PREPARATION OF VIRAL TRANSPORT MEDIUM AND CELL CULTURE MEDIA
Version 1.0

PRINCIPLE: Transport media are used for transport of viral culture samples. They contain proteins to stabilize viruses, buffers to control pH, antibiotics to keep contaminating bacteria and fungi from overgrowing the viruses, and a color pH indicator. Many types of transport media are available commercially. However, suitable transport media can be prepared in-house. The base of one transport medium is Hanks Balanced Salt Solution, which can be purchased in liquid form. Fetal bovine serum and antibiotics are added. The medium is then dispensed into tubes to be used when viral culture specimens are collected. The same instructions for addition of fetal bovine serum and antibiotics can be followed for enriching media for use as cell culture media in the virology laboratory. For cell culture media, Minimum Essential Medium Eagle with Earles Balanced Salt Solution with L-glutamine (or other suitable medium) is used rather than Hanks Balanced Salt Solution.

MATERIALS:

Equipment: individually-wrapped sterile pipettes (10 and 25 ml), sterile 15 ml screw-capped tubes, filters, labels

Reagents: Hanks Balanced Salt Solution (HBSS) 500 ml bottle, Minimum Essential Medium Eagle with Earles Balanced Salt Solution with L-glutamine (EMEM) 500 ml bottle, sterile heat-inactivated fetal bovine serum (FBS), gentamicin sulfate (50 mg/ml), amphotericin B (250 µg/ml) (Fungizone), sheep blood agar plate

PROCEDURE: Preparing Ingredients for Transport Medium and Cell Culture Media

1. Thaw a 500 ml bottle of fetal bovine serum (FBS). Filter sterilize FBS using a .45 µm/500 ml filter unit. Store at 4° C.

2. Thaw a 50 ml bottle of amphotericin B. Add 50 ml gentamicin to the amphotericin B. Filter sterilize this mixture using a .45 µm/150 ml filter unit. Store at 4° C.

PROCEDURE: Preparing Viral Transport Medium

1. Remove plastic seal and loosen lid on one 500 ml bottle of Hanks Balanced Salt Solution.

2. Using a sterile, individually-wrapped pipette, add 10 ml of filter sterilized FBS. Using a sterile, individually-wrapped pipette, add 2 ml of the gentamicin/amphotericin B mixture. The final concentrations of the antibiotics are 100 µg/ml for gentamicin and 0.5 µg/ml for amphotericin B. Cap the bottle securely and mix by inverting the bottle.

3. Label the bottle with the date, additives, and expiration date as follows:

   2% FBS
   100 µg/ml Gentamicin
   0.5 µg/ml Fungizone
   Date: (Insert current date)
   Expires: 1 month from date in use

   This medium is usually dispensed the same day or shortly after preparation–as described in step #4.

4. Aliquot 3 ml of medium into individual sterile screw-capped tubes. Keep lids tightly closed after medium is dispensed.

5. Label each tube with the following information:

   VIRAL TRANSPORT MEDIUM
   For transport of specimens only*
   Not to be taken internally*
   Store at 4° C. DO NOT FREEZE
   Ingredients: Hanks balanced salt solution, fetal bovine serum, gentamicin, amphotericin B
   EXPIRES: (insert current date + 1 year)

6. Store tubes and any medium remaining in the bottle at 2-8°C.

PROCEDURE: Preparing Cell Culture Media for use with Commercially Prepared Cell Cultures.

NOTE: Do not use this medium for cell passages.

1. Remove plastic seals and loosen lids on twelve 500 ml bottles of Minimum Essential Medium Eagle with Earles BSS with L-glutamine (EMEM).

2. Using a sterile, individually-wrapped pipette, add 10 ml of filter sterilized FBS to each bottle of medium. Using a sterile, individually-wrapped pipette, add 2 ml of the gentamicin/amphotericin B mixture to each bottle of medium. The final concentrations of the antibiotics are 100 µg/ml for gentamicin and 0.5 µg/ml for amphotericin B. Cap the bottles securely and mix by inverting the bottles.

3. Label the bottle with the date, additives, and expiration date as follows:

   2% FBS
   100 µg/ml Gentamicin
   0.5 µg/ml Fungizone
   Date: (Insert current date)
   Expires: 1 month from date in use

4. Perform the sterility check as follows: obtain a sheep blood agar plate from the Microbiology specimen receiving/processing area. Using a sterile, individually-wrapped, 1 ml pipette,

withdraw 1 ml of medium from the bottle that was just prepared and apply it to the surface of the sheep blood agar plate. Incubate the plate for 48 hours at 37°C, checking each day for growth. Record the lot information from the bottle of medium in the Quality Control notebook and record the results of the sterility check (i.e. growth or no growth). If bacterial growth should be encountered, take appropriate follow-up actions to remove the bottle of medium from service and discard it.

5. Store medium at 2-8°C.

REFERENCES:

1. Leland, D.S. 1992. Concepts of clinical diagnostic virology, p. 3-43, In E.H. Lennette (ed.), Laboratory Diagnosis of Viral Infections, Second Edition. Marcel Dekker, Inc., New York.