PREPARATION OF VIRAL TRANSPORT MEDIUM

This SOP was adapted from the Indiana University (IU) Health Clinical Virology Laboratory (Indianapolis, IN) protocol entitled *Preparation of Viral Transport Medium and Cell Culture Media,* which was developed by Mr. Fred Joyce (dec.) and shared with the clinical microbiology and public health laboratory communities via private listserv on March 13, 2020 by Dr. Ryan F. Relich, IU Health Clinical Virology Laboratory Director, and laboratory staff.

1.0 Purpose

To provide a standard operating procedure (SOP) for producing viral transport medium (VTM) for specimens for viral culture or other means of viral detection. This SOP provides an alternative to commercially available products. There are many acceptable formulations of this medium that may be suitable for the unique conditions of individual laboratories. The specific needs of the shipping and receiving laboratories should be considered when choosing a VTM formulation.

2.0 Scope

This document applies to VTM produced in support of the Centers for Disease Control and Prevention (CDC) Coronavirus Disease 2019 (COVID-19) outbreak response.

3.0 Responsibility

It is the responsibility of personnel preparing VTM for CDC's COVID-19 outbreak response to follow this SOP accurately. If there are variations from this SOP, the variations should be documented, and data generated to demonstrate equivalent performance of the VTM.

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*

5.5 Smith, K.P. 2020. *Large-Scale, In-House Production of Viral Transport Media to Support SARS-CoV2 PCR Testing in a Multihospital Health Care Network during the COVID-19 Pandemic*. Journal of Clinical Microbiology, volume 58, issue 8

5.6 Joyce, Fred. *Preparation of Viral Transport Medium and Cell Culture Media.* Indiana University (IU) Health Clinical Virology Laboratory

6.0 Equipment/Materials

6.1 Laminar flow hood or biosafety cabinet (workspace capable of maintaining an aseptic environment)
6.2 Calibrated thermometer (for heat inactivation of Fetal Bovine Serum [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 unit
6.9 Cell spreader or equivalent
6.10 Labels

Reagents

6.11 Sterile Hanks Balanced Salt Solution (HBSS) 1X with calcium and magnesium ions, no phenol red, 500mL bottle (or HBSS containing phenol red as a pH indicator)
6.12 Sterile, heat-inactivated fetal bovine serum (FBS)
6.13 Gentamicin sulfate (50mg/mL) (or similar antibiotic at an appropriate concentration to prevent bacterial contamination and growth)
6.14 Amphotericin B (250µg/mL) (Fungizone) (or similar antifungal at an appropriate concentration to prevent fungal contamination and growth)
6.15 Blood agar plate
6.16 Disinfectant, such as 70% ethanol

Note: Gentamicin, and Amphotericin B can be purchased as sterile solutions. The filtration steps in this procedure can be omitted if only sterile solutions are used, provided each of these components is 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 formulation 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), follow manufacturer's recommendations for thawing and storage.

8.2 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.

8.3 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.

8.4 For example, to prepare a 100mL solution:

- 8.4.1 Thaw 50mL of Amphotericin B, follow manufacturer's recommendations for thawing and storage.
- 8.4.2 Add 50mL of Gentamicin to 50mL of Amphotericin B. Use a secondary container, if needed, for combining Amphotericin B and Gentamicin.

8.5 Filter sterilize this antibiotic mixture using a 0.20 to 0.45µm/150mL filter unit.

8.6 Record lot information and preparation in a laboratory-controlled notebook.

Preparation of Viral Transport Medium

Note: Perform in laminar flow hood or biosafety cabinet.

8.7 Clean work surface with appropriate disinfectant.

8.8 Disinfect reagent bottles prior to placing on work surface.

8.9 For example, to prepare a bulk solution of viral transport medium:

- 8.9.1 Remove plastic seal and loosen lid on a 500mL bottle of sterile Hanks Balanced Salt Solution (HBSS).
- 8.9.2 Using a sterile pipette, aseptically add 10mL of the inactivated FBS to the bottle of HBSS.
- 8.9.3 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.

8.10 Record lot information and preparation in a laboratory-controlled notebook.

8.11 Assign laboratory appropriate identification (e.g. lot number).

8.12 Cap the bottle securely and mix thoroughly by inverting the bottle.

8.13 Withdraw 100µL of medium for QC sample. Refer to ***Sterility Check and QC:*** *Sterility Check A: Bulk Product* section below.

8.14 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 of manufacture)
Expires: (Insert date 1 year after manufacture date)
Store at 2-8°C

8.15 Store at 2-8°C until dispensed into aliquots.

8.16 Aliquot 3mL of prepared VTM into individual sterile conical screw-capped tubes (such as a 16x100mm tubes). Keep lids tightly closed after medium is dispensed.

8.17 Label each tube, see *example* below:

VIRAL TRANSPORT MEDIUM
** For transport of specimens only**
Not to be taken internally
Store at 2-8°C (or temperature determined by specific data generated in stability study by manufacturing laboratory).
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)

Note$_1$: Storage temperature and Expiration date need to reflect the specific information gathered from the manufacturing laboratory if varying from this protocol.

Note$_2$: Ingredient list is optional on label if traceability exists from Lab Identification number to manufacturing records.

8.18 Perform sterility check as described in *Sterility Check B: Final Product, Tubes* section below.

8.19 Store tubes and any medium remaining in the bottle at 2-8°C (or temperature determined by specific data generated in stability study by manufacturing laboratory).

Sterility Check and QC

8.20 Perform the sterility checks as follows:

Sterility Check A: Bulk Product

8.20.1 Obtain a blood agar plate or equivalent.

8.20.2 Using a sterile pipette, aseptically withdraw 100µL of medium (as described in step 8.13) and apply it to the surface of the blood agar plate or equivalent.

8.20.3 Using a cell spreader, aseptically spread the media sample across the plate.
8.20.4 Incubate the plate for 48 hours at 37°C ±2°C. Check daily for growth.
8.20.5 Record results of sterility check (growth or no growth) and lot specific information in laboratory-controlled documentation. If growth is observed, take appropriate follow-up actions to remove the bottle of medium and all aliquots from service and dispose of the medium as appropriate.

Sterility Check B: Final Product, Tubes

8.20.6 Refer to *Attachment #1* to determine appropriate number of tubes necessary to incubate overnight at 37°C ±2°C.
8.20.7 Examine tubes the following day for growth of contaminants.
8.20.8 Record results of sterility check (growth or no growth) and lot specific information in laboratory-controlled documentation. If growth is observed, take appropriate follow-up actions to remove the specific batch of tubes from service and dispose of them as appropriate.

8.21 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 Formulation Example (1 Page)

10.0 Revision History

Revision Level	Document Section	Changes Made to Document Section
05	Purpose Responsibility References Equipment/ Materials	Clarified VTM usage Added language to extend flexibility to laboratories if specific requirements are necessary and documented. **5.5** Addition of Journal of Clinical Microbiology article **5.6** Addition of original paper from Indiana University **6.1** Clarified that an aseptic environment must be used. **6.11** Included “sterile” for HBSS **Note statement**-Removed HBSS and FBS

	Procedure	**8.1 and 8.4.1** Added reference to manufacturer's instructions. **8.14** Added storage temp to label example **8.15** Removed Note. **8.16** Added $Note_1$, $Note_2$. Removed "Do Not Freeze" from label example. Noted expanded storage temperature if stability performed in the manufacturing (mfg) lab. **8.19** Noted expanded storage temperature if stability performed in mfg. lab.
	Attachment #2	Updated to reflect changes in procedure. Changed title from "Recipe" to "Formulation"

11.0 Approval Signatures

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

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Viral Transport Medium Formulation Example

Reagents

1. Hanks Balanced Salt Solution (HBSS) 1X with calcium and magnesium ions, no phenol red, sterile 500mL bottle (or HBSS containing phenol red as a pH indicator)
2. Sterile, heat-inactivated fetal bovine serum (FBS)
3. Gentamicin sulfate (50mg/mL) (or similar antibiotic at an appropriate concentration to prevent bacterial contamination and growth)
4. Amphotericin B (250µg/mL) (Fungizone) (or similar antifungal at an appropriate concentration to prevent fungal contamination and growth)

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 sterile 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 Amphotericin B
   Lab ID: (Insert laboratory appropriate identification)
   DOM: (Insert current date)
   Expires: (Insert date 1 year after manufacture date)
   Store at 2-8°C
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 (*or temperature determined by specific data generated in stability study by manufacturing laboratory*)
   Ingredients (*optional on label*): 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 (*or temperature determined by specific data generated in stability study by manufacturing laboratory*).