

**Guidelines for Specimen Collection
At Surveillance Sites
NAVAL HEALTH RESEARCH CENTER
DoD Center for Deployment Health Research
Virology Section**

I. PRINCIPLE:

Proper specimen collection is essential for good viral isolation and identification. When specimen collection is done at the proper time during the infection period, it enhances the chances of virus isolation. Appropriate virus transport media is also required to ensure virus viability.

II. MATERIALS:

A. REAGENT:

VIRAL TRANSPORT MEDIUM (VTM)

1. Purpose:

VTM is used in specimen collection for virus isolation to prevent drying, maintain viral viability between collection and inoculation, and retard bacterial and fungal growth. VTM components are Hank's Balanced Salts, Bovine Serum Albumin, Gelatin, sucrose, L-glutamic acid, Phenol Red (indicator), HEPES buffer, Vancomycin, Amphotericin B and Colistin. **Note:** None of these components will interfere with virus viability or isolation.

2. Storage:

Store unused VTM in a refrigerator with a temperature range of 2-8°C.

3. Availability:

VTM is requested through NHRC. NHRC personnel will then forward to the site upon receipt. The current brand used is Multi-Microbe Media (M4) manufactured by Micro Test, Inc. that comes with a sterile rayon swab.

B. SUPPLIES:

1. **Use only the swab provided by the transport media kit.**

2. Ice cubes contained in disposable cups should always be ready at the point where specimen collection is performed to ensure that specimens are kept cold immediately after collection.

3. Sterile tongue depressors are used to prevent saliva from contaminating swab

III. EQUIPMENT:

A. -70°C freezer to store specimens until it is sent to NHRC.

B. Refrigerator to store VTM and to keep collected specimens, if freezing at -70°C cannot be done immediately.

IV. QUALITY CONTROL PROCEDURE:

A. QUALITY CONTROL OF VIRAL TRANSPORT MEDIA (VTM)

1. Upon receipt at the site, the pH and turbidity of the transport media is evaluated and the lot number is recorded into the Viral transport Media Quality Control Worksheet I following procedures outlined in the Instructions for Quality Control of Viral Transport Media included in the packet. Records of each lot number, expiration date, date VTM opened and date VTM is discarded are kept for quality control purposes.
2. An unused tube of VTM is sent to NHRC from each lot of VTM used in each site. This is inoculated into cell lines in parallel with specimens to check for toxicity of VTM to cells, production of CPE, and hemadsorption it may cause. Upon receipt at NHRC, the site, date received, lot number and expiration date is noted and pH is measured visually (neutral, acid, alkaline.)

B. DOCUMENTATION OF TRAINING FOR SPECIMEN COLLECTION:

1. Documentation is to prove that all persons involved in the collection of throat swabs for respiratory virus isolation at NHRC have been properly trained.
2. Documentation that all SOPs pertinent to specimen collection have been read and understood by the persons involved in collection of throat swabs for respiratory virus isolation at NHRC.
3. Please refer to Virology Lab Manual Binder for the compilation cited in 1 and 2.

C. MONITORING OF EQUIPMENT PERFORMANCE AT THE SITES:

Daily temperature recording of refrigerator and -70°C freezer used to store VTM and specimens are monitored by the persons responsible for collection of samples at the sites. Please refer to the documentation of training of persons performing specimen collection at the sites. Study Coordinator maintains training records of personnel at the sites.

V. PROCEDURE:

A. PATIENT DOCUMENTATION:

1. Fill out a case report form before collection of throat swab specimen.
2. Ascertain the patient's identification by asking the patient's name and social security number as you fill this information in the case report form.
3. Record other information requested on the case report form.
4. Check the form for completeness, and then proceed to collection.

5. In cases where a case report form is not required such as samples collected during outbreaks, a requisition form is filled out, signed and dated by the requesting physician. Please refer to attached copy of requisition and procedure for filling out a case report form.

B. PREPARATIONS BEFORE COLLECTION:

1. Viral Transport Media (VTM) should always be kept refrigerated between 2° to 8° C. A disposable cup with ice cubes should be on hand at bedside or at any point of collection.
2. Before collection, check VTM to be used for color and turbidity. The medium should be clear and salmon pink.
3. Check the integrity of the swab pouch to ensure sterility.

C. SPECIMEN COLLECTION:

1. **Throat swab:**

Using aseptic technique, hold down the tongue to prevent it from contaminating the swab with saliva with a sterile tongue depressor. Vigorously rub the sterile rayon swab across the tonsils and posterior pharynx.

2. **Nasal swab**

- a. Carefully insert the swab at least 1 cm into the nares. Firmly sample the membrane by rotating the swab and leaving it in place for 10 to 15 seconds. Withdraw the swab and place in a tube of viral transport media.
- b. Cap the tube tightly, making sure that fluid will not leak if the tube is accidentally inverted. Place a barcode sticker on the vial, and hand label the tube with the barcode number (a safeguard in case the sticker falls off). Place identical barcode stickers on both the case report form and the log sheet.
- c. Double check if the name and/or the barcode sticker on the tube match those on the case report form.

Notes:

1. Freeze the tube with **the throat swab at -70°C as soon as possible**. If this is not possible, keep it refrigerated but make sure it is frozen at -70°C at the end of the day or within 12 hours of collection.
2. **Always place fresh specimen in wet ice or in cold packs** during transport to the -70 ° C freezer.
3. Keep specimens **stored at -70°C until shipment to NHRC**.

D. SPECIMEN TRANSPORT:

1. Instructions for packing specimens
 - a. Make sure you have **at least 8 lbs. of dry ice available** preferably in pellets. Styrofoam shipping and a sturdy secondary container are necessary to hold the viral transport tubes.

- b. Wrap the viral transport tubes individually with a paper towel and place them in a secondary container.
- c. Place the viral transport tubes inside the secondary container. Place the secondary container with the viral transport tubes inside the Styrofoam box and cover with 8 lbs. Of dry ice.
- d. Place the secondary container with the viral transport tubes inside the Styrofoam box and cover with 8 lbs. of dry ice. Make certain that the viral transport medium does not thaw during packing.

2. Directions for shipping of specimens:

- a. Ship the specimens by Federal Express (Priority Overnight) to NHRC at the end of each 4-week reporting period. The specimens should be packed with dry ice and shipped on a Monday, Tuesday, or Wednesday to make sure they get to NHRC Virology Lab before the weekend.
- b. Include in each shipment, one (1) unused VTM from each lot used in the sample collection for that shipment.
- c. A requisition form should be filled out and signed and dated by the requesting physician.
- d. This requisition form is attached to case report forms corresponding to the specimens to be shipped and is included in the shipment, but should be placed in a plastic Ziplock bag.

VI. PROCEDURE NOTES:

- A. Since viruses are cell-associated organisms, a sufficient amount of infected cells should be present in the sample. To ensure that enough infected cells are collected, make sure that the **swab is vigorously rubbed on the tonsils and the pharynx and not just touched on these areas**. Avoid touching other areas of the mouth, tongue or inner cheeks.
- B. **Viruses are heat labile**, thus should be immediately kept in cold temperatures after collection.
- C. It is also important to remember that viruses lose infectivity with slow freezing and freezing at higher temperatures (e.g., -10 to -20°C). **Specimens should be frozen at -70°C immediately after collection if processing and inoculation into cell cultures cannot be done within 24 hours.**
- D. Safety:
 1. Always wear adequate PPE (laboratory coats and gloves) when handling specimens.
 2. Use bleach (Clorox) or 70% ethanol to decontaminate surfaces.
Note: Both 70% Ethanol and bleach (Na Hypochlorite) are effective disinfectants. Wait 10 minutes when disinfecting using

bleach. 70% Ethanol is fast acting and it is very effective against Influenza, HSV I and II and adenovirus(1).

3. Any contaminated sharp object , if applicable, should be placed in a red biohazard sharp container.
4. Materials to be decontaminated at off-sites from our facility are packaged according to the local, state and Federal regulations.

E. Spills:

1. Bleach contaminated materials for 10 minutes or decontaminate with 70% ethanol any spills that may occur during specimen collection.

REFERENCES:

1. Manual of Clinical Microbiology, 7th edition, 1999, Murray, P; Baron, E.J., et. al; pgs. 147-149.

Method Review

Date Adopted: _____ Date Revised: _____ Supersedes: _____

Reviewed by: _____	Date: _____
Reviewed by: _____	Date: _____