

Patient Preparation and Virus Specimen Collection

Specimen Identification

Each specimen must be correctly labeled. Label information should include: patient name, case number, date of birth, age, sex, date specimen was collected, source of specimen, and the test requested.

Virus transport medium (M4 RT) is recommended for the collection of viral and chlamydial cultures unless otherwise specified. This medium contains antibiotics and an antifungal agent to minimize bacterial and yeast contamination and cannot be used for mycoplasma detection. M4 RT can be stored at room temperature but once inoculated, the tubes must be refrigerated. To obtain viral transport medium, call the Client Services Department.

Swabs. Swab material should be made of sterile cotton, rayon, or dacron. **DO NOT USE CALCIUM ALGINATE SWABS FOR VIRAL OR CHLAMYDIAL ISOLATIONS.** Calcium alginate swabs are toxic for Chlamydia and many enveloped viruses. In addition, specimens collected with calcium alginate swabs cannot be used for PCR, direct fluorescent antibody, or EIA testing. Wooden-shafted swabs are not recommended for culture because wooden swabs can contain toxins and formaldehydes that inhibit the recovery of viruses and Chlamydia. Wooden swabs also absorb transport media thereby reducing the amount of fluid for inoculation onto cell cultures.

Basic concepts for specimen collection:

- Collect the specimen early in the acute phase of infection,
- Use appropriate collection devices and collect a sufficient quantity of material
- Collect an appropriate specimen
- Label the specimen and complete the requisition
- Minimize transport time and keep specimens for virus culture cold in transport

SPECIMEN-SPECIFIC COLLECTION GUIDELINES

Blood

1. Collect blood in a lavender-top tube. Do not use heparin (green) or PPT (white top tubes).
2. Transport to the laboratory at room temperature.

Body Fluids

Cerebrospinal fluid (CSF)

1. Collect the appropriate amount of CSF in a sterile tube.
2. Collect separate specimens for viral and bacterial cultures. If only one specimen is available, do not refrigerate specimen until after bacterial cultures have been plated.

Other Body Fluids

1. Collect body fluid (other than urine) in a sterile container.
2. Whenever possible, collect separate specimens for viral and bacterial cultures. If this is not possible, do not refrigerate specimen until after bacterial cultures have been plated.

Cervical Specimens

1. Use a vaginal speculum to facilitate visualization of the cervix and to prevent the swabs from touching the sides of the vagina.
2. Using a sterile dacron, rayon, or cotton swab, carefully remove the mucus from the endocervix. This swab should be discarded. The cervix of patients with Chlamydia or gonococcal infection is often coated with copious amounts of mucus. This mucus must be removed from the cervix using the absorbent white cleaning swab. **Discard the cleaning swab.** Mucus in the cleaning swab can cause the transport medium to gel. This gel cannot be pipetted and the sample cannot be tested.
3. Using the small blue Dacron swab, carefully sample the transitional zone of the cervix, rolling the swab to assure that the swab contains cervical epithelial cells.
4. Carefully remove the swab to prevent contamination with vaginal flora.

- Place the swab into a vial of appropriate (viral or APTIMA) transport medium and send the specimen to the laboratory.
NOTE: Cervical or vaginal lesions should be swabbed as described for lesions. Lesion swabs should be placed in viral transport medium.

Chlamydia rRNA by TMA

Endocervical Swab Specimens

- Use a vaginal speculum to facilitate visualization of the cervix and to prevent the swabs from touching the sides of the vagina. Using the large white swab from the APTIMA swab collection kit, carefully remove the mucus from the endocervix. **Discard this swab.** Mucus in the cleaning swab can cause the transport medium to gel. This gel cannot be pipetted and the sample cannot be tested.
- Insert the small blue swab into the transitional zone of the endocervix, rolling the swab for 10-30 seconds to ensure adequate sampling.
- Carefully remove the swab to prevent contamination with vaginal flora.
- Place the small swab into the APTIMA transport tube. Break the swab at the score line. Use care to avoid splashing contents.
- Replace the cap securely.



Male Urethral Specimens

- The patient should not have urinated for at least 1 hour prior to specimen collection.
- Position the patient so that the urethra is readily accessible.
- Using the large white swab, remove and discard any mucus present on the external os.
- Insert the small blue-shafted swab 2 to 4 cm into the urethra.
- Carefully rotate the swab for 2-3 seconds to ensure adequate sampling.
- Withdraw the swab carefully.
- Remove the cap of the APTIMA transport tube and place the blue-shafted swab into the transport tube.
- Break the swab at the score line. Use care to avoid splashing contents.
- Replace the cap securely.



Urine Specimens

- Patients should not have urinated for at least 1 hour prior to urine collection.
- Instruct the patient to provide first-catch urine (approximately 20-30 mL of initial stream) into a preservative-free urine collection cup. Collection of larger volumes may result in specimen dilution and reduced test sensitivity. **Female patients should not cleanse the labial area** prior to providing the specimen.
- Remove the cap from the APTIMA urine specimen transport tube and transfer 2 mL of urine into the transport tube using the disposable pipette provided in the collection kit. The correct volume of urine has been added when the fluid level is between the black fill lines on the urine transport tube label.

Eye (Conjunctiva)

1. Remove any exudate from the eye.
Moisten a fine dacron, rayon, or cotton swab with sterile physiologic saline.
2. Gently pull the lower eyelid downward and carefully swab the lower conjunctiva to collect both cells and fluids. The upper eyelid may be swabbed in the same manner.
3. If both eyes are to be cultured, a separate sterile swab should be used for the other eye.
4. Place the swabs in the appropriate (viral or chlamydial) transport vial and send the vial



Human Papillomavirus (HPV) Testing

Biopsy Specimens

1. Aseptically collect cervical or condyloma specimens up to 5 mm in diameter. Biopsies less than 2 mm should not be submitted as they may produce false-negative results.
2. Immediately place the tissue into the 1.0 mL of Digene transport medium. Note: the Digene Cervical Sampler vials contain 1.0 mL of transport medium. Discard the cervical sampler.
3. For best results, the specimen should be frozen and shipped at -20°C or below.

Cervical Brushes

The Digene HPV Test is designed for use with specimens collected and transported using the Digene Cervical Sampler (Digene Cervical Brush and Specimen Transport Medium). Swab specimens will not be tested.

1. Cervical brush specimens should be collected after PAP specimen collection and before colposcopy and the application of acetic acid or iodine.
2. Insert the Digene Cervical Sampler into the cervical os and rotate three times to accumulate cells.
3. Place the sampler into the bottom of the transport medium vial and break the shaft of sampler at the score line.
4. Cap the vial securely and send to the laboratory.

Specimens in Cytoc PreservCyt Solutions

1. Collect specimens using a plastic spatula or cervical broom, the Digene Cervical Sampler, or an endocervical brush.
2. Rinse the collection devices in the PreservCyt Solution. Brushes and samplers should be rotated in the solution at least 10 times while pressing the devices against the inside surface of the vial. Cervical brooms should be pressed against the bottom of the vial 10 times, forcing the bristles apart. Swirl the collection device vigorously to further release material. Discard the collection device.
3. Tighten the cap on the PreservCyt Solution so that the torque line on the cap passes the torque line on the vial.
4. Transport to the laboratory.
Note: The HPV test requires at least 4 mL of PreservCyt Solution.

Specimens in Tripath AutoCyte Solutions

1. Collect specimens using a plastic spatula or cervical broom, the Digene Cervical Sampler, or an endocervical brush.

2. Rinse the collection devices in the AutoCyt Solution. Brushes and samplers should be rotated in the solution at least 10 times while pressing the devices against the inside surface of the vial. Cervical brooms should be pressed against the bottom of the vial 10 times, forcing the bristles apart. Swirl the collection device vigorously to further release material. Discard the collection device.
3. Tighten the cap on the vial and transport to the laboratory.
Note: The HPV test requires at least 2 mL of AutoCyt Solution.

Nasal Washings

Procedure for Infants and Young Children

1. Wipe excess mucus from the nose with facial tissue and discard.
2. Place 5 mL of sterile saline into a clean specimen cup. Using a bulb syringe, suction the fluid into the syringe. Use 1-2 mL of sterile saline for infants, 2-4 mL for larger children or adults.
3. Have the patient lying down with head tilted back (it helps if a second person holds a baby or small child).
4. Insert bulb into the nostril until the opening is occluded.
5. In one motion, gently squeeze wash fluid into the nostril and then release the bulb pressure to collect the fluid again into the bulb.
6. Empty the collected material into a sterile container or back into the nasal wash media tube.
7. Tighten the cap and transport to the laboratory.

Alternate collection procedure for cooperative older children or adults

1. Clean excess mucus with facial tissue and discard.
2. Place 5 mL of sterile saline into a clean cup.
3. Suction the fluid into a needle-less 5cc plastic syringe.
4. Have the patient sit in a chair with the head tilted back.
5. While the patient makes a continuous “k-k-k” sound and holds the cup at mouth level, express the wash fluid into one nostril.
6. Immediately, bring the patient’s head up, making sure the cup is under the nose. As the patient leans forward the fluid will drain into the cup.
7. Empty the material into the wash media tube or send to the laboratory in the sealed cup.
8. Transport specimen to the laboratory.

Nasopharyngeal Aspirate - Pediatric

1. Wipe excess mucus from the nose with a facial tissue and discard.
2. Attach a sterile soft polyethylene #8 French feeding tube to a disposable aspiration trap.
3. Using the tube, measure the distance from the patient's nostril to their ear. Mark the distance on the tube using your thumb and forefinger.
4. Gently insert the tube into the nostril until the thumb and forefinger touch the patient's nose.
5. While applying intermittent suction, slowly remove the tube from the nasopharynx.

Viral or Chlamydial Culture: Place the end of the tube in a vial containing 2-3 mL of viral transport medium and aspirate the contents into the trap.

Bacterial or fungal Culture: Do not add transport media to the trap.

6. Remove the feeding tube and carefully cap all the orifices on the aspiration trap.
7. Send the aspiration trap to the laboratory.

Nasopharyngeal Swab

1. A dry flexible aluminum-shafted, cotton-or rayon-tipped wire swab should be used for this procedure. Separate swabs should be used when specimens are collected from both nostrils.
2. Gently insert the swab into the nostril until resistance is felt.
3. Hold the swab in place for 10-20 seconds to absorb the fluids. Rotate the swab three times to collect respiratory epithelial cells.



4. Withdraw the swab and place the swab in viral transport medium and cut the shaft so that the swab fits into the tube.
5. Transport to the laboratory.

Neisseria gonorrhoeae rRNA by TMA

Endocervical Swab Specimens

1. Use a vaginal speculum to facilitate visualization of the cervix and to prevent the swabs from touching the sides of the vagina.
2. Using the large white swab from the APTIMA swab collection kit, carefully remove the mucus from the endocervix. **Discard this swab.** Mucus in the cleaning swab can cause the transport medium to gel. This gel cannot be pipetted and the sample cannot be tested.
3. Insert the small blue swab into the transitional zone of the endocervix, rolling the swab for 10-30 seconds to ensure adequate sampling.
4. Carefully remove the swab to prevent contamination with vaginal flora.
5. Verify that all of the transport buffer is at the bottom of the APTIMA transport tube. Tap or shake the solution to the bottom of the tube as necessary.
6. Place the small swab into the APTIMA transport tube. Break the swab at the score line. Use care to avoid splashing contents.
7. Replace the cap securely and send to the laboratory.



Male Urethral Specimens

1. The patient should not have urinated for at least 1 hour prior to specimen collection.
2. Position the patient so that the urethra is readily accessible.
3. Using the large white swab, remove and discard any mucus present on the external os.
4. Insert the small blue-shafted swab 2 to 4 cm into the urethra.
5. Carefully rotate the swab for 2-3 seconds to ensure adequate sampling.
6. Withdraw the swab carefully.
7. Remove the cap of the APTIMA transport tube and place the blue-shafted swab into the transport tube.
8. Break the swab at the score line. Use care to avoid splashing contents.
9. Replace the cap securely and transport to the laboratory.



Urine Specimens

1. Patients should not have urinated for at least 1 hour prior to urine collection.
2. Instruct the patient to provide first-catch urine (approximately 20-30 mL of initial stream) into a preservative-free urine collection cup. Collection of larger volumes may result in specimen dilution and reduced test sensitivity. Female patients should not cleanse the labial area prior to providing the specimen.
3. Remove the cap from the APTIMA urine specimen transport tube and transfer 2 mL of urine into the transport tube using the disposable pipette provided in the collection kit. The correct volume of urine has been added when the fluid level is between the black fill lines on the urine transport tube label.
4. Cap securely and transport to the laboratory.



Rectal Swabs

1. Position the patient so that the anus is readily accessible.
2. Insert a dry cotton, rayon, or Dacron swab at least 5 cm into the rectum.
3. Rotate the swab and carefully withdraw it from the rectum. The swab should show signs of fecal material.
4. Place the swab into a vial of viral transport medium and break the shaft of the swab so that it fits into the tube.
5. Cap the vial securely and transport to the laboratory.

Stool

1. Collect 10-30 grams of stool (approximately the size of a walnut) in any clean vessel. Do not use preservatives for virus isolations.
2. Transfer the stool to a screw-top stool transport vial provided by Warde Medical laboratory.
3. Place stool transport vial in a sealed plastic bag.

Stool, Pediatric:

1. Scrape the stool from the diaper (see note below) immediately after the child's bowel movement.
2. Place the specimen in any clean vessel possessing a tight-fitting lid. Do not use preservatives.
3. Transfer the stool to a screw-capped stool transport vial provided by Warde Medical Laboratory.
4. Place stool transport vial into a sealed plastic bag.

NOTE: Highly absorbent paper diapers will quickly soak up liquid stools and cause viruses to bind irreversibly to the paper fibers. This problem can be alleviated by placing a piece of plastic (e.g., a plastic bag) in the diaper. Stool collected on the plastic can be placed into the container as described above. Alternatively, paper diapers can be turned inside out so that the outer plastic cover is next to the child's skin. This procedure can reduce stool leakage to outer garments. Stool specimens collected using this method should be handled as described above.

Throat Swab

1. A dry cotton, Dacron, or Rayon swab or a swab moistened with physiologic saline may be used for this procedure.
2. Rub the swab across the tonsils and posterior pharynx.
3. Place the swab in viral transport medium and cut or break the shaft of the swab so that it fits into the vial.
4. Cap securely and transport to the laboratory.

Tissues

1. Collect tissues aseptically taking care to prevent cross-contamination when specimens are taken from multiple sites.
2. Autopsy specimens should be collected within 24 hours of the time of death.
3. Small tissue specimens may be placed into viral transport medium or sterile saline.
4. Transport the specimens to the laboratory promptly.

Urethral Swabs (Male)

1. Position the patient so that the urethra is readily accessible.
2. Insert a fine, aluminum-shafted cotton or dacron swab 2 to 4 cm into the urethra.
3. Carefully rotate the swab three times and remove the swab from the urethra.



Viral and Chlamydial Cultures. Place the swab in viral transport medium and cut the shaft of the swab so that it fits into the tube. Refrigerate specimens and send them to the laboratory promptly.

Chlamydia and Gonorrhea Tests (Nucleic Acid Tests). Place swab into the APTIMA transport tube and break the shaft so that the swab fits into the tube. Transport to the laboratory. **NOTE: Urethral discharges are not acceptable specimens.**

Urine for Viral Culture/PCR

1. Collect first early morning urine in a sterile container with a tight-fitting lid.
2. Send specimen to the laboratory. Refrigerate specimens and send them to the laboratory promptly. DO NOT FREEZE AT -20°C.

Urine for *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Testing (APTIMA)

1. See individual tests (above).

Vesicle Swab

1. Select a vesicle containing clear fluids. The efficiency of virus recovery decreases significantly when pustular fluids are collected.
2. Carefully unroof the lesion and blot the excess fluid with a swab.
3. Rub the base of the lesion with the swab to collect infected epithelial cells.
4. Place the swab in viral transport medium and break the shaft of the swab so that it will fit into the vial.
5. Cap the vial securely and send to the laboratory.