
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

4.1 **DOM** – Date of Manufacture

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
- 5.4 CLSI Standard, M40-A2; *Quality Control of Microbiological Transport Systems; Approved Standard-Second Edition*

6.0 Equipment/Materials

- 6.1 Laminar flow hood or Biosafety Cabinet (workspace capable of maintaining a clean environment)
- 6.2 Thermometer (for heat inactivation of FBS)
- 6.3 Water bath: 56.0°C +/- 1.0°C (for heat inactivation of FBS, if not purchased as heat-inactivated)
- 6.4 Individual, sterile wrapped pipettes, such as 10 and 25 mL
- 6.5 Pipette aid or pipette bulb
- 6.6 Pipettor, 1mL or 100µL
- 6.7 Sterile conical tubes, such as 16x100mm, or equivalent
- 6.8 0.20µm to 0.45µm filter assembly
- 6.9 Cell spreader or equivalent
- 6.10 Labels

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Reagents

- 6.11 Hanks Balanced Salt Solution (HBSS) 1X with calcium and magnesium ions, no phenol red, 500mL bottle
- 6.12 Sterile, heat-inactivated fetal bovine serum (FBS)
- 6.13 Gentamicin sulfate (50mg/mL)
- 6.14 Amphotericin B (250µg/mL) (Fungizone)
- 6.15 Blood agar plate or equivalent
- 6.16 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

Note: Refer to Attachment #2 for a one-page summary recipe example.

Preparation of Ingredients for Transport Medium

FBS Inactivation, if not purchased as heat-inactivated

- 8.1 Thaw a 500mL 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 a laboratory-controlled notebook.

Antibiotic Preparation

Note: Perform in Laminar flow hood or Biosafety Cabinet.

- 8.2 Prepare volume of antibiotics needed for bulk production to obtain final concentrations in medium of 100µg/mL for Gentamicin and 0.5µg/mL for Amphotericin B.
- 8.3 For example, to prepare a 100mL solution:
 - 8.3.1 Thaw 50mL of Amphotericin B.
 - 8.3.2 Add 50mL of Gentamicin to 50mL of Amphotericin B. Use a secondary container, if needed, for combining Amphotericin B and Gentamicin.
- 8.4 Filter sterilize this antibiotic mixture using a 0.20 to 0.45µm/150mL filter unit.
- 8.5 Record lot information and preparation in a laboratory-controlled notebook.

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Preparation of Viral Transport Medium

Note: Perform in Laminar flow hood or Biosafety Cabinet.

- 8.6 Clean work surface with appropriate disinfectant.
- 8.7 Disinfect reagent bottles prior to placing on work surface.
- 8.8 For example, to prepare a bulk solution of Viral Transport Medium:
 - 8.8.1 Remove plastic seal and loosen lid on a 500mL bottle of Hanks Balanced Salt Solution (HBSS).
 - 8.8.2 Using a sterile pipette, aseptically add 10mL of the inactivated FBS to the bottle of HBSS.
 - 8.8.3 Using a sterile pipette, aseptically add 2mL of the Gentamicin/Amphotericin B mixture from the *Antibiotic Preparation* step to the bottle of HBSS. This results in final concentrations of 100µg/mL for Gentamicin and 0.5µg/mL for Amphotericin B.
- 8.9 Record lot information and preparation in a laboratory-controlled notebook.
- 8.10 Assign laboratory appropriate identification (e.g. lot number).
- 8.11 Cap the bottle securely and mix thoroughly by inverting the bottle.
- 8.12 Withdraw 100µL of medium for QC sample. Refer to ***Sterility Check and QC*** section below.
- 8.13 Label the bottle, see example below:

VIRAL TRANSPORT MEDIUM
2%FBS
100µg /mL Gentamicin
0.5 µg /mL Amphotericin B
Lab ID: (Insert laboratory appropriate identification)
DOM: (Insert current date)
Expires: (Insert date 1 year after manufacture date)
- 8.14 Store at 2-8°C until dispensed into aliquots.

Note: This medium is usually dispensed the same day or shortly after preparation.
- 8.15 Aliquot 3mL of medium into individual sterile conical screw-capped tubes (such as a 16x100mm tubes). Keep lids tightly closed after medium is dispensed.
- 8.16 Label each tube, see example below:

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
Lab ID: (Insert laboratory appropriate identification)
Expires: (Insert date 1 year after the manufacture date)

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- 8.17 Perform sterility check as described *Sterility Check B: Final Product, Tubes* section below.
- 8.18 Store tubes and any medium remaining in the bottle at 2-8°C.

Sterility Checks and QC

- 8.19 Perform the sterility checks as follows:

Sterility Check A: Bulk product

- 8.19.1 Obtain a blood agar plate or equivalent.
- 8.19.2 Using a sterile pipette, aseptically withdraw 100µL of medium (as described in step 8.12) and apply it to the surface of the sheep blood agar plate or equivalent.
- 8.19.3 Using a cell spreader, aseptically spread the media sample across the plate.
- 8.19.4 Incubate the plate for 48 hours at 37°C ±2°C. Check daily for growth.
- 8.19.5 Record results of sterility check (growth or no growth) and lot specific information in laboratory-controlled documentation. If growth should be encountered, take appropriate follow-up actions to remove the bottle of medium from service and dispose of the medium as appropriate.

Sterility Check B: Final Product, Tubes

- 8.19.6 Refer to *Attachment #1* to determine appropriate number of tubes necessary to incubate overnight at 37°C ±2°C.
 - 8.19.7 Examine tubes the following day for growth of contaminants.
 - 8.19.8 Record results of sterility check (growth or no growth) and lot specific information in laboratory-controlled documentation. If growth should be encountered, take appropriate follow-up actions to remove the specific batch of tubes from service and dispose of them as appropriate.
- 8.20 Refer to CLSI standard M40-A2 for quality control procedures to assess media for viral recovery integrity, if required by laboratory Quality systems.

9.0 Attachments

Attachment #1: Sampling Table (1 Page)

Attachment #2: Viral Transport Medium Recipe Example (1 Page)

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10.0 Revision History

Revision Level	Document Section	Changes Made to Document Section
02	<p>Definitions References Equipment/ Material</p> <p>Procedure</p> <p>Attachment #1</p> <p>Attachment #2</p>	<p>4.1 DOM added 5.4 CLSI standard 6.6 Pipettor, 1mL or 100µL 6.7 Conical tubes such as 16x100mm updated 6.9 Cell spreader or equivalent 6.15 Sheep removed 8.2 Note added 8.3 Prep example for 100mL 8.4 Storage temperature removed 8.7 Moved up 8.8 Prep example for bulk 8.9 Documentation requirement added 8.10 Assign Lab ID added 8.12 Changed volume from 1mL to 100µL 8.13 Added VTM title and Lab ID to label 8.16 Added Lab ID to label 8.17 Sterility check added 8.19.2 Changed volume from 1mL to 100µL 8.19.3 Added step 8.19.6-8.19.8 Added second sterility check for final tubes 8.20 Added step Added Sampling table referenced from USP 71 Removed QC plate from “Reagents” Minor edits to align with changes in procedure section of SOP</p>
01	<p>Equipment/ Materials Procedure</p> <p>Attachment #1</p>	<p>6.9 Added Fetal Bovine Serum 8.1 Removed duplicate step 8.4 Updated for clarification 8.18.2 Updated reference to step 8.12 Reagents 5. FBS added</p>
00	New	New Document

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11.0 Approval Signatures

4/15/2020

X Signature on File

DSR Acting Director

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Signed by: PIV

4/15/2020

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Technical Reviewer

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4/15/2020

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DSR Quality Manager

Quality Manager/designee

Signed by: PIV

X Effective Date: 04/15/2020

Signed by: PIV

Final Product Sampling Table

Number of Containers in the Batch	Minimum Number of Containers to be Tested for each Batch
≤ 100 Containers	10% or 4 Items, whichever is greater
101 through < 500 Containers	10 Containers
≥ 500 Containers	2% or 20 Containers, whichever is less

Viral Transport Medium Recipe Example

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 (250µg/mL) (Fungizone)

Procedure

1. Heat inactivate a 500mL bottle of fetal bovine serum (FBS) for 30 minutes in a 56.0°C +/- 1.0°C water bath (or use commercially inactivated FBS).
2. Thaw 50mL of amphotericin B, add 50mL of gentamicin, then filter sterilize through a 0.20 to 0.45µm filter unit (150mL filter unit).
3. Add 10mL of the FBS to one 500mL bottle of Hanks Balanced Salt Solution (HBSS).
4. Add 2mL of the Gentamicin/Amphotericin B mixture to the HBSS with FBS.
5. Securely cap the bottle and mix by inverting the bottle.
6. Label the bottle with the date of production, additives, and expiration date as follows:
VIRAL TRANSPORT MEDIUM
2%FBS
100 µg /mL Gentamicin
0.5 µg /mL Fungizone
Lab ID: (Insert laboratory appropriate identification)
DOM: (Insert current date)
Expires: (Insert Date 1 year after manufacture date)
7. Aliquot 3mL of medium into individual sterile conical screw-capped tubes (such as 16x100mm tubes). Keep lids tightly closed after medium is dispensed.
8. 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
Lab ID: (Insert laboratory appropriate identification)
Expires: (Insert Date 1 year after manufacture date)
9. Store at 2-8°C.