

Section 8

Virology Test Collection

Viral Specimen Collection

Check Before You Collect!

Always check specific test requirements before collecting a specimen. Please provide accurate date, time and source information with each specimen. Viral specimen requirements are listed alphabetically by test in the following pages. For photos and descriptions of containers, see *Container Guide*.

PCR vs. Culture

Polymerase Chain Reaction (PCR) and other molecular methods have become the preferred method for detecting viral diseases. Because PCR tests detect extremely small amounts of viral particles and do not depend on the presence or number of live, viable viral organisms for detection, results are definitive and reliable. PCR tests are also performed in hours instead of days compared to culture methods. If culture is necessary instead of PCR, the specimen should be obtained from patients during the acute stage of illness (when viral shedding is greatest) and transported to the laboratory immediately.

Specimens for PCR Tests

The selection of specimen is based on the clinical syndrome and/or the virus suspected. **Calcium-alginate tipped swabs, wood swabs, or swabs in transport tubes containing gel are NOT ACCEPTABLE for PCR testing. Specimen source is required** on request form.

Specimens for Culture

The selection of specimen is based on the clinical syndrome and/or the virus suspected. Specimens such as throat swabs, spinal fluid, rectal swabs, effusion fluid, lesion, scrapings, and respiratory secretions/washings are usually submitted for viral culture. Urine is acceptable only for MUMPS culture (see virus culture, non-respiratory). **Specimen source is required** on request form. Blood, bone marrow, dermal, genital, and oral specimens are unacceptable for viral culture. Most molecular methods are appropriate for these specimen types.

Viral Transport Media

EMHRL supplies M4 and/or M5 liquid media for viral transport. M4 and M5 media in the conical plastic tube must be stored refrigerated, and does not have an exceedingly long shelf life. Please order sparingly and only in amounts you anticipate using soon.

To inoculate media:

Swabs - Immerse the collection swab into the M4 or M5 liquid immediately after collection. Break off the shaft of the swab in the media tube.

Fluids – Aseptically transfer 0.5 mL fluid to M4 or M5 media tube.

Cap specimen tightly and **label with patient information, source, date & time**. Transport to the lab refrigerated (No ice; do not freeze.)

ADENOVIRUS DNA by PCR

Acceptable Specimens and Specimen Collection

1. **Blood:** Collect whole blood in EDTA (lavender top tube). Centrifuge and separate plasma within 2 hours of draw. Refrigerate in plastic vial.
2. **Body fluids, Respiratory Aspirate or Wash:** Collect in sterile container and aseptically transfer 0.5mL of specimen to M4 viral transport media. Store/transport refrigerated.
3. **Swabs:** Immerse swab in M4 viral transport media; cut or break off swab handle. Store/transport refrigerated.
4. **CSF:** Submit in a sterile container. Store/transport refrigerated

Specimen source is required on request form.

ADENOVIRUS CULTURE: See Virus Culture, Respiratory

CYTOMEGALOVIRUS (CMV) by Rapid PCR

Preferred Specimens and Specimen Collection

1. **Respiratory:** Nasopharyngeal aspirate or washing, transtracheal aspirate, bronchial washing, bronchoalveolar lavage, sputum. 1.5 mL in a sterile container containing 1-2 mL of M5 media. Refrigerate. (No ice. Do not freeze).
2. **Body fluid or Spinal fluid:** Send 0.5 mL in sterile container containing 1-2 mL of M5 media. Refrigerate. (No ice. Do not freeze).
3. **Throat:** Collect with a culture transport swab. Refrigerate. (No ice. Do not freeze).
4. **Blood:** Draw 5 mL blood in lavender top EDTA tube. Refrigerate. (No ice. Do not freeze).
5. **Tissue or Bone Marrow:** Send in sterile container containing 1-2 mL of M5 media. Refrigerate. (No ice. Do not freeze).
6. **Urine:** 2 mL. from a random urine collection in a screw-capped sterile container. Refrigerate.
7. **OTHER:** See Virus Culture, Non-Respiratory

Specimen source is required on request form.

HERPES SIMPLEX VIRUS TYPE 1 & 2 PCR (Polymerase Chain Reaction)

1. **Body Fluid or CSF:** 0.3 mL. Collect in a sterile vial. Refrigerate. (No ice; do not freeze.)
2. **Dermal/Ocular Swab or vesicle aspirate:** Inoculate M4 viral transport media with the swab tip or vesicle aspirate. Store and transport specimens refrigerated. (No ice; do not freeze.)
3. **Genital:** Transport swab in M4 media. Refrigerate.
4. **Throat:** Transport swab in M4 media. Refrigerate.
5. **Tissue:** Send refrigerated in a screw-capped sterile container containing 1-2 mL sterile saline.

Specimen source is required on request form.

HERPES SIMPLEX VIRUS CULTURE: See Virus Culture, Non-Respiratory

MYCOPLASMA HOMINIS CULTURE

Acceptable Specimens

1. **Females:** Vaginal swab, cervical swabs or urethral scrapings, urine, endometrium or fallopian tube.
2. **Males:** Urethral swab or scrapings, urine or prostatic fluid.

Specimen Collection

Collect vaginal or wound specimen by swabbing back and forth over the mucosa or wound surface to maximize recovery of cells. Collect urethra or cervical specimen by inserting swab 1 cm to 3 cm and rotating 360 degrees. After collection, swirl swab into 3 mL of transport media (M5) and express fluid from swab against side of vial. Discard swab and cap vial. **Send M5 transport media frozen. Specimen source is required** on request form.

MYCOPLASMA PNEUMONIAE CULTURE (REF2TC2406)

Acceptable specimens and Specimen Collection

Nasopharynx or throat swab or washing, tracheal aspirate, sputum - Collect specimen in sterile container or swab and transfer swab tip or 0.5mL of fluid to M4 viral transport media. Store and Transport refrigerated. (No ice. Do not Freeze). **Specimen source is required** on request form.

PARAINFLUENZA VIRUS 1,2,3 CULTURE: See Virus Culture, Respiratory

RESPIRATORY VIRUS PANEL by PCR

Acceptable Specimens and Specimen Collection

Nasopharyngeal Swab: Swab nasopharyngeal cavity with sterile mini-tip swab. Immerse swab in M4 viral transport media; cut or break off swab handle. [See graphic below for nasopharyngeal collection instructions.](#) Panel detects Influenza A & B, and Respiratory Syncytial virus (RSV).

RESPIRATORY SYNCYTIAL VIRUS (RSV) ANTIGEN by EIA

Acceptable Specimens and Specimen Collection

1. **Nasopharyngeal Aspirate or Wash:** Collect in sterile container and transfer 0.5 mL to M4 media tube. Cap tube tightly. Store and transport refrigerated. (No ice; do not freeze.)
2. **Nasopharyngeal Swab:** Swab nasopharyngeal cavity with sterile mini-tip swab. Immerse swab in M4 viral transport media; cut or break off swab handle. Cap tube tightly. Store and transport refrigerated. (No ice; do not freeze.)

Specimen source is required on request form.

Additional Information

RSV EIA test results are very specific; any positive results may be considered a final report. Since the RSV EIA method is slightly less sensitive than tissue culture, a negative RSV EIA is considered presumptive and requires a final report based on Respiratory PCR (See 'Respiratory Virus Panel by PCR')

VARICELLA-ZOSTER VIRUS by PCR

1. **Dermal, or Lesion:** Culture transport swab in liquid Stuart media. Refrigerate. (No ice; do not freeze.)
2. **Spinal Fluid:** 0.5 mL spinal fluid in sterile vial. Do not centrifuge. Refrigerate. (No ice; do not freeze.)

Specimen source is required on request form.

VIRUS CULTURE, NON-RESPIRATORY

Preferred Specimens and Specimen Collection

1. **Body Fluid or Spinal Fluid:** 1 mL of fluid. Send specimen refrigerated in a screw-capped, sterile vial. (No ice; do not freeze.) Maintain sterility and forward promptly.
2. **Conjunctival Swab/Corneal Scrapings:** Gently rub the conjunctiva with sterile swab. Inoculate M4 viral transport media, breaking off swab tip into tube. Collect corneal scrapings with a sterile platinum spatula. Rinse spatula in the sterile liquid transport medium of an M4 transport media tube. Store and Transport refrigerated. (No ice; do not freeze.)
3. **Stool or Rectal Swab:** Collect 1-2 gm (mL) of feces (about the size of a thumbnail) in a sterile screw-capped container. Refrigerate. (No ice; do not freeze.) For swab, insert sterile swab well into the rectum and rub mucosa. Repeat until visible fecal material adheres to the swab and inoculate viral transport media. Break off the swab tip in the M4 viral transport media. Transport refrigerated.
4. **Urine (Mumps only):** Collect freshly voided urine in a sterile, leak proof container. Send 0.5 mL refrigerated.

Specimen source is required on request form.

VIRUS CULTURE, RESPIRATORY

Acceptable Specimens and Specimen Collection

Specimens should be collected early in the acute phase of infection.

1. **Bronchial-Alveolar Lavage/ Sputum:** Collect specimen in a sterile, leak proof plastic container. Refrigerate. (No ice; do not freeze.)
2. **Throat swab:** Rub the posterior of the tonsils, soft palate and back wall of the lower pharynx with sterile swab and inoculate M5 viral transport media. Break off the swab tip in the M5 media tube. Store and transport specimens refrigerated.
3. **Tissue (Biopsy, Autopsy):** Sterile instruments should be used for each tissue. Place each tissue into separate M5 viral transport media tubes. Store and transport specimens refrigerated. (No ice; do not freeze.)

Specimen source is required on request form.

Influenza A/B by PCR

Instructions for Specimen Collection



Storage and Use of Transport Media

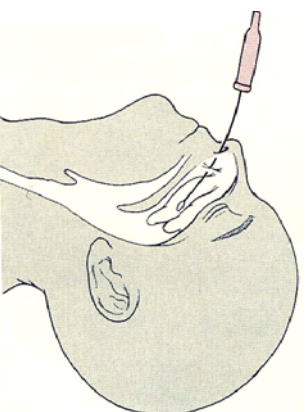
Use Remel M4 or M5 Transport Media (see photo on left). Store in the refrigerator until use. After inoculating media with specimen, keep the specimen refrigerated or on ice.

WATCH EXPIRATION DATES ON MEDIA CONICAL TUBES!



Nasopharyngeal Collection

1. Use one *sterile swab with a flocked nylon or cotton mini-tip* for each patient. Swabs are packaged in either a plastic tube or wrapped in paper. **NOTE: Do not use calcium alginate or wooden shafted swabs as these inhibit viruses.**
2. Immobilize the patient's head and gently pass the swab through the nostril near the septum floor of the nose into the nasopharyngeal cavity to the point of resistance; gently rotate several times and remove.
3. Uncap the viral transport tube and insert swab into the media. Break off the handle by bending back and forth, or use sterile scissors to crop the swab shaft so that the entire swab easily fits inside the tube.
4. Leave the swab immersed in the media for transport and tighten the cap on the tube.
5. Label the specimen with the patient's name, collection date and time of collection.



Transport of Specimen

1. Wrap absorbent material around the specimen tube, place the tube into the biohazard bag, and seal. Transport under refrigeration.
2. Deliver to the Reference Laboratory as soon as possible.

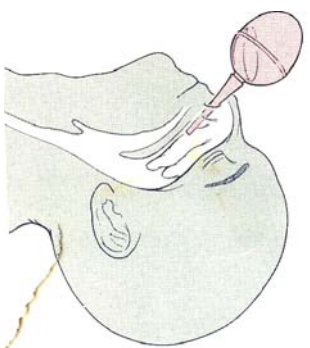
ANY QUESTIONS? PLEASE FEEL FREE TO CALL THE REFERENCE LABORATORY AT (630) 941-4542.

NASOPHARYNGEAL SPECIMEN COLLECTION

Nasal Wash: Bulb Method

Materials: Sterile Saline
1-2 oz., tapered rubber bulb*
Viral Transport Medium (VTM)
Specimen container

1. Suction 3-5 ml saline into a sterile bulb.
2. Insert bulb into one nostril until nostril is occluded.
3. Instill saline into nostril with one squeeze of the bulb and immediately release bulb to collect recoverable nasal specimen.
4. Empty bulb into an M4 Viral Transport Media Vial. Refrigerate.



Nasal Wash: Syringe Method

Materials: Sterile Saline
3-5 ml syringe*
2" 18-20 gauge tubing*
M4 Viral Transport Media

1. Fill syringe with saline; attach tubing to syringe tip.
2. Quickly instill saline into nostril.
3. Aspirate the recoverable nasal specimen. Recovery must occur immediately; as the instilled fluid will rapidly drain. (Alternatively, in appropriate cases, patients may tilt head forward and allow specimen to drain into suitable sterile container).
4. Inject aspirated specimen from syringe into M4 Viral Transport Media. Refrigerate

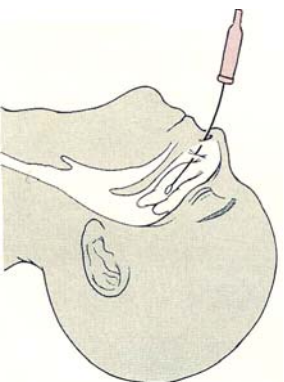
*Length and diameter of syringe, tube or bulb as appropriate for infant, child or adult.



Nasopharyngeal Swab Method

Materials: Nasopharyngeal mini-tip swab
(with synthetic fiber tip)
M4 Viral Transport Media

1. Insert swab into one nostril.
2. Press swab tip on the mucosal surface of the mid-inferior portion of the inferior turbinate, and rub the swab tip several times across the mucosal surface to loosen and collect cellular material.
3. Withdraw swab; break off swab tip into M4 Viral Transport Media Tube. Refrigerate.



For best sample quality

Repeating procedure for the second nostril will deliver optimal combined sample.

Optional: Perform nasopharyngeal swab procedure prior to wash procedures. Samples may be combined.