

2.3 Reagents, Media, and Media Preparation

A. Growth Media

Most of these media are commercially available as pre-made formulas or as bases, which can be easily made in the laboratory. These commercial products are entirely acceptable and should be made and stored according to the manufacturer's recommendations.

1. Brain Heart Infusion Agar (BHIA) and Tryptic Soy Agar (TSA)

These two basic agars are interchangeable for bacterial cultures obtained during an inspection. They are both commercially available.

2. Selective Kidney Disease Medium-2 (SKDM-2) (Austin, et.al.1983)

Used for the selective isolation of *Renibacterium salmoninarum*.

Peptone	10 g
Yeast extract	0.5 g
L-Cysteine HCL	1 g
Agar	15 g
Distilled water	to 1000 mL

Adjust pH to 6.5 before adding agar. Autoclave for 15 minutes at 121°C. Cool to ~ 50°C and add:

Fetal Bovine Serum	200.0 mL
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The following antibiotics can also be added to the SKDM-2 to reduce overgrowth from other bacterial organisms (Austin et. al. 1983).

4.0 mL Cyclohexamide (1.2 g Cyclohexamide in 96 mL dH ₂ O)
1.0 mL D-Cycloserine (0.3 g D-Cycloserine in 24 mL of dH ₂ O)
2.0 mL Polymyxin B-sulfate (0.3 g Polymyxin B-sulfate in 24 mL of distilled H ₂ O)
1.0 mL Oxolinic Acid (0.06 g Oxolinic Acid in 24 mL of 5% NaOH)

B. Media Preparation

1. Plate Media

- a. Prepare media in stainless steel beakers or clean glassware according to manufacturer instructions. Check pH and adjust if necessary. Media must be boiled for one minute to completely suspend agar. Use of a stir bar will facilitate mixing of agar.

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- b. Cover beaker with foil or pour into clean bottles being sure to leave lids loose. Sterilize according to manufacturer's instructions when given or at 121°C for 15 minutes at 15 pounds pressure.
- c. Cool media to 50°C.
- d. Alternatively, media can be autoclaved and cooled to room temperature and refrigerated for later use. Store bottles labeled with media type, date, and initials. When media is needed, boil, microwave, or use a water bath to completely melt the agar. Cool to 50°C. Avoid reheating media multiple times before use.
- e. Before pouring media, disinfect hood or counter thoroughly and place sterile petri dishes on the disinfected surface. Aseptically mix any antibiotic solutions, sheep's blood, or Fetal Bovine Serum into the media at this temperature.
- f. Label the bottom of each plate with medium type and date prepared.
- g. Remove bottle cap and pour plates or dispense with a sterile Cornwall pipette, lifting each petri dish lid as you go. Pour approximately 15 to 20 mL per petri dish. Replace lids as soon as the plate is poured.
- h. Immediately wash medium bottle, cap, and pipette in hot water to remove agar and clean up any spilled agar.
- i. Invert plates when the media has cooled completely (~ 30 to 60 minutes) to prevent excessive moisture and subsequent condensation on the plate lid.
- j. Allow plates to sit overnight at room temperature. Store plates upside down in the refrigerator in a tightly sealed plastic bag or plate storage tin.
- k. Follow manufacturer's recommendation for storage period of prepared media. Each batch should be labeled with date of preparation and/or an expiration date.

2. Tube Media

- a. Prepare media in stainless steel beakers or clean glassware according to manufacturer's instructions. If the medium contains agar, boil for one minute to completely suspend the agar. Use of a stir bar will facilitate mixing of agar.
- b. Media with indicators must be pH adjusted. This can be done when medium is at room temperature; otherwise, compensation for temperature needs to be made.
- c. Arrange test tubes in racks. Disposable screw cap tubes can be used for all tube media.
- d. Use an automatic pipetter or Pipette-aid™ to dispense the medium. If using the Brewer or Cornwall pipette, prime with deionized water and then pump the water out of the syringe prior to pipetting. Discard the first few tubes of media that are dispensed. Dispense approximately 5 to 10 mL media in 16 x 125mm or 20 x 125mm tubes. Close caps loosely.
- e. Immediately after use, rinse the automatic pipetter in hot tap water followed by distilled water to remove all media and prevent clogging of the instrument.

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- f. Follow manufacturer's recommendation for autoclave time and temperature.
- g. If making slants, put tubes in slant racks after autoclaving. Adjust the slant angle to achieve the desired slant angle and butt length (i.e. long butt and short slant for TSI or a standard slant over $\frac{3}{4}$ of the tube length for TSA or BHIA).
- h. Cool completely to room temperature in the slanted position. Tighten caps.
- i. Label the tubes or the tube rack with type of medium and date made.
- j. Store at 2 to 8°C, following manufacturer's recommendation for long-term storage.

C. Reagents

1. 70% Ethanol (EtOH)

Ethanol (95%)	737 mL
Distilled water	to 1000 mL

2. Hanks Balanced Salt Solution (HBSS)

10X HBSS	100.0 mL
Cell culture grade water	895.0 mL
NaHCO ₃ (7.5%)	5.0 mL

If antibiotics are used, subtract 320 mL of water and add in its place:

Penicillin/Streptomycin (16%)	160.0 mL
Penicillin G (10,000 units/mL)	
Streptomycin sulfate (10,000 ug/mL)	
Fungizone	160.0 mL
250 ug/mL Amphotericin B	
205 ug/mL Desoxycholate	

Mix. Filter with 0.22 um filter. Store at 4°C.

3. 10% Neutral Buffered Formalin (10% NBF)

Formalin (37% formaldehyde)	100 mL
Distilled water	900 mL
Sodium phosphate (monobasic)	4 g
Sodium phosphate (dibasic)	6.5 g

Store at room temperature.

4. Davidson's Fixative

95% Ethanol	600 mL
Formalin (37% formaldehyde)	400 mL
Acetic acid (glacial)	200 mL
Distilled water	600 mL

Store at room temperature.