to MS water, bring volume to 1.0 L, and mix thoroughly. Heat the mixture until visual examination shows that it is well dissolved. Distribute into tubes or flasks and sterilize by autoclaving at 118°C - 121°C at 13 psi for 15 minutes. Avoid excessive heating. Pour into sterile Petri dishes or leave in tubes.

Final pH 6.7 ± 0.2 at 25°C.

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**BACILLUS CEREBUS (BC) MOTILITY MEDIUM**

Trypticase	10.0 g
Yeast Extract	2.5 g
Dextrose	5.0 g
Disodium Hydrogen Phosphate	2.5 g
Agar	3.0 g
Distilled water	1.0 L

Dissolve the ingredients in distilled water and heat to boiling to completely dissolve the agar. Mix thoroughly and dispense 2.0 ml into 13X100 mm tubes. Autoclave at 121°C for 15 minutes. Allow medium to solidify and store at room temperature for up to 2 or 3 days for best results.

Final pH 7.4 ± 0.2 at 25°C.

**BAIRD-PARKER MEDIUM**

Basal Medium

Tryptone	10.0 g
Beef Extract	5.0 g
Yeast Extract	1.0 g
Sodium Pyruvate	10.0 g
Glycine	12.0 g
Lithium Chloride 6H <sub>2</sub> O	5.0 g
Agar	20.0 g
MS water	950.0 ml

Suspend ingredients in water. Heat the mixture until visual examination shows that it is well dissolved. Autoclave at 121°C for 15 minutes.

Final pH 7.0 ± 0.2 at 25°C.

Complete medium

- a. Add 50 ml prewarmed (to at least room temperature) Bacto EY tellurite enrichment to 950 ml molten basal medium, which has been tempered to 45-50°C.

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- b. Mix well (avoiding bubbles) and pour 15-18 ml into sterile 100 x 15 mm Petri dishes.
- c. Plates of complete medium should be stored in refrigerator for no longer than 4 weeks before use.
- d. Ensure that the surface of the plate is dry before use.

**BLOOD-FREE BOLTON'S ENRICHMENT BROTH (2X BF-BEB)**

<b>Basal Ingredients</b>	
Meat peptone	20 g
Lactalbumin hydrolysate	10g
Yeast extract	10 g
Sodium chloride	10 g
Sodium pyruvate	1.0 g
α Ketoglutamic acid	2.0 g
Sodium metabisulfite	1.0 g
Sodium carbonate	1.2 g
Haemin	0.02 g
Distilled or DI Water	1.0 L
<b>Supplements (using 4 vials of Oxoid SR0183E)</b>	
Cefoperazone	40 mg
Vancomycin	40 mg
Trimethoprim	40 mg
Cycloheximide †	100 mg

All the above mentioned ingredients/supplements are for the preparation of 1L of double strength blood-free Bolton's enrichment broth (2X BF-BEB).

When rehydrating the supplements, follow the manufacturer's instructions. Use ethyl alcohol (USP grade). Denatured ethanol must not be used because the additives could possibly be toxic to *Campylobacter*.

Add all basal ingredients to water for a 2X BF-BEB solution and mix until ingredients dissolve completely. Autoclave for 15 minutes at 121°C. Cool to at least 50°C if adding supplements at time of preparation. The 2X BF-BEB without supplements is stable for two weeks after preparation stored at 2 – 8°C. To make 500 mL of 2X BF-BEB, add two vials containing all the above mentioned supplements. After supplement addition, the medium is stable up to 48 hours at 2 – 8°C.

Final pH 7.4 ± 0.2 at 25°C.

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NOTE: This 2X BF-BEB formulation is twice as concentrated to meet the specific needs for diluting 1:2 with a 30 mL test portion; *i.e.*, 30 mL of 2X BF-BEB plus 30 mL sample makes 60 mL of 1X BF-BEB sample enrichment for incubation.

**BRAIN HEART INFUSION (BHI) AGAR**

Calf Brain (infusion from 200 g)	7.7 g
Beef Heart (infusion from 250 g)	9.8 g
Proteose peptone or gelysate	10.0 g
NaCl	5.0 g
Na <sub>2</sub> HPO <sub>4</sub>	2.5 g
Dextrose	2.0 g
Agar	15.0 g
MS water	1.0 L

Dissolve ingredients in MS water. Heat the mixture until visual examination shows that it is well dissolved. Dispense as desired and autoclave at 121°C for 15 minutes. This may also be prepared by adding 15 g of agar to each liter of BHI broth,

Final pH 7.4 ± 0.2 at 25°C.

NOTE: Different manufacturers may use different formulations. For non-critical applications any of these formulations may be used.

**BRAIN HEART INFUSION (BHI) BROTH**

Pancreatic digest of gelatin	14.5 g
Brain heart, solids from	6.0 g
Peptic digest of animal tissue	6.0 g
NaCl	5.0 g
Glucose	3.0 g
Na <sub>2</sub> HPO <sub>4</sub>	2.5 g
MS water	1.0 L

Add components to MS water. Mix thoroughly. Dispense and autoclave at 121°C for 15 minutes.

Final pH 7.4 ± 0.2 at 25°C.

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NOTE: Different manufacturers may use different formulations. For **non-critical** applications any of these formulations may be used.

**BRILLIANT GREEN SULFA AGAR (BGS)**

Yeast Extract	3.0g
Polypeptone	10.0 g
Sodium Chloride	5.0 g
Lactose	10.0 g
Sucrose	10.0 g
Phenol Red	0.08 g
Agar	20.0 g
Sulfapyridine	1.0 g
Brilliant Green	0.0125 g
MS water	1.0 L

Mix thoroughly and heat with frequent agitation to dissolve. Autoclave at 121°C for 15 minutes. Cool to approximately 50°C and pour approximately 20 ml into sterile 100 x 15 mm Petri dishes.

Final pH 6.9 ± 0.2 at 25 C

**BROMCRESOL PURPLE (BCP) DEXTROSE BROTH**

Peptone	10.0 g
Beef Extract (optional)	3.0 g
Sodium Chloride	5.0 g
Bromcresol Purple (0.16 g/ 10.0 ml of 95% ethanol).	2.0 ml
MS water	1.0 L

Combine the above ingredients with 5 g dextrose per liter. (Other carbohydrates such as adonitol, arabinose, mannitol, maltose, sucrose, lactose, sorbitol, cellobiose, salicin or trehalose may also be used individually at a quantity of 5 g per liter to prepare these individual BCP carbohydrate fermentation broths). Adjust to pH 7.0. Dispense 8.0 ml aliquots into 16 x 150 mm tubes containing inverted 12 x 75 mm fermentation tubes. Autoclave for 10 minutes at 121°C.

Final pH 6.9 ± 0.1 at 25°C.

NOTE: Dehydrated prepared medium not available commercially.

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**BUFFERED PEPTONE WATER (BPW)**

Peptone	10.0 g
Sodium Chloride	5.0 g
Sodium Phosphate, dibasic	3.5 g
Potassium Phosphate, monobasic	1.5 g
MS water	1.0 L

Dissolve dry ingredients in MS water, dispense into appropriate containers, and sterilize in the autoclave at 121°C for 15 minutes.

Final pH 7.2 ± 0.2 at 25°C.

**CAMPY-CEFEX AGAR**

Pancreatic Digest of Casein	10.0 g
Peptic Digest of Animal Tissue	10.0 g
Dextrose	1.0 g
Yeast Extract	2.0 g
Sodium Chloride	5.0 g
Ferrous Sulfate	0.5 g
Sodium Bisulfite	0.3 g
Pyruvic Acid (Sodium Pyruvate)	0.5 g
Cycloheximide †	0.2 g
Agar	15.0 g
Deionized Water	1.0 L
<b>Supplement</b>	
Lysed Horse Blood	50 mL
Cefoperazone	33 mg

Suspend all ingredients in 1 L of deionized water and heat to boiling using a hotplate or equivalent. Autoclave for 15 minutes at 121°C and cool to 50°C. Add supplements. Dispense into petri dishes (20 mL/plate).

Final pH 7.0 ± 0.2 at 25 °C.

After media plate preparation, plates may either be held up to 90 days in 2 - 8 °C away from direct light or held in the dark at room temperature for 2 – 4 days to allow sufficient time for drying. Media should not be used if there are any signs of deterioration, hemolysis, and contamination prior to the expiration date.

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NOTE: Two milligrams of Amphotericin B may be used in place of 0.2 grams of Cycloheximide. Store lysed horse blood frozen for up to 1 year.

**CARBOHYDRATE FERMENTATION BROTH (EWING)**

Fermentation Broth Base

Peptone	10.0 g
Meat Extract	3.0 g
Sodium Chloride	5.0 g
Andrade's indicator	10.0 ml
MS water	1.0 L

Adjust pH to 7.1-7.2. Dispense in tubes with inverted insert tubes and sterilize at 121°C for 15 minutes. (See exceptions)

Dextrose, lactose, sucrose, and mannitol are employed in a final concentration of 1%. Other carbohydrates such as galactitol, salicin, etc., may be used in a final concentration of 0.5%. Dextrose, mannitol, galactitol, salicin, adonitol, and inositol may be added to the basal medium prior to sterilization. Medium containing neutral glycerol should be sterilized at 121°C for 10 minutes. Disaccharides such as lactose, sucrose, and cellobiose (10% solution in MS water, neutral pH) should be sterilized by filtration or at 121°C for 10 minutes and added to previously sterilized basal medium. Arabinose, xylose, and rhamnose also should be sterilized separately. If basal medium is tubed in 3.0-ml amounts, add 0.3 ml of sterile aqueous carbohydrate solution, i.e., one-tenth the volume. The natural occurring forms of the carbohydrates are used.

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**DEY-ENGLEY (DE) NEUTRALIZING BROTH**

Tryptone	5.0 g
Yeast Extract	2.5 g
Glucose	10.0 g
Sodium thioglycollate	1.0 g
Sodium thiosulfate	6.0 g
Sodium bisulfite	2.5 g
Polysorbate 80	5.0 g
Lecithin (soy bean)	7.0 g
Brom cresol purple	0.02 g
MS water	1.0 L

Heat to dissolve ingredients in MS water, dispense into appropriate containers and sterilize in the autoclave at 121°C for 15 minutes.

Final pH  $7.6 \pm 0.2$  at 25°C.

**DOUBLE MODIFIED LYSINE IRON AGAR (DMLIA)**

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
MS water	1.0 L
Sodium Novobiocin	0.015 g

Suspend all ingredients except Sodium Novobiocin in 1.0 L MS water and heat to boiling using a hotplate or equivalent (or heat to 100°C for 10 minutes). **DO NOT HEAT ABOVE 100°C.** Cool to approximately 50°C and add Sodium Novobiocin from a filter-sterilized stock solution. Pour 15-20 ml/ plate. Stored refrigerated for up to 3 weeks.

Final pH  $6.7 \pm 0.2$  at 25°C.

This medium is also commercially available as a dehydrated powder with a separate novobiocin supplement.

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**E BUFFER**

Bovine Albumin (Sigma # A7906-500G or current)	0.5 g
Tween-20	50 µl
Buffered Peptone Water (BPW)	100 ml

Prepare by mixing Bovine Albumin and Tween-20 into Buffered Peptone Water (BPW). Filter sterilize (0.2 µm) and store at 2-8°C.

Final pH 7.2 ± 0.2 at 25°C.

**EY-FREE TRYPTOSE SULFITE CYCLOSERINE (TSC) AGAR**

The above medium is made exactly as that shown for Tryptose Sulfite Cycloserine (TSC) Agar except, omit the 50 ml addition of sterile egg yolk emulsion. Add 50 ml MS water instead of the egg yolk emulsion.

Final pH 7.6 ±0.2 at 25°C.

**FRASER BROTH**

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 <sub>2</sub> PO <sub>4</sub>	1.35 g
Na <sub>2</sub> HPO <sub>4</sub>	12.0 g
Esculin	1.0 g
Naladixic Acid † (2% in 0.1 M NaOH)	1.0 ml
Acriflavin	25mg
Lithium Chloride	3.0 g
MS water	1.0 L

Acriflavin Stock

Acriflavin Hydrochloride (Sigma)	13 mg
MS water	10 ml

Dissolve and add to 1 L of Fraser Broth.

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Ammonium iron (III) citrate (Ferric Ammonium Citrate)

Ammonium iron (III) citrate (Sigma)	5 g
MS water	100 ml

In a 100 ml volumetric flask, dissolve 5g of ammonium iron (III) citrate (Sigma) in MS water. Bring to volume and filter sterilize. Store at 2-8°C.

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

Mix well to resuspend the media and dispense into test tubes. Sterilize at 121°C for 15 minutes. Store in the refrigerator.

Just before use, 0.1 ml of ammonium iron (III) citrate in MS water to each 10 ml tube.

Final pH 7.2 ± 0.2 at 25°C.

**HORSE BLOOD OVERLAY MEDIUM (HBO or HL)**

a. Base Layer

Columbia Blood Agar Base	1.0 L
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Prepare according to manufacturer's specifications and sterilize at 121°C for 15 minutes. Pour 10 ml per 100 mm diameter Petri dish. Allow to solidify, overlay with blood agar as described below.

b. Top Layer

Add 4 ml of sterile horse blood to each 100 ml of melted/tempered Columbia Blood Agar Base which has been cooled to 46°C. Stir or swirl to mix evenly. Quickly place 5 to 6 ml on top of the base layer and tilt the plates to spread top layer evenly. Store plates refrigerated up to 2 weeks. Discard any plates which become discolored.

Final pH 7.2 ± 0.2 at 25°C.

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**KF BROTH**

Pancreatic digest of casein	5.0 g
Peptic digest of animal tissue	5.0 g
Yeast Extract	10.0 g
Sodium Chloride	5.0 g
Sodium Glycerophosphate	10.0 g
Maltose	20.0 g
Lactose	1.0 g
Na <sub>2</sub> CO <sub>3</sub>	0.636 g
Sodium Azide†	0.4 g
Phenol Red	0.018 g
MS water	990.0 ml

Stock 2,3,5-triphenyltetrazolium chloride solution

Place 0.1 g 2,3,5-triphenyltetrazolium chloride in MS water to make a total volume of 10 ml. Filter sterilize through a 0.2 µm filter.

Place the above components, except for the 2,3,5-triphenyltetrazolium chloride solution, in MS water, bring volume to 990.0 ml, and mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 minutes at 121°C. Cool to 45° - 50°C and aseptically add the 10 ml sterile, stock 2,3,5-triphenyltetrazolium chloride solution to the base medium. Mix thoroughly. Aseptically distribute in 5 - 8 ml volumes in sterile tubes.

Final pH 7.2 ± 0.2 at 25°C.

**LYSINE IRON AGAR (LIA)**

Peptone	5.0 g
Yeast Extract	3.0 g
Dextrose	1.0 g
L-lysine HCl	10.0 g
Ferric Ammonium Citrate	0.5 g
Sodium Thiosulfate	0.04 g
Bromcresol Purple	0.02 g
Agar	15.0 g
MS water	1.0 L

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Dispense into tubes and autoclave for 12 minutes at 121°C. Slant with deep butt and short slant.

Final pH  $6.7 \pm 0.2$  at 25°C

**MANNITOL YOLK POLYMYXIN (MYP) AGAR**

Preparation A

Beef Extract	1.0 g
Peptone	10.0 g
D-Mannitol	10.0 g
NaCl	10.0 g
Phenol Red	0.025 g
Agar	15.0 g
MS water	900.0 ml

Preparation B

Egg yolk Enrichment 50%

Preparation C

Polymyxin B Sulfate - Dissolve 500,000 units of sterile polymyxin B sulfate (Sigma-Aldrich, St. Louis, Missouri or equivalent product) in 50.0 ml of sterile MS water. Filter sterilize the solution and store in the dark at 4°C. If the solution is prepared under sterile conditions, the filter sterilizing step may be omitted.

Mix the ingredients (Preparation A) in MS water. Heat the mixture until visual examination shows that it is well dissolved. Adjust the pH to  $7.2 \pm 0.1$ , and dispense. Autoclave at 121°C for 20 minutes, cool to 50°C in a waterbath, and add 50 ml of Preparation B and 10 ml of Preparation C. Mix well, pour into Petri dishes, allow to solidify, and dry for 24 h at room temperature. Plates may be stored at 2° to 8°C for 30 days.

Final pH  $7.1 \pm 0.1$  at 25°C.

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**MODIFIED COOKED MEAT MEDIUM**

- a. Cooked Meat Medium (dehydrated prepared medium available commercially)

Beef Heart	454.0 g
Proteose Peptone	20.0 g
Dextrose	2.0 g
Sodium Chloride	5.0 g

- b. Diluent (not available commercially)

Trypticase or Tryptone	10.0 g
Sodium Thioglycollate	1.0 g
Soluble Starch	1.0 g
Dextrose	2.0 g
Neutral Red (1% aqueous)	5.0 ml
MS water	1.0 L

Adjust to pH  $6.8 \pm 0.2$ . Add about 1 gram of (a) and 16 ml of (b) to screw-capped tubes no smaller than 20 x 150 mm. Tighten caps, vortex tubes to disperse meat, loosen caps, and autoclave at 121°C for 15 minutes. Note: b. may be heated to dissolve starch if necessary.

Final pH  $6.8 \pm 0.2$  at 25°C.

**MODIFIED OXFORD MEDIUM (MOX)**

MOX Agar Base

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
MS water	1.0 L

Rehydrate commercial Modified Oxford Agar Base with constant stirring using a magnetic mixer. Autoclave this base at 121°C for 15 minutes, mix again, and cool to 45° to 50°C in a water bath. Add 2 ml of 1% filter sterilized Moxalactam Solution to make the complete MOX medium, mix well, and pour 12 ml per plate.

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Final pH 7.0 ± 0.2 at 25 °C.

1% Moxalactam Solution or use commercially available supplement at same level

Sodium (or Ammonium) Moxalactam (Sigma)	1.0 g
0.1 M Potassium Phosphate Buffer, pH 6.0	100.0 ml

Dissolve, sterilize by filtration, dispense in small quantities for use and store in freezer at -10°C or below. Refreezing may decrease potency.

CAUTION: DO NOT use the Modified Oxford Antibiotic Supplement since it contains both moxalactam and colistin.

**MODIFIED RAINBOW AGAR O157 (mRBA)**

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
MS water	1.0 L

Potassium Tellurite Solution

Potassium tellurite	0.010 g
MS water	10.0 ml

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

Sodium Novobiocin Solution

Sodium novobiocin	0.4 g
MS water	100 ml

Dissolve the sodium novobiocin in the MS water. Filter sterilize. Store in the dark up to one year at 2 to 8°C.

Cefixime Solution

Cefixime Trihydrate	0.050 g
Methanol	10 ml

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Dissolve the cefixime trihydrate in the methanol. Dilute the cefixime solution by adding 1 ml of it to 9 ml of water for a working concentration of 0.5mg/ml. The methanol solution can be stored at -20°C for six months. The 1:10 dilution should be filter sterilized and made on day of use.

Add 60 g of Rainbow agar base (Biolog Inc., Hayward California, 94545) to 1 liter of MS water. Boil gently until dissolved. Autoclave for 10 minutes at 121°C. Cool to 50°C. Add 1.25 ml of sodium novobiocin solution, 0.15 ml of potassium tellurite solution, and 0.1ml of cefixime solution and mix well. Dispense approximately 20 ml per plate into petri plates. Store in a closed container in the dark. Shelf life of the prepared medium is two weeks if stored under refrigeration in sealed container such as sealed plastic bags.

Final pH 7.9 ± 0.2 at 25°C

**MODIFIED TRYPTONE SOYA BROTH WITH NOVOBIOCIN (mTSB+n) with 8 mg/L of SODIUM NOVOBIOCIN**

<b>Basal Ingredients</b>	
Modified Tryptone Soya Broth (Oxoid Product # CM0989B or current)	33.0 g
Casaminoacids (casein acid hydrolysate)	10.0 g
MS water	1.0 L
<b>Supplement</b>	
Sodium novobiocin solution (concentration of 4 mg/mL)	2 mL

The use of other manufacturer's modified Tryptone Soya broth or Trypticase™ (Tryptic) Soy Broth base (other than Oxoid) is permitted if the formula is equivalent.

Rehydrate the basal ingredients of the mTSB by stirring, then autoclave for 20 minutes @ 121°C. Let media cool to approximately 50°C. Add 2 mL of filter sterilized, aqueous sodium novobiocin solution prepared at a concentration of 4 mg/mL (adjusted for potency; Sigma N1628) for each liter of medium. If refrigerated, media must be pre-warmed to 18-35°C prior to use.

Final pH 7.4 ± 0.2 at 25°C.

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**MODIFIED TRYPTONE SOYA BROTH WITH NOVOBIOCIN (mTSB+n) with 20 mg/L of SODIUM NOVOBIOCIN**

<b>Basal Ingredients</b>	
Modified Tryptone Soya Broth (Oxoid Product # CM0989B or current)	33.0 g
Casaminoacids (casein acid hydrolysate)	10.0 g
MS water	1.0 L
<b>Supplement</b>	
Sodium novobiocin solution (concentration of 4 mg/mL)	5 mL

The use of other manufacturer's modified Tryptone Soya broth or Trypticase™ (Tryptic) Soy Broth base (other than Oxoid) is permitted if the formula is equivalent.

Rehydrate the basal ingredients of the mTSB by stirring, then autoclave for 20 minutes @ 121°C. Let media cool to approximately 50°C. Add 5mL of filter sterilized, aqueous sodium novobiocin solution prepared at a concentration of 4mg/mL (adjusted for potency; Sigma N1628) for each liter of medium (a total of 20 mg/L). If refrigerated, media must be pre-warmed to 18-35°C prior to use.

Final pH 7.4 ± 0.2 at 25°C.

**MODIFIED UVM BROTH**

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 <sub>2</sub> PO <sub>4</sub>	1.35 g
Na <sub>2</sub> HPO <sub>4</sub>	12.0 g
Esculin	1.0 g
Naladixic Acid (2% in 0.1 M NaOH)	1.0 ml
Acriflavin	12.0 mg
MS water	1.0 L

Sterilize at 121°C for 15 minutes. Store in the refrigerator.

Final pH 7.2 ± 0.2 at 25°C.

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**MORPHOLINEPROPANESULFONIC ACID-BUFFERED LISTERIA ENRICHMENT BROTH (MOPS-BLEB)**

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
MS water	1.0 L g

Listeria Enrichment Broth (LEB)

Use commercial powdered media or the following ingredients:

Pancreatic Digest of Casein	17.0g
Soytone	3.0 g
Dextrose	2.5 g
Sodium Chloride	5.0 g
Dipotassium Phosphate	2.5 g
Yeast Extract	6.0 g
Cycloheximide †	0.05 g
Acriflavine HCL	0.015 g
Nalidixic Acid	0.04 g

Weigh out ingredients as listed above for MOPS-BLEB and mix well to dissolve. Dispense and sterilize for 15 minutes at 121°C.

Final pH 7.3 ± 0.2 at 25°C.

**MOTILITY-NITRATE MEDIUM (BUFFERED)**

Beef Extract	3.0 g
Peptone	5.0 g
Potassium Nitrate	1.0 g
Disodium Phosphate	2.5 g
Agar	3.0 g
Galactose	5.0 g
Glycerol	5.0 g
MS water	1.0 L

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Dissolve the ingredients, except agar, in MS water, and adjust the pH to 7.4. Add the agar, and heat the mixture until visual examination shows that it is well dissolved. Dispense and sterilize by autoclaving for 15 minutes at 121°C, and cool quickly in cold water. If the medium is not used within 4 h after preparation, heat for 10 minutes in boiling water or flowing steam and chill in cold water before use.

Final pH 7.4 ± 0.2 at 25°C

**MOTILITY TEST MEDIUM (EWING)**

Meat Extract	3.0 g
Peptone	10.0 g
Sodium Chloride	5.0 g
Agar	4.0 g
MS water	1.0 L

Adjust to pH 7.4. Add agar. Heat the mixture until visual examination shows that it is well dissolved. Dispense and sterilize at 121°C for 15 minutes.

Final pH 7.3 ± 0.2 at 25 °C.

**MR-VP MEDIUM (EWING)**

Buffered Peptone	7.0 g
Dextrose	5.0 g
K <sub>2</sub> HPO <sub>4</sub>	5.0 g
MS water	1.0 L

Dissolve, dispense and sterilize at 121°C for 15 minutes.

Final pH 6.9 ± 0.2 at 25°C.

**MUELLER HINTON AGAR**

Beef Extract	2.0 g
Acid hydrolysate of casein	17.5 g
Starch	1.5 g
Agar	17.0 g
MS water	1.0 L

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Suspend ingredients and heat the mixture until visual examination shows that it is well dissolved. Dispense and autoclave at 121°C for 15 minutes.

Final pH  $7.3 \pm 0.1$  at 25°C.

**NEUTRALIZING BUFFER**

Monopotassium Phosphate	42.5 mg
Sodium Thiosulfate	0.16 g
Sulfonate Complex	5 g
MS water	1.0 L

Dissolve all ingredients in MS water. Dispense and autoclave for 15 minutes at 121°C.

Final pH  $7.2 \pm 0.2$  at 25°C

**NITRATE BROTH**

Beef Extract	3.0 g
Peptone	5.0 g
Potassium Nitrate	1.0 g
MS water	1.0 L

Suspend above ingredients in MS water and heat the mixture until visual examination shows that it is well dissolved. Dispense and autoclave for 15 minutes at 121°C.

Final pH  $7.0 \pm 0.2$  at 25°C.

**NUTRIENT AGAR**

Beef Extract	3.0 g
Peptone	5.0 g
Agar	15.0 g
MS water	1.0 L

Heat the mixture until visual examination shows that it is well dissolved. Dispense into tubes or flasks. Autoclave 15 minutes at 121°C.

Final pH,  $6.8 \pm 0.2$  at 25°C.

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**NUTRIENT BROTH, SEMI-SOLID (Holding Media)**

Beef Extract	3.0 g
Peptone	5.0 g
Agar	7.5 g
MS water	1.0 L

Heat the mixture until visual examination shows that it is well dissolved. Dispense and autoclave 15 minutes at 121°C.

Final pH,  $6.8 \pm 0.2$  at 25°C

**PLATE COUNT AGAR (STANDARD METHODS AGAR)**

Pancreatic digest of casein USP	5.0 g
Yeast Extract	2.5 g
Dextrose	1.0 g
Agar	15.0 g
MS water	1.0 L

Suspend ingredients in MS water. Heat the mixture until visual examination shows that it is well dissolved. Sterilize at 121°C for 15 minutes.

Final pH  $7.0 \pm 0.1$  at 25°C.

**RAINBOW AGAR O157**

Rainbow agar base	60.0 g
Potassium Tellurite solution	0.8 ml
Sodium Novobiocin solution	2.5 ml
MS water	1.0 L

**Potassium Tellurite Solution**

Potassium tellurite	0.010 g
MS water	10.0 ml

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

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Sodium Novobiocin Solution

Sodium novobiocin	0.40 g
MS water	100 ml

Dissolve the sodium novobiocin in the MS water. Filter sterilize. Store in the dark up to one year at 2 to 8°C.

Add 60g of Rainbow agar base (Biolog Inc., Hayward California, 94545) to 1 liter of MS water. Boil gently until dissolved. Autoclave for 10 minutes at 121°C. Cool to 50°C. Add 2.5 ml of sodium novobiocin solution and 0.8 ml of potassium tellurite solution and mix well. Dispense approximately 20 ml per plate into petri plates. Store in a closed container in the dark. Shelf life of the prepared medium is two weeks if stored under refrigeration in sealed container such as sealed plastic bags.

Final pH  $7.9 \pm 0.2$  at 25°C

**RAPPAPORT-VASSILIADIS R10 BROTH (Available from Difco)**

Pancreatic Digest of Casein	4.54 g
Sodium Chloride	7.20 g
Potassium Dihydrogen Phosphate	1.45 g
Magnesium Chloride, Anhydrous	13.4 g
Malachite Green Oxalate	0.036 g
MS water	1.0 L

Suspend the ingredients in MS water. Heat the mixture until visual examination shows that it is well dissolved. Dispense and sterilize at 115-116°C for 15 minutes.

Final pH  $5.1 \pm 0.2$  at 25°C

**RVS BROTH (Available from Oxoid Unipath or EMD Science)**

	EMD Science	Oxoid
Magnesium Chloride	29 g (hexahydrate)	13.58g (anhydrous)
Sodium Chloride	8.0 g	7.2 g
Peptone from soymeal	4.5 g	4.5 g
Potassium Dihydrogen Phosphate	0.6 g	1.26 g
Dipotassium Hydrogen Phosphate	0.4 g	0.18 g
Malachite Green	0.036 g	0.036 g
MS water	1.0 L	1.0 L

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Add ingredients to MS water. Mix thoroughly. Dispense and autoclave for 15 minutes at 10 psi – 115°C.

Final pH 5.2 ± 0.2 at 25°C.

**RAPPAPORT-VASSILIADIS BROTH, modified (Available from Fluka)**

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
MS water	1.1L

Add ingredients to MS water. Heat gently if necessary to dissolve the medium completely. Sterilize by autoclaving at 115°C, 10 psi for 15 minutes.

Final pH 5.2 ± 0.2 at 25°C.

**SOB + Ampicillin MEDIUM**

Bacto-tryptone	20.0 g
Bacto-yeast extract	5.0 g
NaCl	0.5 g
Bacto-agar (For SOB agar only)	15.0 g
MS water	950.0 ml

Shake and mix until all solutes have dissolved. Add 10 ml of a 250 mM solution of KCl. Adjust the pH to 7.0 with 1 N NaOH (less than two ml). Adjust the volume of the solution to 1 liter with MS water. Sterilize by autoclaving for 20 minutes at 15 psi on liquid cycle.

To the autoclaved and tempered medium, add 5 ml of a sterile solution of 2 M MgCl<sub>2</sub>, 10 ml of a sterile solution of 2M MgSO<sub>4</sub>, and a filter sterilized solution of ampicillin (sodium salt) to give a final concentration of 100 µg/ml. Once ampicillin is added to the SOB base, the shelf-life is 30 days.

Final pH 7.0 ± 0.2 at 25°C.

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250 mM KCL

KCL	1.86 g
MS Water	100.0 ml

2M MgCL<sub>2</sub>

MgCL <sub>2</sub>	19.0 g
MS Water	100.0 ml

Sterilize by autoclaving for 20 minutes at 15 psi on liquid cycle.

2M MgSO<sub>4</sub>

MgSO <sub>4</sub>	24.1 g
MS Water	90.0 ml

Adjust the volume of the solution to 100 ml with MS water. Sterilize by autoclaving for 20 minutes at 15 psi on liquid cycle.

**TRIPLE SUGAR IRON (TSI) AGAR**

Beef Extract	3.0 g
Yeast Extract	3.0 g
Pancreatic Digest of Casein	15.0 g
Proteose Peptone No. 3	5.0 g
Lactose	10.0 g
Sucrose	10.0 g
Dextrose	1.0 g
Ferrous Sulfate	0.2 g
Sodium Chloride	5.0 g
Sodium Thiosulfate	0.3 g
Agar	12.0 g
Phenol Red	0.024 g
MS water	1.0 L

Heat the mixture until visual examination shows that it is well dissolved. Dispense and autoclave at 121°C for 15 minutes. Slant tubes for generous butt.

Final pH 7.4 ± 0.2 at 25°C.

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**TRYPTICASE™ SOY AGAR (TRYPTIC SOY AGAR)**

Trypticase™ (Tryptic-pancreatic digest of casein)	15.0 g
Phytone (papain digest of soybean meal)	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g
MS water	1.0 L

Add components to MS water and bring volume to 1.0 liter. Mix thoroughly. Heat the mixture until visual examination shows that it is well dissolved. Autoclave 121°C for 15 minutes. Do not overheat. Pour into sterile petri dishes or leave in tubes.

Final pH 7.3 ± 0.2 at 25°C.

**TRYPTICASE™ SOY AGAR with 5% SHEEP BLOOD (TSA-SB, SHEEP BLOOD AGAR or SBA)**

Trypticase™ (Tryptic)	15.0 g
Phytone	5.0 g
Sodium Chloride	5.0 g
Agar	15.0 g
MS water	1.0 L

Suspend ingredients in water. Heat the mixture until visual examination shows that it is well dissolved. Sterilize at 121°C for 15 minutes. If desired, cool to approximately 50°C, add 5% sterile, defibrinated, sheep blood and swirl. Avoid bubble formation. Pour 15 mL quantities into sterile 100 x 15 mm Petri dishes. For *Listeria monocytogenes* CAMP test usage, pour 9 ± 1 mL quantities into sterile 100 x 15 mm Petri dishes for ease of plate interpretation.

Final pH 7.3 ± 0.2 at 25°C.

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**TRYPTICASE™ SOY AGAR-YEAST EXTRACT (TSA-YE)**

Trypticase™ (Tryptic)	15.0 g
Phytone	5.0 g
Sodium Chloride	5.0 g
Yeast Extract	6.0 g
Agar	15.0 g
MS water	1.0 L

Suspend the above ingredients in MS water. Heat the mixture until visual examination shows that it is well dissolved. Autoclave for 15 minutes at 121°C. Temper the medium to 45 - 50°C and pour into sterile Petri dishes.

Final pH 7.3 ± 0.2 at 25°C.

**TRYPTICASE™ SOY BROTH**

Trypticase™ (Triptic)	17.0 g
Phytone™	3.0 g
Sodium Chloride	5.0 g
Dipotassium Phosphate	2.5 g
Dextrose	2.5 g
MS water	1.0 L

Dispense into tubes and sterilize at 121°C for 15 minutes.

Final pH 7.3 ± 0.2 at 25°C.

**TRYPTICASE™ SOY BROTH (TSB) WITH 10% SODIUM CHLORIDE AND 1% SODIUM PYRUVATE (PTSBS)**

Sodium Chloride	100.0 g
Trypticase™ (Tryptic) Pancreatic Digest of Casein	17.0 g
Phytone (Papaic Digest of Soya Meal)	3.0 g
K <sub>2</sub> HPO <sub>4</sub>	2.5 g
Dextrose	2.5 g
Sodium Pyruvate	10.0 g
MS water	1.0 L

To make from commercial TSB, add 95 g of NaCl to 30 g of dry ingredients, and dissolve in 1.0 L MS water. Dispense and sterilize at 121°C for 15 minutes.

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Final pH  $7.3 \pm 0.2$  at 25°C.

NOTE: Dehydrated complete medium is not available commercially

**TRYPTOSE SULFITE CYCLOSERINE (TSC) AGAR**

Tryptose	15.0 g
Agar	14.0 g
Beef Extract	5.0 g
Pancreatic digest of soybean meal	5.0 g
Yeast Extract	5.0 g
Ferric Ammonium Citrate	1.0 g
Na <sub>2</sub> S <sub>2</sub> O <sub>5</sub>	1.0 g
Egg Yolk Enrichment (50%)	50.0 ml
Cycloserine † Solution	10.0 ml
MS water	940.0 ml

NOTE: First 7 ingredients available commercially as SFB Base

Cycloserine Solution

D-Cycloserine †	0.4 g
MS water	10.0 ml

Add cycloserine to MS water, bring volume up to 10.0 ml, mix thoroughly and filter sterilize through a 0.2 µm filter.

To prepare this medium, add the above components, except for the egg yolk emulsion and the cycloserine solution, to MS water and bring volume up to 940.0 ml. Mix thoroughly. Gently heat and bring to boiling. Autoclave for 15 minutes at 121°C. Cool to 45 - 50°C and aseptically add 50 ml of the prepared egg yolk emulsion and the sterile 10 ml cycloserine solution. Mix thoroughly and pour into sterile Petri dishes.

Final pH  $7.6 \pm 0.2$  at 25°C.

See preparation of Egg Yolk Free Tryptose Sulfite Cycloserine Agar (EY-free TSC).

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**TT BROTH (HAJNA AND DAMON, 1956)**

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
MS water	1.0 L

Dissolve and heat to boiling using a hotplate or equivalent. DO NOT AUTOCLAVE. Cool below 50°C. Add 40 ml iodine solution. Do not heat after the addition of iodine. Dispense into sterile containers while keeping the solution well mixed and use the day it is prepared.

Final pH 7.6 ± 0.2 at 25°C after addition of iodine.

**Iodine Solution**

Potassium Iodide	8 g
Iodine crystals †	5 g
MS water	20 ml

Dissolve potassium iodide in 20 ml MS water. Add iodine crystals and stir until completely dissolved. Add MS water to volume of 40 ml. Mix thoroughly. Store in the dark at 2-8°C.

Final pH 7.6 ± 0.2 at 25°C after addition of iodine.

**WANG'S FREEZING/STORAGE MEDIUM**

Brucella broth powder	28 g
Glycerol	200 ml
DI water	750 ml
<b>Supplement</b>	
Lysed horse blood	5 ml/100 ml

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Add Brucella broth powder to water and bring to a boil to dissolve completely. Add 200 mL of glycerol to the homogenous mixture and mix well. Dispense 95 ml of the mixture into individual 100 ml bottles. Autoclave for 15 minutes at 121°C and cool it to 50°C. Add 5 ml lysed horse blood into each bottle and mix thoroughly. Dispense 1 mL Wang's storage medium into a 2 mL cryovial.

NOTE: Once prepared, expiration date for Brucella broth prior to addition of horse lysed blood is 90 days. Lyse horse blood by subjecting it to two freeze/thaw cycles. Once the horse lysed blood has been added to the prepared Brucella broth, Wang's storage medium can be stored up to 3 weeks at 2 – 8°C in a flask or dispensed into cryovials.

**WANG'S TRANSPORT MEDIUM (SEMISOLID)**

Purified grade agar	4 g
Brucella broth powder	28 g
DI water	950 ml
<b>Supplement</b>	
Lysed horse blood	5 ml/100 ml

Add Brucella broth powder and purified grade agar to water and bring to a boil to dissolve completely. Dispense 95 ml of the mixture into individual 100 ml bottles. Autoclave for 15 minutes at 121°C and cool it to 50°C. Add 5 ml lysed horse blood into each bottle and mix thoroughly. Dispense 1 ml Wang's transport medium into a 2 ml cryovial.

NOTE: Once prepared, expiration date for Brucella broth prior to addition of horse lysed blood is 90 days. Lyse horse blood by subjecting it to two freeze/thaw cycles. Once the horse lysed blood has been added to the prepared Brucella broth, Wang's transport medium can be stored up to 3 weeks at 2 – 8°C in a flask or dispensed into cryovials.

**XLT4 AGAR**

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 Chemical Co, formally Tergitol 4)	4.6 ml
MS water	1.0 L

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- a. Dissolve Niaproof 4 in distilled or deionized water in a 2 L or larger Erlenmeyer flask and mix with a magnetic stir-bar.
- b. Add other ingredients, mix well using stir-bar and Heat the mixture until visual examination shows that it is well dissolved.
- c. Cool to 45 - 50°C in a water bath and mix again gently.
- d. Pour plates fairly thick (about 5 mm deep). The plates may appear dark at first but should lighten up after cooling overnight. Allow plates to remain at room temperature overnight to dry, then refrigerate (in plastic bags or containers) at 3-8°C.
- e. Remove plates from the refrigerator 24 h prior to use for further drying.
- f. pH of XLT4 plates =  $7.5 \pm 0.2$  (usually no adjustment is necessary).

NOTE: Poured XLT4 plates have a shelf life of at least 3 months when stored refrigerated in closed plastic bag or other container.

Neither XLD agar nor Tergitol 7 can be used in place of plain XL agar base or Tergitol 4, respectively.

CAUTION: Consult a Material Safety Data Sheet (MSDS) before working with KCN.

Do not dispose of hazardous fluids such as sodium azide by pouring down sink drains. Accumulation of sodium azide in lead drains may result in an explosion.

Collect sodium azide wastes and dispose of in accordance with the standard chemical waste management procedures for your laboratory.

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**APP 1.3 REAGENTS**

**ANDRADE'S INDICATOR (EWING)**

Acid fuchsin	0.2 g
MS water	100.0 ml
Sodium hydroxide (1.0 N)	16.0 ml

The fuchsin is dissolved in the MS water, and the sodium hydroxide is added. If, after several hours, the fuchsin is not sufficiently decolorized to a golden color, add an additional 1 or 2 ml of alkali. Sterilize by filtration. The dye content of different samples of acid fuchsin varies quite widely, and the amount of alkali that should be used with any particular sample usually is specified on the label. The reagent improves somewhat on aging and should be prepared in sufficiently large amounts to last for several years (up to ten years). The indicator is used in the amount of 10 ml per liter of medium.

**BUFFERED GLYCEROL SALT SOLUTION**

Glycerol (glycerin)	100.0 ml
Dipotassium Phosphate (anhydrous)	12.4 g
Monopotassium Phosphate (anhydrous)	4.0 g
Sodium Chloride	4.2 g
MS water	900.0 ml

Dissolve the sodium chloride in part of the water, and make up to 900.0 ml. Add the glycerol and phosphates, and adjust the pH to 7.2. Autoclave for 15 minutes at 121°C. For double strength (20%) glycerol solution, use 200 ml of glycerol and 800.0 ml of MS water.

**BUTTERFIELD'S PHOSPHATE DILUENT**

a. Stock solution

Dissolve 34 g  $\text{KH}_2\text{PO}_4$  in 500 ml MS water, adjust to pH 7.2 with ca. 175 ml 1 N NaOH, and dilute to 1 liter. Store under refrigeration.

b. Diluent

Dilute 1.25 ml stock solution (a) to 1 liter with MS water. Readjust the pH to 7.2, if necessary, by the drop-wise addition of 0.1 N HCl or 0.1 N NaOH. Autoclave at 121°C for 15 minutes.

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**ENDOSPORE STAIN**

a. Solution A

Dissolve 5.0 g of Malachite green in 100 ml MS water. Filter to remove undissolved dyes.

b. Solution B

Dissolve 0.5 g Safranin O in 100 ml of MS water.

**GRAM STAIN (HUCKER MODIFICATION)**

a. Crystal violet solution

Crystal Violet (90% dye)	2.0 g
Ethanol (95%)	20.0 ml

b. Oxalate solution

Ammonium Oxalate	0.8 g
MS water	80.0 ml

Working crystal violet solution

Mix the above two solutions together and store in a glass-stoppered bottle.

c. Gram's iodine solution

Iodine crystals	1.0 g
Potassium Iodide	2.0 g
MS water	300.0 ml

Dissolve potassium iodide completely in 5 ml MS water, dissolve the iodine crystals, and then bring to volume with MS water. Mix well and store in an amber glass bottle.

d. Decolorizer

Ethanol, 95% 500.0 ml  
Store in glass-stoppered bottle.

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e. Stock safranin (Counterstain)

Safranin O (2.5% solution in 95% ethanol)	10.0 ml
MS water	100.0 ml

Mix well and store in a glass-stoppered bottle.

**OXIDASE REAGENT**

Tetramethyl-p-phenylenediamine dihydrochloride	1.0 g
MS water	100.0 ml

Prepare fresh daily or refrigerate for not longer than 1 week. Alternatively, use commercial oxidase reagents.

**KOVAC'S REAGENT (EWING)**

Pure Amyl or Isoamyl Alcohol	150.0 ml
Paradimethylaminobenzaldehyde	10.0 g
Concentrated HCl	50.0 ml

Dissolve aldehyde in alcohol and slowly add acid. The dry aldehyde should be light in color. Prepare reagent in small quantities. Store in refrigerator.

**METHYL RED REAGENT (EWING)**

Methyl Red	0.1 g
Ethyl Alcohol (95-96%)	300.0 ml

Dissolve dye in the alcohol and then add MS water to make 500 ml. Use 5 or 6 drops per 5.0 ml of culture.

**NITRATE REDUCTION REAGENTS**

Solution A

Sulfanilic Acid	0.5 g
Glacial Acetic Acid	30.0 ml
MS water	120.0 ml

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Solution B

N(1-naphthyl)ethylenediamine dihydrochloride ( Marshal's Reagent)	0.2 g
Glacial Acetic Acid	30.0 ml
MS water	120.0 ml

Cleve's acid (5-amino-2 naphthalene sulfonic acid) may be substituted for Marshal's Reagent.

**PEPTONE WATER DILUENT (0.1%)**

Peptone	1.0 g
MS water	1.0 L

Dissolve peptone in MS water and adjust pH to  $7.0 \pm 0.1$ . Prepare dilution blanks with this solution, dispensing a sufficient quantity to allow for loss during autoclaving. Autoclave at 121°C for 15 minutes.

**PHOSPHATE BUFFERED SALINE (PBS)**

Anhydrous Na <sub>2</sub> HPO <sub>4</sub>	12.0 g
NaH <sub>2</sub> PO <sub>4</sub> ·H <sub>2</sub> O	2.2 g
NaCl	85.0 g

Dissolve dry ingredients in MS water and bring volume to 1 L (**10X PBS**). Adjust pH to 7.4 with 0.1 N HCl or 0.1 N NaOH. To make 1X PBS, dilute 100 ml 10X PBS in 900 ml MS water. Check and adjust pH (7.4) if necessary. Sterilize at 121°C for 15 minutes.

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**0.15 M PHOSPHATE BUFFERED SALINE at pH 7.2 (PBS)**

“Acid” solution

Anhydrous Na <sub>2</sub> HPO <sub>4</sub>	10.36 g
NaCl	4.38
RO water	1.0 L

“Base” solution

NaH <sub>2</sub> PO <sub>4</sub> .H <sub>2</sub> O	10.65 g
NaCl	4.38 g
RO water	1.0 L

Prepare ‘acid’ and ‘base’ solutions by added ingredients to RO water. Dissolve completely. While mixing with a magnetic stirrer and monitoring the pH on a pH meter, add a sufficient quantity of the ‘acid’ solution to the ‘base’ solution to achieve a final, stabilized pH of 7.2. Dispense into glass containers. Autoclave at 121°C for 15 minutes. Store at room temperature.

**PHOSPHATE BUFFERS**

0.1 M phosphate buffer, pH 4.5 (± 0.1)

Dissolve 13.6 g of potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>) in about 800 ml of laboratory grade water. Check the pH of the solution. Adjust, if necessary, by the dropwise addition 0.1 N HCl or NaOH. Dilute to 1 liter. Autoclave for 15 minutes at 121°C.

0.1 M phosphate buffer, pH 6.0 (± 0.1)

Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	11.2 g
Dipotassium hydrogen phosphate (K <sub>2</sub> HPO <sub>4</sub> )	2.8 g

Dissolve in laboratory grade water. Check the pH of the solution. Adjust, if necessary, by the dropwise addition of 0.1 N HCl or NaOH. Dilute to 1 liter. Autoclave for 15 minutes at 121°C.

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0.1 M phosphate buffer, pH 8.0 (± 0.1)

Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	0.523 g
Dipotassium hydrogen phosphate (K <sub>2</sub> HPO <sub>4</sub> )	16.73 g

Dissolve in about 800 ml of laboratory grade water. Check the pH of the solution. Adjust if necessary by the dropwise addition of 0.1 N HCl or NaOH. Dilute to 1 liter. Autoclave for 15 minutes at 121°C.

0.2 M phosphate buffer, pH 8.0 (± 0.1)

Potassium dihydrogen phosphate (KH <sub>2</sub> PO <sub>4</sub> )	1.046 g
Dipotassium hydrogen phosphate (K <sub>2</sub> HPO <sub>4</sub> )	33.46 g

Dissolve in about 800 ml of laboratory grade water. Check the pH of the solution. Adjust if necessary by the dropwise addition of 0.1 N HCl or NaOH. Dilute to 1 liter. Autoclave for 15 minutes at 121°C.

**HYPOTONIC SALINE SOLUTION 0.45% (STERILE)**

Sodium Chloride	4.5 g
MS water	1.0 L

Dissolve salt completely in MS water and autoclave at 121°C for 15 minutes.

**PHYSIOLOGICAL SALINE SOLUTION 0.85% (STERILE)**

Sodium Chloride	8.5 g
MS water	1.0 L

Dissolve salt completely in MS water and autoclave at 121°C for 15 minutes.

**TRIS BUFFER (0.02 M, pH 7.75)**

Trishydroxymethylaminomethane	7.5 g
MS water	3.0 L

Dissolve tris completely in MS water and adjust pH to 8.5 with 20% HCl. Dispense into 150 ml portions and autoclave at 115°C for 15 minutes.

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**V-P REAGENT OF O'MEARA, MODIFIED (EWING)**

Potassium Hydroxide	40.0 g
Creatine	0.3 g
MS water	100.0 ml

Dissolve alkali in water. Add creatine. Keep refrigerated. Make new reagent every 3 weeks. Use equal parts of reagent and culture. Aerate by shaking. Place test tube at 37°C. Read in 4 hours.

**APP 1.4      Reference(s):**

Atlas, Ronald M. 2004. Handbook of Microbiological Media, 3<sup>nd</sup> Ed. CRC Press, Boca Raton, FL.

Difco & BBL Manual: Manual of Microbiological Culture Media. 2003. Published by Becton, Dickinson and Company.

Downes, F. P. and K. Ito (Editors). 2001. Compendium of Methods for the Microbiological Examination of Foods. Fourth Edition. Published by American Public Health Association, Washington, D.C.

Edwards, P.R. and M.A. Fife. 1961. Lysine-Iron agar in the detection of Arizona cultures. Appl. Microbiol. **9**: 478-480.

Ewing, W. H. 1986. Edwards and Ewing's Identification of Enterobacteriaceae, 4<sup>th</sup> Edition. Elsevier Science Publishing Co., Inc., New York.

Hajna, A., and S. R. Damon. 1956. New enrichment and plating media for the isolation of *Salmonella* and *Shigella* organisms. Appl. Microbiol. **4**:341-345.

Holdeman, L. V., E. P. Cato, and W. E. C. Moore. 1977. *Campylobacter*, p.114-115. In Anaerobe Laboratory Manual. 4th Edition. Published by Virginia Polytechnic Institute and State University, Blacksburg, Va.

Hunt, J. M, C. Abeyta and T. Tran. 2001. Chapter 7: "*Campylobacter*" In FDA Bacteriological Analytical Manual *Online*.

Hutchinson, D. N., and F. J. Bolton. 1984. Improved blood free selective medium for the isolation of *Campylobacter jejuni* from faecal specimens. J. Clin. Pathol. **37**: 956-957.

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Osborne, W. W. and J. L. Stokes. 1955. A modified selenite brilliant-green medium for the isolation of salmonellae from egg products. *Appl. Microbiol.* **3**: 295-299.

Palumbo, S. A., F. Maxino, A. C. Williams, R. L. Buchanan, and D. W. Thayer. 1985. Starch-ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. *Appl. Environ. Microbiol.* **50(4)**:1027-1030.

Wang, W. L. L., N. W. Luechtefeld, L. B. Reller, and M. J. Blaser. 1980. Enriched *Brucella* medium for storage and transport of cultures of *Campylobacter fetus* subsp. *jejuni*. *J. Clin. Microbiol.* **12**:479-480.