

## Specimen Collection -- Supplies

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### INTRODUCTION

The quality of results from laboratory testing depends greatly on the proper collection and handling of the specimen submitted for analysis. Correct patient preparation, specimen collection, specimen packaging and transportation are essential factors.

Specific specimen requirements for each determination, including sample size, are provided in the Alphabetical Listing of Tests Section. To avoid additional expense and inconvenience, please make sure you have submitted at least the quantity specified for the test requested. As a rule of thumb, the volume of blood drawn should equal 2½ times the amount of serum or plasma required. For example, to obtain 4 ml serum, draw at least 10 ml of whole blood. We will mark the notation "Q.N.S." (Quantity Not Sufficient) on reports for sample quantities that were insufficient to analyze.

When inappropriate specimens are submitted, they will be stored in the laboratory and the client can be contacted and requested to recollect the specimen. A written report explaining the issue will also be issued.

When test requisitions with unclear (nonspecific) test requests are received, CompuNet will contact your office to clarify the physician's orders.

### HEALTH AND SAFETY PRECAUTIONS

All specimens should be handled as if they are infectious. The greatest dangers to health care workers exposed to blood and body fluids are the Human Immunodeficiency Virus (HIV) and the hepatitis viruses. CompuNet has an extensive safety policy which adheres to the following guidelines:

Joint Advisory Notice, **Protection Against Occupational Exposure to Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV)**, Department of Labor/Department of Health and Human Services, Oct. 19, 1987.

**1988 Agent Summary Statement for Human Immunodeficiency Virus and Report on Laboratory-Acquired Infection**, Department of Health and Human Services, April 1, 1988.

**The Occupational Safety and Health Administration (OSHA) Standard on Bloodborne Pathogens** 29 CFR 1910.1030.



The following safety guidelines need to be followed when preparing specimens for transport to CompuNet:

- Specimen container must be properly sealed. A leaking container not only compromises the specimen integrity but poses a health hazard for those handling the specimen. A leaking specimen can also contaminate the requisition which is a safety hazard throughout the process.
- **Only Arterial Blood Gas Specimens will be accepted in a syringe. The needle must be removed before transport to the laboratory.** All other specimens collected in a syringe should be transferred to the appropriate container for the testing being performed. If there is a question as to container type, consult your Directory of Services or call CompuNet **(937)-297-8260** to clarify.
- All specimens must be transported in a plastic ziplock bag as secondary containment. This protects anyone handling the specimen in case of leakage or breakage.
- All specimens are transported to CompuNet in coolers with the Biohazard label. This meets the requirements of CFR 1910.1030. “(G) Individual containers of blood or other potentially infectious materials that are **placed in a labeled container during storage, transport, shipment, or disposal** are exempted from the (individual specimen) labeling requirement.”
- If a specimen has broken in the centrifuge and the centrifuge is being returned for replacement, the centrifuge must be decontaminated before its return.

### **Exposure Accident Protocol**

- Any facility where employees are at risk for exposure to biohazards is required to have an “Exposure Follow up” procedure in place (OSHA Bloodborne Pathogens CFR 1910.1030).
- Refer to the Ohio Administrative Code 3701-3-11, concerning requirements for HIV consent.
- In the event of an exposure incident, STAT HIV testing is available for the "Source Patient" only. Required - A physician name for contact.
- The most effective way to maintain patient confidentiality is submission of the specimen with the name as a “code” with instructions directing the results to the person responsible for accident follow up.
- As with other testing, a requisition with the requested tests must be presented in order for the blood to be drawn at a CompuNet Patient Service Center.



## **SPECIMEN COLLECTION SUPPLIES**

Selected supplies necessary to draw and submit specimens are provided to clients as part of our testing services. To order supplies, include a completed supply order form with your next specimen submission or call the Client Response Center at **(937)-297-8260**.

Specimen collection devices supplied by CompuNet are to be used only for the collection of specimens that will be submitted to CompuNet. Such supplies are not to be used to store or dispose of biological materials, including sharp instruments, or for any activity not connected with the collection of specimens for CompuNet.

CompuNet is required by the Office of Inspector General to monitor supplies, ensuring that supplies requested match testing submitted. Therefore, supply orders may be adjusted to reflect the client's current usage.

### **Alphabetical Listing of Specimen Collection:**

#### **Anaerobic Transport Tube (Port-A-Cul)**

For transport of swab, or fluid specimens for aerobic and/or anaerobic culture. Tube of gel medium. Swabs should be placed into gel for transport. Maintains microorganism viability but does not promote growth of microorganisms. See *Microbiology Specimen Collection* for specific instructions.

#### **Anaerobic Transport Vial**

For transport of fluid material aspirated with needle and syringe for anaerobic culture. See *Microbiology Specimen Collection* for specific instructions.

#### **Biopsy Tissue Specimen Container – NOT FOR CULTURE**

Plastic screw top container (7.5 ml or 30ml size) which contains 10% formalin.

#### **Blood Collection Vials - evacuated tube method**

##### **Blue (Light) Top**

Contains buffered sodium citrate solution as anticoagulant. Available in 2.7 ml or 4.5 ml sizes. Use for coagulation studies.

##### **Blue (Royal) Top**

Contains sodium heparin as anticoagulant (green label) for whole blood or red blood cells analysis; or comes without anticoagulant (red label) for serum analysis. Available in 7 ml size. Use for trace element determinations.

Brown (Tan) Top

Contains sodium heparin as anticoagulant. Available in 5 ml size. Glass has a lead content less than 0.05 µg per tube. Use for whole blood lead analysis.

Gray Top

Contains sodium fluoride as a preservative and potassium oxalate as an anticoagulant. Available in 3 ml, 5 ml and 7 ml sizes. Use for glycolytic inhibition.

Dark Green Top

Contains sodium heparin as anticoagulant. Available in 5 ml and 10 ml sizes. Use for preparing heparinized plasma, whole blood or bone marrow specimens.

Light Green Top (Plasma Separator Tubes)

Contains Gel and lithium heparin. Available in 3 ml size. Use for limited chemistry testing.

Lavender Top

Contains di-potassium ethylene diaminetetraacetate (K<sub>2</sub> EDTA) as anticoagulant. Available in 3 ml (clear over-cap) and 4 ml (lavender over-cap) sizes. The two tubes are the same size, but since they have different vacuums they fill to different levels. Use for hematology studies.

Red Top

Contains no anticoagulant or preservative. Available in 4 ml and 10 ml sizes. Use for determinations requiring serum (therapeutic drug monitoring, blood banking).

Red/Gray Mottled Top (Serum separator tube)

Contains no anticoagulant or preservative. Tube walls are coated with a clot activator; a polymer barrier material is in the bottom of the tube. Available in 9.5 ml size. Used for one-step collection of serum or clotted whole blood. Instructions for centrifugation appear in the *Blood and Urine Collection* section.

Yellow Top (ACD-A)

Contains Acid Citrate Dextrose solution A. Available in 12.5 ml size.

Yellow/Red Speckled Top (Ascorbic Acid Tube)

Contains EDTA (Na<sub>2</sub>) and Ascorbic Acid. Available in 14 ml size.

**Blood Collection Vials - fingerstick method**

Available in both Serum Separator (red top) and EDTA (lavender) types.

**Blood Culture Bottle Sets**



Each set contains two bottles of special media and atmosphere for culturing blood specimens both aerobically and anaerobically. Caution: Do not vent bottles. Store at room temperature. See *Microbiology Specimen Collection* for specific instructions.

### **Chlamydia Amplified DNA Kit**

Female (pink) kit contains a large-tipped cleaning swab and a smaller swab for specimen collection. Male (blue) kits contain a mini-tipped swab. Other swabs/collection kits are not acceptable. See *Virology/Molecular Diagnostics Specimen Collection* section for specific instructions.

### **Chlamydia Culture Transport Vial**

Contains media for the collection and transport of specimens for **chlamydial** isolation, Store at 4°C. See *Virology/Molecular Diagnostics Specimen Collection* for specific instructions.

### **Cytology (non-GYN) Fixative**

100 ml screw-cap containers with 30 ml 50% CytoLyt Fixative.

### **Cytology Screen, (Conventional Pap Smear) Gynecology Kit**

Contains instructions, slide, fixative, cervical scrapers or cytobrush and submission folder.

### **Cytology, Thinprep Pap**

Vial contains PreservCyt fixative solution, scraper and cytobrush.

### **Drug Testing Kit**

Chain-of-Custody contains supplies necessary for collecting samples and maintaining sample integrity of specimens submitted for drugs of abuse testing.

### **Hemocult Kit**

Contains applicator, instructions and sample submission card for detection of fecal occult blood. (Does not contain developer.)

### **Human Papilloma Virus (HPV) Collection Kit**

Kit contains swab, collection tube, sample collection and transport instructions.

### **Isostat Isolator Tube (10 ml)**

Sterile collection tube containing sodium polyanetholsulfonate and sodium chloride. Use when determining the presence of fungus or mycobacterium in blood or body fluids. See *Microbiology Specimen Collection* for specific instructions.

### **Mycoplasma Transport Vial**

See Viral Transport Medium.

### **Neisseria gonorrhoeae (GC) Amplified DNA Kit**

Female (pink) kit contains a large-tipped cleaning swab and a smaller swab for specimen collection. Male (blue) kits contain a mini-tipped swab. Other swabs/collection kits are not acceptable. See *Virology/Molecular Diagnostics Specimen Collection* section for specific instructions.

### **Neisseria gonorrhoeae (GC) Culture Plate**

For isolation of Neisseria gonorrhoeae. See *Microbiology Specimen Collection* for specific instructions.

### **Neonatal Testing Kit - PKU**

Contains filter paper and test request form.

### **Parasite Examination Kit, Feces (Para-Pak)**

Kit has two specimen collection vials:

Formalin Vial (Pink): Contains formaldehyde solution for preservation of stool specimens for ova and parasite examination.

PVA Vial (Blue): Contains polyvinyl alcohol (PVA) for preservation of stool specimens for protozoan cysts and trophozoites.

See *Microbiology Specimen Collection* for specific instructions.

### **Pinworm Collection System**

Plastic tube containing a sticky paddle for collection of specimens for pinworm (Enterobius vermicularis) examination.

### **Plastic Containers (120 cc Screw-Cap; Sterile or Nonsterile)**

For urine aliquot submissions. Contains no preservative. Tighten securely.

### **Plastic Vials (10 ml Volume)**

Screw-cap, leakproof vial for transfer of serum, plasma or whole blood from blood collection tubes.

### **Stool Culture Transport Vial**

Screw cap vial (orange) with 15 ml special transport media. Use for culture of stool for enteric pathogens. See *Microbiology Specimen Collection* for specific instructions.

### **Tissue Immunofluorescence Kit**

Contains vial and instructions for the preparation and transportation of tissue specimens for Tissue Immunofluorescence.

**Transport Swab for Bacterial Culture**

Contains one rayon tipped swab and a special holding medium for bacterial transport which maintains microorganism viability but does not promote growth of microorganisms. (Mini-tip size available for collection/transport of nasopharyngeal, urethral, eye specimens, etc.) See *Microbiology Specimen Collection* for specific instructions.

**Ureaplasma Transport Vial**

See Viral Transport Medium.

**Urine Container (24 hour)**

For tests requiring 24-hour urine collection.

**Urine Culture Transport Kit (Gray topped tube)**

Contains special transport media for urine culture. Follow the instructions provided for collection of specimen. See *Microbiology Specimen Collection* for specific instructions.

**Urine Chemistry Transport Tube (Yellow topped tube)**

Contains special transport media to stabilize urine for chemical analysis.

**Viral Transport Medium**

Contains swab and media for the collection and transport of specimens for **viral** isolation and DFA testing. Also used for Chlamydia, Ureaplasma and Mycoplasma culture. Store at 4°C. See *Virology/Molecular Diagnostics Specimen Collection* for specific instructions.

## Specimen Collection -- Blood and Urine

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### INTRODUCTION

Specific instructions for storage and shipment of specimens for individual tests are listed under specimen requirements in the alphabetical listing of laboratory tests. Please follow them carefully.

After the specimen is collected:

- ✓ **Label the specimens** -- Label each specimen with the patient's full name (complete last name and complete first name) and the collection date at time of collection. Note on the specimen container if the contents are plasma. Make sure all containers are tightly closed.

All Blood Bank specimens must be labeled with: last name, first name, middle initial; date of birth and specimen collection date.

- ✓ **Package the specimens** -- Fold each laboratory requisition in half, with the front sides (test information) showing, and place in a zip-lock bag along with the matching specimens for that requisition. Place only one requisition into each zip-lock bag.
- ✓ **Store the specimens** -- Maintain the specimens at room temperature or refrigerator, unless otherwise noted in the specimen requirements.

### CAUSES OF INACCURATE TEST RESULTS AND UNACCEPTABLE SPECIMENS

Specific instructions for storage and shipment of specimens for individual tests are listed under specimen requirements in the alphabetical listing of laboratory tests. Please follow them carefully.

Occasionally, blood specimens are contaminated with substances that interfere with accurate sample analysis:

- **Hemolysis** -- Hemolysis occurs when the membrane surrounding red blood cells is disrupted and hemoglobin and other intracellular components escape into the serum or plasma. (This may occur with a difficult draw.)

Hemolyzed serum or plasma varies in color from faint pink to bright red, rather than the normal straw color. Even a slight hemolysis will alter certain test results. Grossly or moderately hemolyzed specimens may be rejected.

- **Hyperbilirubinemia** -- Icteric serum or plasma varies in color from dark to bright yellow, rather than the normal straw color. Icterus may affect certain test results. Upon receipt of such specimens, we may request a new sample to assure results of diagnostic value.
- **Radioisotope Interference** -- Diagnostic procedures or therapy involving radioactive compounds may invalidate radioisotope assays. Please obtain specimens for anticipated radioisotope assays before administering isotopes to patient.
- **Turbidity (Lipemia)** -- Turbid, cloudy, or milky serum (lipemia serum) may be produced by the presence of fatty substances (lipids) in the blood. Bacterial contamination may also cause cloudy serum. Moderately or grossly lipemic specimens may alter certain test results. A recent meal produces transient lipemia; therefore, we recommend that patients fast 12-14 hours before a blood specimen is obtained.
- **Unspun/Partially Spun** -- Erroneous results may be produced by not centrifuging specimens in SST (Serum Separator) tubes within 1 hour of collection. The same may be produced with poorly spun (incomplete gel separation) specimens.

## INSTRUCTIONS FOR BLOOD AND URINE SPECIMEN COLLECTION

### Blood, Plasma

Plasma contains fibrinogen and other clotting factors when separated from the red cells. Evacuated tubes used to collect plasma specimens contain an anticoagulant and frequently a preservative. The additive in the tube is specified and correlates to the color of the tube stopper (refer to Supplies - Blood Collection Vials). Consult the individual test specimen requirement to determine the correct additive/tube to use. If the plasma is removed from the original vacutainer vial, the transfer tube and requisition must be labeled to indicate that the specimen is plasma.

### Blood, Serum

We recommend the use of serum separator collection tubes for most analyses. Please check individual specimen requirements for restrictions. When using a serum separator tube, follow these instructions:

1. Perform venipuncture as with any other blood collection tube.
2. Invert the tube gently at least ten times.
3. Do not remove the stopper at any time. Allow the blood to clot at least 30 minutes (but not more than 60 minutes) before spinning. Do **not** centrifuge immediately after drawing blood.
4. Centrifuge > 3000 RPM for 10 minutes.

5. Normally the specimen is transported to CompuNet in the original collection tube. Only transfer the clear serum to a plastic vial when immediate freezing is necessary. Do not freeze the glass tubes.

**Note: Do not use serum separator tubes for therapeutic drug monitoring or toxicological analyses.** The plastic serum separator material extracts lipophilic substances (most drugs) resulting in a falsely low drug-concentration result. Instead, collect the specimen in an evacuated tube (plain red top) containing no anticoagulants or preservatives. Follow the above guidelines for centrifugation, then use a pipette to transfer the serum to a plastic vial for shipment to the laboratory.

### **Blood, Serum or Plasma Frozen**

Plasma or serum specimens need to be frozen only if specifically stated in the specimen requirement. However, in these cases it is essential to freeze the specimen as soon as it is separated from the cells. Always freeze specimens in plastic tubes unless specifically instructed otherwise. Lay the tube at a 45° angle to avoid tube breakage caused by expansion during freezing.

Label the plastic vial with patient's full name, specimen type and collection date. Write directly on the plastic vial as extreme cold causes labels to become brittle and detach from the specimen tube. If the specimen is plasma, note this on both the vial and requisition.

**Note:** If more than one test is requested on a frozen specimen, please split the sample prior to freezing, and submit separately. Please use separate requisition forms for those tests requiring a frozen sample.

### **Blood, Whole**

Collect whole blood according to the instructions provided for the individual test. Thoroughly mix the blood with the additives by gently inverting the tube at least ten times. Maintain the specimen at room temperature before shipping to our laboratory unless instructed otherwise by the specimen requirements. **Never** freeze whole blood unless specifically instructed by the specimen requirements.

### **Urine, Chemistry**

The normal composition of urine varies considerably during a 24-hour period. Most reference values are based on analysis of the first urine voided in the morning. This specimen is preferred because it has a more uniform volume and concentration, and its lower pH helps preserve the formed elements.

To reduce contamination, the specimen submitted for urinalysis should be a clean catch "midstream sample." Submit a first morning specimen whenever possible.

**Urine may be submitted in a evacuated tube urinalysis tube. This tube has a preservative that stabilizes the urine chemistry for up to 72 hours.**

Urine for pregnancy testing should be a first morning voiding, or a random specimen with a specific gravity of at least 1.010. Note the time of collection of the specimen on the test requisition form and on the label of the container.

If a frozen specimen is required, freeze the urine immediately after collection. Pack in dry ice for shipment to the laboratory.

### **Urine, Cultures**

See *Microbiology Specimen Collection* section for specific instructions.

### **Urine, 24 Hour Collection**

Because proper collection of 24-hour urine specimens is essential for accurate test results, patients should be carefully instructed in the correct procedure. Printed instructions for the patient are available from the laboratory.

1. Unless the physician indicates otherwise, instruct the patient to maintain the usual amount of liquid intake but to consume no alcoholic beverages.
2. During the collection period, place the 24-hour urine container provided by CompuNet in a refrigerator or cool place, to prevent growth of microorganisms and possible decomposition of urine constituents.
3. Have the patient empty his/her bladder in the morning into the toilet (**not** to be included in the 24-hour collection). Write the date and time of voiding on the container label.
4. Collect the patient's next voiding and add it as soon as possible to the 24-hour container.
5. Add all subsequent voidings to the container as in Step 4. The last sample collected should be the first specimen voided the following morning at the same time as the previous morning's first voiding.

# Specimen Collection -- Microbiology Cultures

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## INTRODUCTION

Quality results in microbiology depend on both the client and the laboratory. The many factors contributing to the successful isolation of potential pathogens range from specimen selection and collection to proper transport and timely delivery to the laboratory. Specific plating media and procedures depend on proper specimen handling for pathogen isolation. It is extremely important to refer to specimen collection and submission instructions to ensure that we receive the most suitable or appropriate specimens for laboratory analysis.

The following section describes a series of anatomic sites and patient diseases. **Always indicate the source of the specimen** on the test request form to guide us in our efforts to assist you in your diagnosis and treatment.

Because not all specimens contain clinically significant pathogens, antimicrobial susceptibility studies will be performed only on appropriate isolates and at an additional charge. Isolates are held for 7 days in the laboratory in case susceptibilities are deemed necessary on selected isolates.

## SPECIMEN COLLECTION

Three general categories of specimen collection are presented in this section, based on the relationship of the site to indigenous flora. To obtain a quality specimen, first carefully select the optimal site for the disease process. Next, prepare the site and remove the material according to one of the three general categories of specimen handling instructions provided in the paragraphs that follow. Consult the test sections for additional information.

### **Category 1 - Specimens from a Normally Sterile Site (blood, CSF, aspirates of body fluids)**

Specimen collection from these sites requires a needle puncture or a surgical procedure. The following procedures are designed to reduce the risk of contamination with skin flora.

#### **A. Decontamination of skin**

This procedure must be performed prior to the collection of specimens such as blood, CSF and aspirates.

1. Clean the puncture site with 70% alcohol

2. Clean the puncture site with a tincture of iodine preparation. Allow to remain on the skin for at least 1-2 minutes. Do **not** probe with your finger after puncture site has been decontaminated.

## **B. Collection of Clinical Material**

### **1. Blood Culture**

Since blood cultures are processed using special media, it is necessary to submit all blood cultures in the appropriate bottles supplied by CompuNet. Most often, two separate sets of blood cultures will suffice. Because collection of just one set supplies inadequate blood volume for detection of many organisms, a single set should not be submitted.

#### Timing of Collection:

Whenever possible, blood cultures should be obtained prior to the start of antimicrobial agents. Two or three sets of cultures provide adequate blood for detection of pathogens provided that 20 ml of blood is collected for each set (adult patients).

In patients with probable intermittent bacteremia (patients with abscesses, pneumonia, meningitis, osteomyelitis etc.) obtain 2 sets of blood cultures 1-2 hours apart (if antibiotics must be started, collection times may be compressed). Repeat cultures after 24 - 48 hrs if negative.

In patients with suspected endocarditis obtain 2 - 3 sets of blood cultures over 1-2 hours. Notify laboratory of suspected diagnosis (bottles will be incubated an extended period of time). Repeat cultures after 24 - 48 hrs if negative.

#### Collection Process:

- a) Disinfect the skin with tincture of iodine prior to collecting blood.
- b) Before injecting the blood into the culture bottles, decontaminate the diaphragm tops by swabbing with 70% alcohol and allow to dry.
- c) Draw the sample for each set of blood cultures with a needle and syringe. 20 ml of blood should be collected for each set of cultures. For pediatric patients, collection of 1-5 ml of blood is acceptable.
- d) After collection, immediately transfer 10 ml of blood into the aerobic and anaerobic bottles. These bottles must have a minimum of 5 ml of blood in each.

- e) Following inoculation, all bottles should be held at room temperature and transported to the laboratory as soon as possible.

For mycobacterial or fungal blood cultures, 8-10 ml of blood should be inoculated into an Isostat Isolator Tube. Clean the top of the isolator tube with alcohol prior to injecting blood.

## 2. Cerebrospinal Fluid (CSF)

Submit a separate sterile, screw-capped tube containing as much fluid as possible. For routine bacterial culture, at least 1 ml of fluid should be submitted. If additional cultures are desired, submit 1 - 2 ml/culture. For optimal results, submit the second or third tube collected.

## 3. Other Body Fluids

Follow standard procedures and collect the specimen by aspiration. If anaerobic culture is desired, the specimen **must** be submitted in an anaerobic transport device.

## C. Specimens for Anaerobic Culture

Specimens **must** be transported in anaerobic transport devices.

### 1. Specimens from the following sites are acceptable for anaerobic culture:

- Aspirated pus from abscesses
- Bronchoscopy specimens collected by double lumen technique
- Exudates, aspirated pus from deep wounds
- Genital specimens from the following sites only: cul-de-sac aspirate, culdocentesis, fallopian tubes, percutaneous transfundal aspirate, placenta, prostatic or seminal fluid, septic abortion
- Normally sterile body fluids
- Suprapubic urine specimens
- Surgical Specimens
- Transtracheal aspirate

**Note:** Specimens not mentioned above may be processed anaerobically only after consultation with CompuNet Clinical Laboratories Microbiology Manager or Director.

### 2. Specimens from the following sites are **not** acceptable for anaerobic culture at any time:

- Throat and nasopharyngeal swabs

- Sputum or bronchoscopy specimens (except double lumen protected specimens)
- Feces and rectal swabs
- Voided or catheterized urines
- Vaginal or cervical swabs
- Specimens from sites contaminated with intestinal contents: colostomy site, draining pilonidal sinus, traumatic perforation
- Superficial wounds

## **Category II - Specimens Routinely Collected through Contaminated Pathways (urine, sputum, nasopharyngeal swabs)**

### **A. Urine**

#### 1. Clean Catch: Patient guidelines

##### Females

Prepare a sterile gauze pad for washing by wetting it and placing a small amount of soap on the surface. Prepare two more sterile gauze pads for rinsing by moistening. Finally, open a fourth gauze pad, but do not moisten.

Wash the vaginal area from the front to the back, using the soapy gauze pad. Discard the gauze in the wastebasket. Rinse the area from front to back, using first one moistened pad and then the second. Last, dry the area from the front to the back with the dry pad.

Lean forward slightly so that the urine flows directly down into the toilet without running along the skin. After voiding the first portion of urine, place the clean container under the stream of urine and collect the rest of the urine into the container. Transfer the urine immediately into the urine transport tube.

##### Males

Prepare a sterile gauze for washing by wetting it and placing a small amount of soap on the surface. Prepare two more sterile gauze pads for rinsing by moistening. Finally, open a fourth gauze pad, but do not moisten.

Use the soapy gauze to wash the end of the penis. Discard the gauze into the wastebasket.

Rinse, using first one moistened gauze pad and then the other, discarding them in the wastebasket. Begin to urinate into the toilet. After voiding the first part, place the clean

container under the stream of urine and collect the rest of the urine into the container. Transfer immediately into the urine transport tube.

## 2. Indwelling Catheter

Obtain the specimen with a needle and syringe. Select the puncture site 1-2 inches away from the catheter tube entry point. Cleanse the area to be punctured with 70% alcohol. Aspirate 5 ml of urine with a sterile needle and syringe. Transfer the specimen to a urine transport tube.

Specimens obtained from the collection bag are **not** suitable for culture. Foley tips will not be accepted.

## 3. Cystoscopic or Suprapubic Aspiration.

These specimens should be submitted in both aerobic and anaerobic transport systems.

## B. Sputum

The preferred specimen is an early morning expectorated sputum obtained after a deep cough. Do not pool multiple samples collected during a 24 hour period. The patient should rinse his/her mouth with water before the specimen is collected. If mycobacterial cultures are desired, the patient should rinse with sterile water to avoid non-pathogenic mycobacteria which can contaminate tap water. Instruct the patient to avoid adding saliva or nasopharyngeal discharge to the sputum sample. Generally, better specimens are obtained if the collection of the sputum is observed.

The specimen should be collected in a leak-proof sterile container and refrigerated until it is picked up.

## C. Nasopharyngeal Specimens

Nasopharyngeal secretions obtained by aspiration or washings are the preferred specimen.

If unobtainable, the specimen may be obtained using a swab on a fine wire. Immobilize the patient's head and insert the swab into the nostril to the posterior nares. Leave it in place for a few seconds and remove. Submit swab in transport medium.

## Category III - Specimens from Sites Usually Containing Commensal Microorganisms (stool, throat, genitals, superficial skin)

## A. Stool (Fecal) Specimen

Have patient pass stool directly into a wide-mouth container or bedpan, and transfer specimen into appropriate transport devices.

The specimen should not be collected with toilet paper (which may contain inhibitory substances) or contaminated with urine. Specimens collected within 7 days of a barium enema are unsuitable for examination.

If multiple specimens are desired, these should be collected at 1-3 day intervals.

Transport devices are available for bacterial culture and for ova and parasite examination (see Transport Section).

## B. Throat

Depress tongue gently with tongue depressor. Extend sterile swab between the tonsillar pillars and behind the uvula (avoid touching the cheeks, tongue, uvula, or lips). Sweep the swab back and forth across the posterior-pharynx, tonsillar areas, and any inflamed or ulcerated areas to obtain sample.

## C. Genital Specimens

Note: DNA tests for Chlamydia and Gonorrhea require specific collection devices, see Transport Device Section.

### Female

#### 1. Cervix

Do not use lubricant during procedure. Wipe the cervix clean of vaginal secretions and mucus. Rotate a sterile swab, and obtain exudate from the endocervical glands (for Chlamydia, cervical cells should be obtained). If no exudate is seen, insert a sterile swab into the endocervical canal, and rotate the swab.

#### 2. Vaginal Secretions

Use a speculum without lubricant. Collect secretions from the mucosa high in the vaginal canal with sterile pipette or swab.

### Male

### 1. Prostatic Massage

Perform a digital massage through the rectum. Collect the specimen in a sterile tube or on a sterile swab.

### 2. Urethra

Collect specimen at least 2 hours after patient has urinated. Insert a thin urethrogenital swab 2-4 cm into the endourethra, gently rotate it, leave it in place for 1-2 sec., and withdraw it.

## **D. Superficial lesions/wounds (including conjunctiva)**

### 1. Conjunctiva

Optimal specimens are obtained by scraping the conjunctiva with a sterilized spatula. Alternatively, a swab may be used to swab the inferior tarsal conjunctiva (inner surface of the eyelid) and the fornix of the eye.

### 2. Superficial wound - bacterial

Syringe aspiration is always superior to swab collection. The surface should be disinfected with 70% alcohol followed by tincture of iodine.

Using a 3-5 ml syringe with a 22-23 gauge needle, the physician should aspirate the deepest portion of the lesion. The material should be transferred into an anaerobic transport vial. If no material is obtained, sterile, 0.85% saline may be injected into the lesion, then aspirated.

If a swab must be used, the superficial area should be cleaned as described above, and any overlying debris removed. The base of the lesion or ulcer should then be sampled with a sterile swab.

### 3. Superficial lesions - fungal

Clean the surface with sterile water. Using a scalpel blade, scrape the periphery of the lesion border. Samples from scalp lesions should contain hair that is selectively collected for examination. If there is nail involvement, obtain scrapings of debris or material beneath the nail plate. Transport in a sterile container at room temperature. If possible, also include scalpel blade used for collection.

# Specimen Collection and Transport – Virology/Molecular Diagnostics

## INTRODUCTION

The ability to isolate viruses from specimens requires proper collection from appropriate sites, and maintaining specimens at refrigerated temperatures. Viruses die rapidly at room temperature, so it is imperative to keep specimens at refrigerated temperature after collection.

## COLLECTION OF SPECIMENS FOR VIRAL CULTURE AND DFA

For specimens not included in this table, contact the Virology lab at 937-297-8338

Type of specimen	Means of collection	Comments
<b>Blood</b>	Collect 8-10 ml in anticoagulant sodium citrate (yellow top) or EDTA (purple top)	Useful for CMV
<b>Bone marrow</b>	Collect at least 2 ml in anticoagulant sodium citrate (yellow top) or EDTA (purple top)	Useful for CMV
<b>Fluids</b>		
CSF	Collect 2-5 ml in sterile container	Recovery of herpes viruses is rare. DNA testing (PCR) is preferred.
Urine	Collect 10-20 ml of midstream, clean-voided urine in sterile container <b>without</b> preservatives. For neonates, submit as much urine as possible.	Useful for CMV, HSV, adenoviruses
<b>Gastrointestinal</b>		
Rectal swab	Insert swab 4-6 cm into rectum and roll against mucosa. Place swab in tube of VTM (viral transport medium)	Stool specimen is preferred.
Stool	Place walnut-sized amount, or several mls of liquid stool into sterile container.	Useful for enteroviruses, adenoviruses
<b>Genitourinary</b>		
Cervical swab	Remove exocervical mucus with clean swab. Insert fresh swab at	Useful for HSV (herpes)

	least 1 cm in cervical canal and rotate for 15-30 seconds. Place swab in tube of VTM.	
Urethral swab	Express and discard exudate. Insert flexible, narrow-shaft swab 2-4 cm into urethra. Place swab in tube of VTM.	Patient should not urinate for at least 1 hour prior to collection.
<b>Lesion, dermal</b>		
Swab	Disrupt vesicle and collect fluid with swab. Using same swab, rub the base of the lesion. For nonvesicular or dry lesions, premoisten swab with sterile saline prior to rubbing base of lesion. Place swab in tube of VTM.	Recovery of herpes simplex virus diminishes in ulcerative and crusted lesions. Useful for HSV, VZV, enteroviruses (coxsackie, echo)
Vesicle aspirate	Aspirate fluid from vesicle using narrow gauge needle and syringe. Place fluid in tube of VTM. Rinse syringe with VTM.	
<b>Lesion, mucosal (oral, genital)</b>		
Swab	Swab lesion and place swab in tube of VTM	Anogenital: HSV Oral: HSV, coxsackie virus
<b>Ocular</b>		
Conjunctival swab	Swab lower conjunctiva with swab premoistened with sterile saline. Place swab in tube of VTM.	Useful for adenoviruses, enteroviruses, HSV, Chlamydia
Corneal or conjunctival scraping	Place scraping into tube of VTM.	Useful for HSV, VZV
<b>Respiratory</b>		
Nasopharyngeal swab	Insert flexible, narrow-shafted wire swab through nostril into posterior nasopharynx until resistance is felt. Rotate for several seconds. Place swab in tube of VTM.	Anterior nasal swabs are inferior for detection of viruses. Useful for influenza, RSV, adenovirus, parainfluenza.
Throat swab	Vigorously swab tonsillar areas. Place swab in tube of VTM.	Nasopharyngeal swabs or aspirates are preferred for detection of respiratory viruses.

		Useful for enteroviruses.
Nasopharyngeal aspirate	Insert catheter tubing into posterior nasopharynx. Using syringe or mucus collection device, apply suction. Put aspirate material into empty tube or tube of VTM. Use VTM or saline to wash material through tubing.	Preferred specimen for RSV
Nasopharyngeal wash	With patient's head tilted back, instill 2-5 ml saline into nostrils. Instruct patient not to swallow. Return patient's head upright and collect material in cup placed under nose. Instruct patient to "snort" into cup. Place contents of cup into empty tube or tube of VTM.	
Bronchoalveolar lavage	Place 8-10 ml in sterile container.	Useful for CMV, HSV, etc.
<b>Tissue</b>	Place small samples into tube of VTM or sterile saline to prevent drying.	

**HANDLING OF SPECIMENS FOR VIRAL CULTURE AND DFA**

All specimens for viral culture, herpes culture, mycoplasma/ureaplasma culture, and chlamydia culture must be kept at refrigerated temperature immediately after collection. Place specimens in a refrigerator or on frozen cold packs until courier pickup.

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**COLLECTION AND HANDLING OF SPECIMENS FOR CHLAMYDIA, MYCOPLASMA, UREAPLASMA CULTURE**

For anogenital tract specimens, swab the female endocervix, male urethra or rectum. Place swab in tube of Viral Transport Medium (also appropriate for mycoplasma, chlamydia). For diagnosis of congenital chlamydia infection, swab the conjunctiva and/or throat and place swab in tube of VTM.



All specimens for mycoplasma/ureaplasma culture and chlamydia culture must be kept at refrigerated temperature immediately after collection. Place specimens in a refrigerator or on frozen cold packs until courier pickup.

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### COLLECTION OF SPECIMENS FOR CHLAMYDIA/GC AMPLIFIED DNA

Note: this test is not FDA approved for vaginal or labial swabs. It is not approved for non-genital sites such as eye, rectum and throat. The performance characteristics for these specimens are unknown.

In cases of suspected sexual abuse, and for other medico-legal situations, it is recommended to collect specimens for chlamydia and gonorrhea **culture**, in addition to DNA testing.

Type of specimen	Means of collection	Comments
Female endocervical swab	Using large-tipped swab provided in kit, remove excess mucus from the cervical os. Insert the pink culturette swab into the cervical canal and rotate for 15-30 seconds. Place the swab in the transport tube.	Inadequate removal of mucus can result in false negative test results. Failure to swab the cervix for 15-30 seconds can cause false negative test results.
Male urethral swab	Insert the blue mini-tip swab 2-4 cm into urethra and rotate for 3-5 seconds. Place the swab in the transport tube.	
Urine	Patient should not have urinated for a least 1 hour prior to specimen collection. Collect 15-20 ml of <b>first void</b> urine in sterile container <b>without</b> preservatives.	Urine is preferred over swabs for male specimens.

### HANDLING OF SPECIMENS FOR CHLAMYDIA/GC AMPLIFIED DNA TESTS

Swabs are stable at room temperature. Urine must be kept at refrigerated temperature.