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## PREPARATION OF VIRAL TRANSPORT MEDIUM

### 1.0 Purpose

The purpose of this procedure is to describe the process for producing viral transport medium (VTM) for transport of specimens.

### 2.0 Scope

This document applies to the Centers for Disease Control and Prevention (CDC) Coronavirus outbreak response.

### 3.0 Responsibility

3.1 It is the responsibility of personnel preparing viral transport medium in response to the CDC Coronavirus outbreak to follow this procedure accurately.

### 4.0 Definitions

N/A

### 5.0 References

- 5.1 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.
- 5.2 Johnson, F.B. 1990. Transport of Viral Specimens. Clinical Microbiology Reviews 3(2):120-131.
- 5.3 Biosafety in Microbiological and Biomedical Laboratories (BMBL), current edition

### 6.0 Equipment/Materials

- 6.1 Laminar flow hood or Biosafety Cabinet (workspace capable of maintaining a clean environment)
- 6.2 Thermometer
- 6.3 Water bath set for 56.0°C +/- 1.0°C (For heat inactivation of FBS)
- 6.4 Individually sterile wrapped pipets (10 and 25 mL)
- 6.5 Pipet aid or pipet bulb
- 6.6 Sterile 15mL centrifuge tubes or equivalent
- 6.7 0.20 to 0.45 micron filter assembly
- 6.8 Labels

#### *Reagents*

- 6.9 Fetal Bovine Serum (FBS)
- 6.10 Hanks Balanced Salt Solution (HBSS) 1X with calcium and magnesium ions, no phenol red, 500mL bottle
- 6.11 Sterile, heat-inactivated fetal bovine serum (FBS)
- 6.12 Gentamicin sulfate (50mg/mL)

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- 6.13 Amphotericin B (250ug/mL) (Fungizone)
- 6.14 Sheep blood agar plate or equivalent quality control plate
- 6.15 Disinfectant (such as 70% ethanol)

***Note: HBSS, FBS, Gentamicin, and Amphotericin B can be purchased as sterile solutions. The filtration steps in this procedure can be omitted provided each of these components are manipulated using aseptic techniques and sterility is maintained for each component.***

## 7.0 Safety Precautions

- 7.1 Follow standard biological or clinical laboratory practices.

## 8.0 Procedure

### ***Preparation of Ingredients for Transport Medium***

#### *FBS Inactivation*

- 8.1 Thaw a 500 mL bottle of fetal bovine serum (FBS). Heat inactivate the FBS at 56°C for 30 minutes in a 56.0°C +/- 1.0°C water bath. Record lot information and preparation in laboratory-controlled notebook.

#### *Antibiotic Preparation*

- 8.2 Thaw 50mL of Amphotericin B.
- 8.3 Add 50mL of Gentamicin to the Amphotericin B.
- 8.4 Filter sterilize this antibiotic mixture using a 0.20 to 0.45µm/150mL filter unit. Store at 2-8°C.
- 8.5 Record lot information and preparation in laboratory-controlled notebook.

### ***Preparation of Viral Transport Medium***

- 8.6 Disinfect work surface with disinfectant.
- 8.7 Remove plastic seal and loosen lid on one 500 mL bottle of Hanks Balanced Salt Solution (HBSS).
- 8.8 Disinfect reagent bottles with disinfectant.
- 8.9 Using a sterile, individually wrapped pipette, add 10 mL of the heat inactivated FBS to the bottle of HBSS.
- 8.10 Using a sterile, individually wrapped pipette, add 2 mL of the gentamicin/amphotericin B mixture from the *Antibiotic Preparation* step to the bottle of HBSS. This results in final concentrations of 100 µg/mL for gentamicin and 0.5 µg/mL for amphotericin B.
- 8.11 Cap the bottle securely and mix by inverting the bottle.
- 8.12 Withdraw 1mL of medium for QC sample. Refer to ***Sterility Check*** section below.

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- 8.13 Label the bottle with the date, additives, and expiration date as follows:  
2%FBS  
100µg /mL Gentamicin  
0.5 µg /mL Fungizone  
Date: (Insert current date)  
Expires: (1 year after manufacture date)  
**Note: This medium is usually dispensed the same day or shortly after preparation.**
- 8.14 Store at 2-8°C until dispensed.
- 8.15 Aliquot 3 mL of medium into individual sterile 15mL screw-capped tubes. Keep lids tightly closed after medium is dispensed.
- 8.16 Label each tube with the following information:  
VIRAL TRANSPORT MEDIUM  
\*\* For transport of specimens only\*\*  
\*\*Not to be taken internally\*\*  
Store at 2-8°C. DO NOT FREEZE  
Ingredients: Hanks balanced salt solution, fetal bovine serum, gentamicin, amphotericin B  
Expires: (1 year after the manufacture date)
- 8.17 Store tubes and any medium remaining in the bottle at 2-8 °C.

***Sterility Check***

- 8.18 Perform the sterility check as follows:
- 8.18.1 Obtain a sheep blood agar plate or equivalent quality control plate.
- 8.18.2 Using a sterile, individually wrapped, 1 mL pipette, withdraw 1mL of medium as described in step 8.12 and apply it to the surface of the sheep blood agar plate or equivalent quality control plate.
- 8.18.3 Incubate the plate for 48 hours at 37°C ±2C. Check daily for growth.
- 8.18.4 Record results of sterility check (growth or no growth) and lot specific information in laboratory-controlled documentation. If bacterial growth should be encountered, take appropriate follow-up actions to remove the bottle of medium from service and dispose of the medium as appropriate.
- 8.18.5 Store medium at 2-8°C.

**9.0 Attachments**

Attachment #1: Viral Transport Medium Recipe (1 Page)

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10.0 Revision History

Revision Level	Document Section	Changes Made to Document Section
01	Equipment/Materials Procedure	<b>6.9</b> Added Fetal Bovine Serum <b>8.1</b> Removed duplicate step <b>8.4</b> Updated for clarification <b>8.18.2</b> Updated reference to step 8.12
	Attachment #1	<b>Reagents 5.</b> FBS added
00	New	New Document

11.0 Approval Signatures

3/19/2020

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**Viral Transport Medium Recipe**

**Reagents**

1. Hanks Balanced Salt Solution (HBSS) 1X with calcium and magnesium ions, no phenol red, 500mL bottle
2. Sterile, heat-inactivated fetal bovine serum (FBS)
3. Gentamicin sulfate (50mg/mL)
4. Amphotericin B (250ug/mL) (Fungizone)
5. Fetal Bovine Serum (FBS)
6. Sheep blood agar plate or equivalent quality control plate

**Procedure**

1. Heat inactivate a 500 ml bottle of fetal bovine serum (FBS) for 30 minutes in a 56.0C +/- 1.0°C water bath.
2. Thaw 50mL of amphotericin B, add 50mL of gentamicin, then filter sterilize through a 0.20 to 0.45 µm filter unit (150 ml filter unit)
3. Add 10 ml of the FBS to one 500 ml bottle of Hanks Balanced Salt Solution (HBSS)
4. Add 2 ml of the gentamicin/amphotericin B mixture to the HBSS w/FBS
5. Securely cap the bottle securely and mix by inverting the bottle.
6. Label the bottle with the date, additives, and expiration date as follows:
7. 2%FBS
  - 100 µg /mL Gentamicin
  - 0.5 µg /mL Fungizone
  - Date: (Insert current date)
  - Expires: (1 year after manufacture date)
8. Aliquot 3 mL of medium into individual sterile 15mL screw-capped tubes. Keep lids tightly closed after medium is dispensed.
9. Label each tube with the following information:
  - VIRAL TRANSPORT MEDIUM
  - \*\* For transport of specimens only\*\*
  - \*\*Not to be taken internally\*\*
  - Store at 2-8° C. DO NOT FREEZE
  - Ingredients: Hanks balanced salt solution, fetal bovine serum, gentamicin, amphotericin B
  - Expires: (1 year after manufacture date)
10. Store at 2-8 °C.