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Food Safety and Inspection Service
Office of Public Health Science
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Laboratory Guidebook
Notice of Change

Chapter new, revised, or archived: MLG Appendix 1.09

Title: Media and Reagents

Effective Date: 12/29/2017

Description and purpose of change(s):

This chapter was revised to incorporate the following material:

General heating instructions were updated.
Recommendation for media expiration dates/storage times was added.
Neutralizing buffered peptone water (nBPW) recipe was added.
XLT4 recipe updated to incorporate the change to XLT4 agar base.
Sodium Novobiocin Solution recipe was added to Double modified lysine iron agar (DMLIA).
Neutral Red (1% aqueous solution), and Bacto brom thymol blue was added to the reagents.

The following media were removed: AK Agar #2 (Sporulating Agar), Antibiotic medium #2 with Dextrose, Carbohydrate fermentation broth (Ewing), KF broth, MR-VP Medium (Ewing), Mueller Hinton agar, Trypticase soy agar-yeast extract (TSA-YE), Trypticase™ Soy Broth (TSB) with 10% Sodium Chloride and 1% Sodium Pyruvate (PTSBS), Neutral Red, and Andrade's indicator (Ewing).

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. Method validation is necessary to demonstrate the equivalence of alternative tests as detailed in the document titled "FSIS Guidance for Evaluating Test Kit Performance" on the FSIS website.

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 Safety Data Sheets. Chemicals with '†' are of particular concern.

- Media and reagents may be filter-sterilized.

- Any departures from manufactures' 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. Heating prior to sterilization can be disregarded when using an autoclave with stirring capabilities and also may not be necessary with smaller volumes. Agars must be heated with frequent agitation before dispensing into bottles or tubes prior to the sterilization step. 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 preparation, specified pH, time, and temperature of sterilization, etc. Dehydrated media of any brand equivalent to the provided formulations and procedures may be used unless instructions indicate otherwise.

- Medium should not be used if there are signs of deterioration, hemolysis, or contamination prior to the expiration date.

- It is recommended that expiration dates/storage times are applied to media prepared within the laboratory from dehydrated media. The expiration date of the prepared medium should be based on the prepared product and not the expiration dates of the components added to the medium. However, all components added to the medium should be within their date of expiration. If a change in color, a precipitate, or drying is observed, then the product is discarded regardless of the expiration date. Recommendations of expiration dates/storage times can be obtained from manufacturers of dehydrated product(s). A study is recommended to determine the shelf-life of a medium or to extend it past the recommended guidelines.

- Microbiologically 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 megaohms-cm resistance at 25ºC 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.

APP 1.2 MEDIA PREPARATION INSTRUCTIONS

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

Autoclave at 121°C for 15 minutes.

Final pH 6.5 – 6.6 at 25ºC.

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

Autoclave at 121°C for 15 minutes.

Final pH 7.8 – 8.0 at 25ºC 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	15.0 g
MS water	1.0 L

Autoclave at 121°C for 15 minutes. Final pH 5.8 – 5.9 at 25ºC or as specified by the manufacturer if using commercial dehydrated medium.

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

Autoclave at 121°C for 15 minutes.

Final pH 7.95 ± 0.05 at 25ºC 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_2HPO_4	5.0 g
Sodium Citrate	5.0 g
Na_2CO_3	1.25 g
$MnCl_2.4H_2O$	0.14 g
$MgSO_4.7H_2O$	0.8 g
Polysorbate 80	0.2 g
$FeSO_4.7H_2O$	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 118ºC – 121°C at 13 psi for 15 minutes. Avoid excessive heating. Dispense into sterile Petri dishes or leave in tubes.

Final pH 6.7 ± 0.2 at 25ºC.

BACILLUS CEREUS (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
MS 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 13 x 100 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 $6H_20$	5.0 g
Agar	20.0 g
MS water	950.0 ml

Suspend ingredients in water. Autoclave at 121°C for 15 minutes.

Final pH 6.9 ± 0.1 at 25°C.

Complete medium

a. Add 50 ml pre-warmed (to at least room temperature) Bacto EY tellurite enrichment to 950 ml base medium which has been tempered to 45 – 50°C.
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 ENRICHMENT BROTH (2X BF-BEB)

Basal Ingredients	
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
MS Water	1.0 L
Commercial Supplements:	
Cefoperazone	40 mg
Vancomycin	40 mg
Trimethoprim or Trimethoprim lactate	40 mg
Amphotericin B or Cycloheximide†	20 mg 100 mg

Laboratory Made Supplements:

Amphotericin B

Amphotericin B	1.0 g
MS water	100.0 ml

Dissolve 1.0 g of Amphotericin B in 100 ml MS water in a volumetric flask and filter sterilize. Store up to 3 weeks at 2 – 8°C. Add 2 ml/L to yield a final concentration of 20 mg/L.

Cefoperazone

Cefoperazone sodium salt	2.0 g
MS water	100.0 ml

Dissolve 2.0 g of cefoperazone in 100 ml MS water in a volumetric flask and filter sterilize. Store up to 5 days at 2 – 8°C; 14 days at ≤ -20°C; or 5 months at ≤ -70°C. Freeze 2 ml aliquots in sterile plastic tubes. Add 2 ml/L to yield a final concentration of 40 mg/L.

Trimethoprim

Trimethoprim	0.50 g
Hydrochloric acid , 0.05N	0.30 ml
MS water	100.0 ml

In a volumetric flask, pre-warm the portion of hydrochloric acid to 50°C on a hot plate with a magnetic stirrer. Add trimethoprim and stir until dissolved. Remove stirrer and adjust volume to 100 ml of MS water and filter sterilize. Store up to 1 year at 2 – 8ºC. Add 8 ml/L to yield a final concentration of 40 mg/L.

Trimethoprim Lactate

Trimethoprim lactate	0.66 g
MS water	100.0 ml

Dissolve 0.66 g of trimethoprim lactate in 100 ml MS water in a volumetric flask and filter sterilize. Store up to 1 year at 2 – 8°C. Add 8 ml/L to yield a final concentration of 40 mg/L.

Vancomycin

Vancomycin	2.0 g
MS water	100.0 ml

Dissolve 2.0 g of vancomycin in 100 ml MS water in a volumetric flask and filter sterilize. Store up to 2 months at 2 – 8°C. Add 2 ml/L to yield a final concentration of 40 mg/L.

All the above mentioned ingredients/supplements are for the preparation of 1 L 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 only). 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 – 8ºC. 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 – 8ºC.

Final pH 7.4 ± 0.2 at 25ºC.

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_2HP0_4	2.5 g
Dextrose	2.0 g
Agar	15.0 g
MS water	1.0 L

Dissolve ingredients in MS water. Dispense as desired and autoclave at 121ºC 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 25ºC.

BRAIN HEART INFUSION (BHI) BROTH

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_2HP0_4	2.5 g
Dextrose	2.0 g
MS water	1.0 L

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

Final pH 7.4 ± 0.2 at 25ºC.

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

Autoclave at 121°C for 15 minutes. Cool to approximately 50ºC and dispense 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 121ºC.

Final pH 6.9 ± 0.1 at 25ºC.

NOTE: Dehydrated prepared medium not available commercially.

BRUCELLA BROTH

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
Sodium Bisulfite	0.10 g
MS water	1.0 L

Dissolve the dehydrated ingredients in MS water. Dispense as desired and autoclave at 121ºC for 15 minutes.

Final pH 7.0 ± 0.2 at 25ºC

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 121ºC for 15 minutes.

Final pH 7.2 ± 0.2 at 25ºC.

CAMPY-CEFEX AGAR

Basal Ingredients	
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
MS Water	1.0 L
Supplement	
Lysed Horse Blood	50 ml
Cefoperazone	33 mg

Suspend all ingredients in l L of MS water and heat with frequent agitation to dissolve. Autoclave for 15 minutes at 121ºC and cool to 50ºC. Add supplements. Dispense into petri dishes (20 ml/plate).

Final pH 7.0 ± 0.2 at 25 ºC.

After media plate preparation, plates may either be held up to 90 days in 2 – 8ºC away from direct light or held in the dark at room temperature for 2 – 4 days to allow sufficient time for drying.

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.

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 121ºC for 15 minutes.

Final pH 7.6 ± 0.2 at 25ºC.

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

Sodium Novobiocin Solution

Sodium Novobiocin	1.5 g
MS water	100 ml

Dissolve the sodium novobiocin in the MS water. Filter sterilize. Store at 2-8ºC in the dark for up to 6 months

Suspend all ingredients in MS water and heat to boil to 100ºC for 10 minutes. DO NOT HEAT ABOVE 100ºC. Cool to approximately 50ºC, add 1 ml of Sodium Novobiocin from a filter-sterilized stock solution. Dispense 15 – 20 ml/plate. Store refrigerated for up to 3 weeks.

NOTE: If the MRBA Novobiocin stock is used, add 3.75 ml/L of DMLIA.

Final pH 6.7 ± 0.2 at 25ºC.

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

E BUFFER

Bovine Albumin	5 g
Tween-20	0.5 ml
Buffered Peptone Water (BPW)	20 g
MS Water	1L

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 25ºC.

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 25ºC.

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_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 mg
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.

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

Fraser 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 re-suspend the media and dispense into test tubes. Sterilize at 121ºC 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 25ºC.

HORSE BLOOD OVERLAY MEDIUM (HBO or HL)

a. Base Layer

Columbia Blood Agar Base	1.0 L

Prepare according to manufacturer's specifications and sterilize at 121ºC for 15 minutes. Dispense 10 ml per Petri dish. Allow to solidify, overlay with blood agar as described below.

b. Overlay

Prepare according to manufacturer's instructions. Add 4 ml of sterile defibriated horse blood per 100 ml of sterilized Columbia Blood Agar Base which has been cooled to 46ºC. Stir or swirl to mix evenly. Dispense 5 to 6 ml on top of the base layer and tilt the plates to spread top layer evenly. Refrigerate blood plates up to 2 weeks. Discard any plates which become discolored.

Final pH 7.2 ± 0.2 at 25ºC.

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

Heat to dissolve the agar completely. Dispense into tubes and autoclave for 12 minutes at 121°C. Slant with deep butt and short slant.

Final pH 6.7 ± 0.2 at 25ºC

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 ± 0.1, and dispense. Autoclave at 121ºC for 15 minutes, cool to 50ºC in a waterbath, and add 50 ml of Preparation B and 10 ml of Preparation C. Mix well, dispense 18 – 20 ml into Petri dishes, allow to solidify, and dry for 24 h at room temperature. Plates may be stored at 2º – 8ºC for 30 days.

Final pH 7.2 ± 0.1 at 25ºC.

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 ± 0.2. Add about 1 gram of Cook Meat Medium (a) and 16 ml of diluent (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: The diluent may be heated to dissolve starch if necessary. Steam tubes of Cook Meat Medium for 10 minutes and cool just prior to use.

Final pH 6.8 ± 0.2 at 25ºC.

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	15.0 g
Colistin	0.01
MS water	1.0 L

Autoclave 121ºC for 15 minutes, mix again, and cool to 45º – 50ºC in a water bath. Add 2 ml of 1% filter sterilized Moxalactam solution to make the complete MOX medium, mix well, and dispense 12 – 14 ml per plate.

Final pH 7.0 ± 0.2 at 25 ºC.

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

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

Dissolve, filter sterilize, dispense in small quantities for use and store in freezer at -10ºC 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 6 months at 2 – 8°C.

Cefixime Solution

Cefixime Trihydrate	0.050 g
Methanol	10 ml

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 to 1 liter of MS water. 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 21 days if stored under refrigeration in sealed container such as sealed plastic bags.

Final pH 8.1 ± 0.2 at 25ºC

MODIFIED TRYPTONE SOYA BROTH (mTSB)

Base Ingredients	
Modified Tryptone Soya Broth (Oxoid Product # CM0989B or equivalent	33.0 g
Casaminoacids (casein acid hydrolysate)	10.0 g
MS water	1.0 L

Suspend all ingredients in MS Water and autoclave for 15 minutes at 121°C.
If refrigerated, media must be pre-warmed to 18 – 35ºC prior to use.

Final pH 7.4 ± 0.2 at 25ºC.

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

Base Ingredients	
Modified Tryptone Soya Broth	33.0 g
Casaminoacids (casein acid hydrolysate)	10.0 g
MS water	1.0 L
Supplement	
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.

Suspend all ingredients in MS Water and autoclave for 15 minutes at 121°C.

Let media cool to at least 50ºC. 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 – 35ºC prior to use. Store Novobiocin stock solution at 2-8 ºC for up to 6 months

Final pH 7.4 ± 0.2 at 25ºC.

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_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	12.0 mg
MS water	1.0 L

Sterilize at 121ºC for 15 minutes. Store in the refrigerator.

Final pH 7.2 ± 0.2 at 25ºC.

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

Dehydrated 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 dehydrated 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

Suspend all ingredients in 1 liter MS water. Dispense and sterilize for 15 minutes at 121°C.

Final pH 7.3 ± 0.2 at 25ºC.

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

Dissolve the ingredients, except agar, in MS water, and adjust the pH to 7.4 ± 0.2. 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 121ºC, 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 25ºC

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 121ºC for 15 minutes.

Final pH 7.3 ± 0.2 at 25ºC.

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 121ºC.

Final pH 7.2 ± 0.2 at 25ºC

NEUTRALIZING BUFFERED PEPTONE WATER (nBPW)

Buffered Peptone (Difco or equivalent)	20.0 g
Refined Soy Lecithin (MP Biomedicals, item # 102147, store at -20^{0}C or Alfa Aesar #36486, CAS 8002-43-5) or equivalent	7 g
Sodium Thiosulfate	1 g
Microbiology Suitable (MS) water	1 L
Sodium Bicarbonate	12.5g

Mix Buffered Peptone, soy lecithin, and sodium thiosulfate in 833 ml of MS water. Autoclave at 121°C for 15 minutes. Dissolve the sodium bicarbonate in 167 ml MS water, filter sterilize and add to autoclaved broth after it cools to at least 55°C. Constant agitation while sterilely dispensing this medium is recommended. Volumes may be adjusted for larger or smaller batches.

Final pH 7.7 ± 0.5 at 25°C after the addition of sodium bicarbonate.

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. Dispense and autoclave for 15 minutes at 121ºC.

Final pH 7.0 ± 0.2 at 25ºC.

NUTRIENT AGAR

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

Dispense into tubes or flasks. Autoclave 15 minutes at 121ºC.

Final pH 6.8 ± 0.2 at 25ºC.

NUTRIENT BROTH, SEMI-SOLID (Holding Media)

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

Dispense and autoclave 15 minutes at 121ºC.

Final pH 6.8 ± 0.2 at 25ºC.

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. Sterilize at 121°C for 15 minutes.

Final pH 7.0 ± 0.1 at 25ºC.

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. Dispense and sterilize at 115 – 116ºC for 15 minutes.

Final pH 5.1 ± 0.2 at 25ºC

RVS BROTH (Available from Oxoid Unipath or EMD Science)

	EMD Science	Oxoid
Magnesium Chloride	29 g (hexahydrate)	13.58 g (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

Add ingredients to MS water. Mix thoroughly. Dispense and autoclave for 15 minutes at 115ºC.

Final pH 5.2 ± 0.2 at 25ºC.

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 at 115ºC for 15 minutes.

Final pH 5.2 ± 0.2 at 25ºC.

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

Dispense ingredients in MS water.
Add 10 ml of a 250 mM solution of KCl. Adjust the pH to 7.0 with 1 N NaOH (less than 2 ml). Adjust the volume of the solution to 1 liter with MS water. Sterilize at 121°C for 15 minutes

To the autoclaved and tempered medium, add 5 ml of a sterile solution of 2 M $MgCl_2$, 10 ml of a sterile solution of 2M $MgSO_4$, 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 25ºC.

250 mM KCl

KCl	1.86 g
MS Water	100.0 ml

2M $MgCl_2$

Sterilize by autoclaving for 15 minutes at 121°C on liquid cycle.

2M $MgSO_4$

$MgSO_4$	24.1 g
MS Water	90.0 ml

Adjust the volume of the solution to 100 ml with MS water. Sterilize by autoclaving for 15 minutes at 121ºC.

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

Dispense and autoclave at 121ºC for 15 minutes. Slant tubes for generous butt.

Final pH 7.4 ± 0.2 at 25ºC.

TRYPTICASE™ SOY AGAR (TRYPTIC SOY AGAR)

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

Suspend ingredients in MS water. Autoclave at 121°C for 15 minutes. Dispense as desired.

Final pH 7.3 ± 0.2 at 25ºC.

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. Sterilize at 121°C for 15 minutes. Cool to approximately 50ºC, 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.

TRYPTICASE™ SOY BROTH

Trypticase™ (Tryptic)	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.

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
$Na_2S_2O_5$	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 121ºC. Cool to 45 – 50ºC 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 ± 0.2 at 25ºC.

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

TT BROTH (HAJNA AND DAMON, 1956)

TT 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
MS water	1.0 L

Dissolve and heat to boiling using a hotplate or equivalent. DO NOT AUTOCLAVE. Cool below 50°C. Add 4% iodine solution per final volume. 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. The base may be stored at 2 – 8°C for up to six months prior to the addition of iodine.

Final pH 7.6 ± 0.2 at 25ºC 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. Under a vent hood, add iodine crystals and stir until completely dissolved. Add MS water to volume of 40 ml. Mix thoroughly. Store in the dark at 2-30 ºC.

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

WANG'S FREEZING/STORAGE MEDIUM

B Ingredients	
Brucella broth powder	28 g
Glycerol	200 ml
MS water	750 ml
Supplement	
Lysed horse blood	5 ml/95 ml

Add Brucella broth powder to MS water and heat the mixture until visual examination shows that it is well dissolved. Add 200 ml of glycerol to the homogenous mixture and mix well. Dispense 95 ml of the mixture into individual bottles. Autoclave for 15 minutes at 121ºC and cool it to 50ºC. Add 5 ml lysed horse blood per 95 ml bottle and mix thoroughly. Dispense 1 ml Wang's storage medium into a 2 ml cryovial.

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

Final pH 7.0 ± 0.2 at 25ºC before addition of supplement.

WANG'S TRANSPORT MEDIUM (SEMISOLID)

Bas Ingredients	
Purified grade agar	4 g
Brucella broth powder	28 g
MS water	950 ml
Supplement	
Lysed horse blood	5 ml/95 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 bottles. Autoclave for 15 minutes at 121ºC and cool it to 50ºC. Add 5 ml lysed horse blood to each 95ml bottle and mix thoroughly. Dispense 1 ml Wang's transport medium into a 2 ml cryovial.

NOTE: Once prepared, expiration date for the base medium prior to addition of lysed horse blood is 90 days. Lyse horse blood by subjecting it to two freeze/thaw cycles.

Once the lysed horse blood has been added to the prepared base medium, Wang's transport medium can be stored up to 3 weeks at 2 – 8ºC in cryovials.

Final pH 7.0 ± 0.2 at 25ºC before addition of supplement.

XLT4 AGAR

XLT4 Agar Base* (BD# 223420)	59.0 g
XLT4 Agar Supplement (A 27% solution (approximate) of the surfactant Tergitol™ 4)	4.6 ml
MS water	1.0 L

*XLT4 Agar Base (approximate Formula Per liter)	
Proteose Peptone No. 3	1.6g
Yeast Extract	3.0g
L-Lysine	5.0g
Xylose	3.75g
Lactose	7.5g
Saccharose	7.5g
Ferric Ammonium Citrate	0.8g
Sodium Thiosulfate	6.8g
Sodium Chloride	5.0g
Agar	18.0g
Phenol Red	0.08g

Dissolve 59.0 g of XLT4 Agar Base in 1.0 L MS water and mix with a magnetic stir-bar. Add 4.6 ml of XLT 4 agar supplement. Heat to boiling to dissolve completely. Cool to 45 - 50ºC in a water bath and mix again gently. Dispense approximately 20 ml into sterile 100 x 15 mm Petri dishes.. Allow plates to dry at room temperature overnight and then refrigerate (in plastic bags or containers) at 2-8ºC. pH of XLT4 plates = 7.5 ± 0.2

NOTE: XLT4 plates have a shelf life of 10 weeks when stored refrigerated in closed plastic bag or other container.

APP 1.3 REAGENTS

BACTO BROM THYMOL BLUE

Dissolved 0.1 g dye in 18.5 ml 0.01N NaOH. Dilute to 250 ml with MS water.

Final pH range 5.2 – 6.8.

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 121ºC. 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 KH_2P0_4 in 500 ml MS water, adjust to pH 7.2 with approximately 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 121ºC for 15 minutes.

CALCIUM CARBONATE

Transfer the needed aliquot into an autoclavable container. Sterilize the aliquot for 15 min at 121ºC using dry cycle parameters. Use the expiration date specified by manufacturer.

CRYSTAL VIOLET (1% AQUEOUS)

Steam at 100°C for 15 minutes or filter sterilize.

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.

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.

NEUTRAL RED (1% AQUEOUS)

Dissolved 0.1 gm in 60 ml of ethanol and diluted to 100 ml of MS water as required.

Final pH range 6.8 – 8.0

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

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 ± 0.1. Autoclave at 121°C for 15 minutes. Dispense as desired into sterile containers.

PHOSPHATE BUFFERED SALINE (PBS)

Anhydrous Na_2HPO_4	12.0 g
$NaH_2PO_4.H_2O$	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.

0.15 M PHOSPHATE BUFFERED SALINE at pH 7.2 (PBS)

"Acid" solution

Anhydrous Na_2HPO_4	10.36 g
NaCl	4.38g
MS water	1.0 L

"Base" solution

$NaH_2PO_4.H_2O$	10.65 g
NaCl	4.38 g
MS water	1.0 L

Prepare 'acid' and 'base' solutions by added ingredients to MS 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_2PO_4) in about 800 ml of MS water. Check the pH of the solution. Adjust, if necessary, by the dropwise addition 0.1 N HCl or NaOH. Dilute to l liter. Autoclave for 15 minutes at 121°C.

0.1 M phosphate buffer, pH 6.0 (± 0.1)

Potassium dihydrogen phosphate (KH_2PO_4)	11.2 g
Dipotassium hydrogen phosphate (K_2HPO_4)	2.8 g

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

0.1 M phosphate buffer, pH 8.0 (± 0.1)

Potassium dihydrogen phosphate (KH_2PO_4)	0.523 g
Dipotassium hydrogen phosphate (K_2HPO_4)	16.73 g

Dissolve in about 800 ml of MS 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_2PO_4)	1.046 g
Dipotassium hydrogen phosphate (K_2HPO_4)	33.46 g

Dissolve in about 800 ml of MS 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 121ºC 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 121ºC 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 115ºC for 15 minutes.

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.

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