Chapter 5

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Appendix

a) Preparation of Microbial Media:

Brilliant Green Agar (BGA); (Difco, Sparks,MD) with Naladixic Acid (Sigma, St LouisMO)

- *Prepare the BGA* by combining the correct amount of media concentrate and de-ionized water as indicated on the bottle.
- Bring to the boil to dissolve thoroughly.
- Pour into suitable glass bottles and autoclave at 121°C for 20 minutes.
- Allow to cool to 50°C.
- Add Naladixic Acid Stock Solution at an inclusion of 3ml per liter of media.
- Pour the plates in a sterile hood and allow to set, protected from light.

- *Prepare the Naladixic Acid Stock Solution* by combining 100ml 0.05M NaOH (Sigma, St Louis MO) and 1g Naladixic Acid.
- Leave on stirring plate for 30min to dissolve completely, covered with aluminum foil.
- Pour into a centrifuge tube using a sterile filter.
- Store in cold room.

Xylose Lysine Deoxycholate (XLD) (Difco, Sparks,MD)

- Combine the correct amount of media concentrate powder and de-ionized water as indicated on the bottle
- Bring media to the boil and allow to boil for 1 minute
- Pour into sterile glass bottle, using a sterile glass funnel.
- Allow to cool to 55°C and pour plates.

Triple Sugar Agar (TSI) (Difco, Sparks,MD)

- Combine the correct amount of media concentrate powder and de-ionized water as indicated on the bottle.
- Bring media to the boil.
- Distribute 12ml each into test tubes.
- Autoclave at 121°C for 20 minutes.
- Allow to cool at an angle, forming a deep butt.

- Place in cold room as soon as it has set.

0.1% Peptone water

- Per liter of de-ionized water, add 10g peptone (Difco, Sparks,MD) and 5g NaCl (Sigma, St Louis MO)
- Stir for 15 minutes on cold plate
- Autoclave at 121°C for 20 minutes
- Pour 80ml each into bladed jars
- Store in cold room

Maximum Recovery Diluent (MRD)

- Combine 8.5g NaCl (Sigma, St Louis MO) and 1g peptone (Difco, Sparks,MD) with 1L de-ionized water.
- Stir for 15 minutes on cold plate.
- Pipette out into test tubes, 9ml per tube.
- Autoclave at 121°C for 20 minutes.
- Store in cold room.

Rappaport – Vassiliadis R10 Broth (Difco, Sparks,MD)

- Combine the correct amount of media concentrate powder and de-ionized water as indicated on the bottle.
- Heat gently to dissolve
- Pipette out into test tubes, 9ml per tube.
- Autoclave at 116°C for 15 minutes
- Allow to cool, and then place in the cold room.

Lactose broth (Difco, Sparks,MD)

- Combine the correct amount of media concentrate powder and de-ionized water as indicated on the bottle.
- Heat gently without boiling.
- When dissolved, pipette out into test tubes, 9ml each.
- Autoclave at 121°C for 20 minutes.
- Allow to cool, and then place in the cold room.

Violet Red Bile Agar (Difco, Sparks,MD)

- Combine the correct amount of media concentrate powder and de-ionized water as indicated on the bottle
- Bring media to the boil and allow to boil for 1 minute
- Pour into sterile glass bottle, using a sterile glass funnel.
- Allow to cool to 55°C and pour plates.

Violet Red Bile Agar (Difco, Sparks,MD) with 30ppm tetracycline(Sigma, St Louis MO)

Prepare tetracycline stock solution:
Add 100mg tetracycline to 10ml deionised water
Stir on stirring plate until dissolved
Filter sterilize (0.22µ Fisherbrand filters) into a 15ml sterile centrifuge tube.

Add stock solution at an inclusion of 3ml per liter VRBA.

b) Preparation of staining solutions

Alcian blue solution:

Make up a 3% acetic acid solution from Acetic Acid Glacial (Fisher Scientific, Fair Lawn, NJ).
Combine: 50ml 3% Acetic Acid
0.5g Alcian Blue 8GX (Sigma-Aldrich, St Louis, MO)

Periodic Acid Solution:

Combine: 0.5g Periodic Acid (Sigma-Aldrich, St Louis, MO)
50ml De-ionised water