

Specimen Collection

To provide the most accurate test results, we rely on you to supply us with the best possible representative specimens. To ensure quality specimens, please adhere to the guidelines in this section.

The Clinical Laboratory Improvement Amendment of 1988 and our regulatory agencies require a physician's order or an order from another authorized source before laboratory testing can be performed. A completed Sentara Reference Laboratory requisition form provides this order. Perform specimen collections with the Sentara Reference Laboratory supplies provided. To request routine supplies, fax a client Supply Order Form to our supply center at (757) 965-0044. A copy of the Supply Order Form is located in the Policies and Procedures chapter of this guide. Should specialty containers, tubes or other supplies be required or if you have questions about specimen collection or related supplies, contact Client Services at (757) 388-3621.

Sentara Reference Laboratory requires that OSHA standards be followed to insure the safety of your staff and ours, and requires each individual patient specimen be placed in a **biohazard bag** for transport. Packaged this way, a broken or leaking specimen does not contaminate others.

Contaminated specimens may not be used. Sentara Reference Laboratory will contact you if it is necessary for a contaminated specimen to be recollected.

Specimen Identification

Sentara Reference Laboratory is proud of our commitment to quality laboratory service. Together, with our clients, we work hard to maintain the highest level of patient safety. To insure accurate processing and testing, a Sentara Reference Laboratory requisition form must accompany each specimen.

Sentara Reference Laboratory specimen identification requires at least two unique identifiers for each specimen received at the laboratory. These identifiers must be present on the container holding the specimen.

1. Clearly label each specimen with the patient's complete name – first and last – exactly as it appears on the test requisition.
2. Other examples of identifiers are date of birth, Social Security number or another patient assigned number such as a medical record number. Each of these must match the information submitted on the requisition.
3. Sentara Reference Laboratory requisitions include specimen identification number and stickers at the top of the form. This may be used as an identifier – but note that this alone will not identify the specimen.

Specimens submitted without two identifiers may be rejected.

In addition, samples poured into transfer tubes must include specimen type indicated on the transfer tube. Source information is also required on tubes or containers for anatomical pathology and microbiology specimens.

The requisition accompanying each specimen must be complete with:

- ◆ Patient Social Security number/Medical Record number
- ◆ Patient name (written exactly as on specimen)
- ◆ Sex
- ◆ Race (required for AFP testing eGFR)
- ◆ Date of birth
- ◆ Ordering physician's name
- ◆ Collection date and time
- ◆ Identification of collector
- ◆ Source – anatomical pathology and microbiology specimens

Specimens may be rejected and testing delayed if all of the above information is not provided. For specimens that cannot be recollected, you may be requested to complete a waiver accepting responsibility for specimen and patient identification.

Results will not be released until all missing information is provided. If a specimen is rejected, your office will be notified on a lab report via auto fax, printer or courier delivery the next business day after receipt of the specimen in the laboratory.

If you have any questions concerning our specimen identification procedures, please contact Vicki Pierce, Clinical Specialist, Specimen Processing at (757) 388-1486.

Blood Collection, Preparation and Handling

It is a fact that laboratory results are relied on to diagnose and manage medical care. Therefore, it is critical that specimens are not altered during the collection process. *Accurate* patient preparation, specimen collection, and handling (processing, storage, and transportation) must be followed to ensure *accurate* testing. The following table summarizes the types of errors often overlooked and organizes them into those that occur before, during and after the actual venipuncture. Specimens may be rejected and a recollection requested if any of the following errors occur.

Before Collection

Patient misidentification
 Improper time of collection
 Wrong tube
 Inadequate fast
 Prolonged fast
 Exercise
 Patient posture
 Poor coordination with other treatments
 Nonsterile site preparation
 Not coordinating with medication

During Collection

Prolonged tourniquet time
 Hemolysis
 Order of Draw
 Failure to invert tubes
 Faulty technique
 Underfilling tubes
 (QNS)

After Collection

Improper centrifugation process (too soon, incomplete, etc)
 Failure to separate serum from cells
 Improper use of serum separators
 Processing delays
 Exposure to light
 Improper storage conditions
 Rimming clots

Tube Code

Perform specimen collection with the Sentara Reference Laboratory supplies provided. Should the test specimen collection instruction not be included in this guide, or should you have any questions, please contact Client Services at 757/388-3621 or 1-800/822-0468. The following provides a guide for the types of tubes available for specimen collection. Refer to the alphabetical test listing to determine the tube required for testing.

Note: Red, Lavender, and green microtainers have the additives shown in the following tube code chart. Microtainers must be filled to the lines indicating accurate volumes.

Hemogard Closure	Conventional Stopper	Additive	Inversions at Collection**	Laboratory Use	Effects of Underfilling
Gold	Red/Black	Clot activator & gel for serum separation	5	Tube for serum determinations in chemistry. Tube inversions ensure mixing of clot activator with blood. Blood clotting time: 30 minutes.	Poor barrier formation. Insufficient sample
Light green	Green/Gray	Lithium heparin and gel for plasma separation	8	Tube for plasma determinations in chemistry. Tube inversions prevent clotting.	Erroneous results due to excessive heparin.
Red	Red	None (glass), Clot activator (plastic)	0 5	For serum determinations in chemistry, serology and immunohematology (ABO grouping, Rh typing, antibody screening, red cell phenotyping, and DAT testing). Tube inversions ensure mixing of clot activator with blood and clotting within 60 minutes.	Insufficient sample
Green	Green	Sodium Heparin, Lithium Heparin	8 8	For plasma determinations in chemistry. Tube inversions prevent clotting	Erroneous results due to excessive heparin
Lavender		Liquid K ₂ EDTA (glass), Spray coated K ₂ EDTA	8 8	K ₂ EDTA for whole blood hematology determinations. K ₂ EDTA for whole blood hematology determinations and immunohematology testing (ABO grouping, Rh typing, antibody screening). Tube inversions prevent clotting.	Erroneous low hematocrits, cell counts and; morphologic changes to RBC's
Light Blue	Light Blue	Sodium Citrate	3-4	For coagulation determinations. Note: Certain tests may require chilled specimens. Tube inversions prevent clotting	Coagulation results are erroneously prolonged.
	Dark Blue Top	EDTA	8	For trace element, toxicology and nutritional-chemistry determinations.	
	Yellow	Acid citrate dextrose (ACD) *Solution A – 22.0 g/l trisodium citrate 8.0 g/l citric acid 24.5 g/l dextrose *Solution B – 13.2 g/l trisodium citrate 4.8 g/l citric acid 14.7 g/l dextrose	8 8	ACD for use in blood bank studies, HLA phenotyping, DNA and paternity testing	
Pink		Spray coated K ₂ EDTA	8	For whole blood determinations in immunohematology (ABO grouping, Rh typing, antibody screening) Tube inversions prevent clotting	

** invert gently, do not shake
Reference: BD Diagnostics

Patient Preparation

The quality of your sample is directly related to the instructions provided to your patient before collection of the specimen. Diet, medication and/or other procedures being performed can all affect the specimen you collect and may result in questionable results. Therefore, it is important to discuss these factors with the patient prior to collection of the specimen.

Collection of Specimen

This section is present only as a guide for those already trained in the venipuncture technique. The National Committee for Clinical Laboratory Standards (CLSI) is an excellent resource for information on the collection, storage and handling of specimens for laboratory testing. Carefully review the specimen type, volume, collection materials and the storage and handling instructions before collecting the specimen. Have all necessary supplies ready before performing the venipuncture (evacuated tubes, safety needles, alcohol prep, gauze, needle holder, tourniquet, and Band-Aid or tape). Gloves are worn for the phlebotomy process.

1. Identify the patient before obtaining the specimen.
2. Have the patient sit or lie down (as your collection area allows)
3. Choose an appropriate venipuncture site on the arm, preferably in the antecubital area. Sites to avoid: scarred areas, arm on side of mastectomy, hematoma and arms with fistulas or vascular grafts.
4. Place the arm in a downward position. It is sometimes helpful to have the patient form a fist, but there must not be vigorous hand pumping.
5. Prepare the venipuncture site with an appropriate antiseptic. Allow the antiseptic to dry. Apply a tourniquet - do not leave the tourniquet on the patient's arm more than 1 minute.
6. Remove the needle cap. Visually inspect the point for burrs.
7. Perform the venipuncture. After anchoring the vein, insert the needle at a 15-30o angle, lined up with the vein and the bevel facing up. If multiple samples are collected, carefully remove first tube and invert to prevent clotting (see note below). Insert the next tube by pushing forward and initiating vacuum suction.
8. Follow the correct order of draw (see next section)
9. Release the tourniquet.
10. When the desired quantity has been collected, withdraw the needle.
11. Immediately after withdrawing the needle and initiating safety features, dispose of needle and holder in sharps container. Do not recap, cut or bend needles.
12. Apply direct pressure on the venipuncture site with clean gauze until the bleeding stops.
13. Label the specimen with patient's complete name as it appears on the requisition. Small barcode label from requisition should also be applied to specimen as a second form of identification. If label is not available, hand write requisition number on tube. Record date and time of collection on requisition. (See Specimen Identification)

Note: Proper mixing of tubes

Many tubes contain an additive (anticoagulant) or clot activator that needs to be mixed with the blood sample immediately after drawing. This is achieved by holding the tube upright and gently inverting 180o and back. Repeat this movement as prescribed for each tube (see Tube Guide).

Order of Draw

The order in which the tubes must be filled was established by NCCLS (CLSI) to avoid crosscontamination of anticoagulants or bacterial contamination of blood cultures.

1. Tubes or bottles for blood culture
2. Light-blue stopper tubes. Tubes for coagulation studies
3. Red-stopper tubes or tubes without additives
4. Gel separator tubes (May contain a clot activator or heparin)
5. Green-stopper tubes (Contain heparin)
6. Lavender-stopper tubes; pink stopper tubes. (Contain EDTA)
7. Other tubes (eg gray stopper, etc)

Preparation of Serum

Serum is obtained from clotted blood that has not been mixed with an anticoagulant.

1. After collection of Serum Separator Tube, **gently** invert gel tube five (5) times to mix clot activator with blood.
2. Allow blood to clot a minimum of 30 minutes, but no longer than 1 hour.
3. Centrifuge for 15 minutes at 1000-1300 G's (normal speed of centrifuge provided by Sentara Reference Laboratory)
4. Remove from centrifuge. Barrier will have formed, separating cells and serum
5. Sample in gel tube, along with the appropriate requisition form, is now ready to be transported to the laboratory.
6. If the test requested requires a frozen specimen, it is necessary to pour the specimen into a plastic transport tube. Cap the tube and place in the freezer. Do not freeze the gel tube. Label according to guidelines provided in this manual.
7. If a plain red top tube is used for collection instead of the gel, it is necessary to remove the serum from the cells with a disposable pipet after centrifugation is complete. Place the serum into a plastic transport tube. Label according to guidelines provided in this manual. (See Specimen Identification) Cap and store for pickup.
8. Store blood specimen in refrigerator until delivery to laboratory unless other instructions are given.

Preparation of Plasma

Plasma is obtained from blood that has been mixed with an anticoagulant in the collection tube, and therefore, has not clotted. Always use the correct tube for tests requiring anticoagulant.

1. Fill the tube completely – failure to do so will cause an improper blood to anticoagulant ratio and may result in questionable results.
2. Gently invert the tube after collection to mix the blood with the anticoagulant.
3. After centrifugation of specimen, carefully remove from centrifuge being careful not to disturb the contents.
4. Carefully remove the plasma with a disposable pipet and transfer into a plastic transport tube. Label according to guidelines provided in this manual and also include type of plasma submitted (i.e. citrate, EDTA, heparin etc)
5. Store at required temperature.

Specimen Collection for Cytology

Collection of GYN Smears (Conventional PAP Smears) - One Slide Technique

1. Have fixative available.
2. Print patient's full name on slide using pencil.
3. Take cervical smear with cervical spatula by thoroughly scraping the entire ectocervix, rotating 360° with special emphasis on the squamo-columnar junction. Using a single stroke, spread material evenly on one half of slide and spray fix slide immediately using 3–4 rapid pump sprays 6–8 inches from the slide. **Have other half of slide covered with cardboard while spraying.**
4. Optimal smears are achieved if obtained mid-cycle, not during menstruation, at least 24 hours after douching, use of lubrication or intercourse.
5. Take endocervical smear with cervical brush by rotating clockwise one-quarter turn in the endocervical canal. Smear endocervical material evenly on the other half of slide by gently rolling the brush across the slide counter clockwise once to insure deposition of all cells. Immediately spray fix slide. After preparation, the slide is to be completely dry before being placed in slide container/folder for submission to cytology.
6. For maturation index take smear from lateral vaginal wall using vaginal end of spatula. Spread material evenly over a separate slide and spray fix immediately. Designate this as vaginal.

Collection of Liquid-Based GYN Cytology

Cytec™Thin Prep PAP Preparation

Broom-like Device - Obtain an adequate sampling from the cervix using the broom-like device.

Insert the central bristles of the broom into the endocervical canal deep enough to allow the shorter bristles to fully contact the ectocervix. Push gently and rotate the broom in a clockwise direction five times. Rinse the broom into the PreservCyt Solution vial by pushing the broom into the bottom of the vial 10 times, forcing the bristles apart. As a final step, swirl the broom vigorously to further release material. Discard the collection device. Replace lid and tighten the cap so that the torque line on the cap passes the torque line on the vial. Label the vial with patient first and last name, Social Security number, and date of collection.

Endocervical Brush/Spatula Protocol -

Obtain an adequate sampling from the ectocervix using a plastic spatula. Rinse the spatula into the PreservCyt solution vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula. Obtain an adequate sampling from the endo cervix using the endocervical brush device. Insert the brush into the cervix until only the bottom most fibers are exposed. Slowly rotate ¼ or ½ turn in one direction. Do not over-rotate. Rinse the brush in the PreservCyt Solution by rotating the device in the solution 10 times while pushing against the PreservCyt vial wall. Swirl the brush vigorously to further release material. Discard the brush. Tighten the cap so that the torque line on the cap passes the torque line on the vial. Label the vial with patient first and last name, Social Security number and date of collection.

Collection of Non-GYN Cytology

All body fluid specimens (urine, sputum, cyst fluid, spinal fluid, etc.) should be placed in cytology fixative containers provided by Sentara Reference Laboratory. It is preferable to completely fill a cytology container with well-mixed specimen. Sputum specimens should be deep-cough first-morning specimens. For urine, discard first morning void and collect subsequent void.

Specimens submitted on labeled glass slides – i.e., brushes, lesion scrapings, Tzanck smears, nipple smears and oral smears – must be fixed immediately upon preparation of the slide(s). Scrape the material from the lesion site with a blade or needle onto the labeled slide and then fix immediately. Spray fixative is

applied by using 3–4 rapid pump sprays 6–8 inches from the slide. Slides are to be thoroughly dry before being placed in slide containers/folders for submission to cytology. The recommended number of slides per specimen is 1–4.

Fine Needle Aspiration (FNA)

Fine needle aspiration specimens (FNA) may be submitted as mirror-image sets of slides and/or needle rinses in cytology fixative. When slides are made, it is optimal to submit one to two sets of frosted-end labeled slides, with one of each set air-dried and labeled “AD” and one immediately spray-fixed and labeled “FX.” After preparation all slides are to be completely dry before being placed in slide containers/folders for submission to cytology. Blood clots and/or tissue fragments and needle rinses should be submitted in a labeled cytology fixative container. If the FNA specimen is cyst fluid, place all of the fluid in a labeled cytology fixative container.

Specimen Collection for Cytogenetics

The Cytogenetics Laboratory is open Monday – Friday from 8:00am – 5:00pm.

Cytogenetics is the study of chromosomes and the related disease states caused by numerical and structural chromosome abnormalities. Different types of tissue are used to obtain chromosome preparations. Examples include peripheral blood, bone marrow, amniotic fluid, and products of conception. Although specific techniques differ according to the type of tissue used, the basic requirements for obtaining chromosome preparations is as follows:

Routine Blood/STAT Blood High Resolution Blood

Collect whole blood into a green top, sodium heparin vacutainer.

ADULTS: 3.0 cc of blood

NEWBORN/INFANT: 1.0 –2.0 cc of blood

Amniotic Fluid

Collect 20.0 cc whole amniotic fluid into two sterile conical 15cc centrifuge tubes.

Fibroblast/Product of Conception/Skin Biopsy

Tissue/Skin Biopsy

Collect approximately 0.5cm into sterile specimen cup containing sterile transport medium or Hanks Solution.

Products of Conception – Chorionic villi (approximately 20mg) and/or a tissue sample (0.5cm) such as lung, skin or pericardium in a sterile container cup containing sterile transport medium or Hanks Solution. Must indicate source and gestational age on specimen requisition.

Leukemic Blood

Collect blood into a green top sodium heparin vacutainer. The patient should have a white blood cell count of 15,000 or higher with approximately 10% immature myeloid or lymphoid cells.

ADULTS: 5.0 cc of blood

NEWBORN/INFANTS: 1.0 – 2.0 cc of blood

Bone Marrow

Collect bone marrow in a green top sodium heparin vacutainer or aspirate into a sterile syringe containing 0.1 cc of preservative – free sodium heparin.

Chorionic Villi Samples (on-going pregnancies):

Collect 15 – 30 mg of Chorionic villi in a 15 ml sterile, conical centrifuge tube containing transport media supplied by the Cytogenetics Laboratory.

Labeling - Appropriate information is critical to proper processing of specimens. The following information should be provided on the lab requisition:

1. Patient Social Security number/medical record number
2. Patient name (written exactly as on specimen)
3. Sex
4. Race (required for AFP testing)
5. Date of birth
6. Ordering physician's name
7. Collection date and time
8. Identification of collector
9. Source – anatomical pathology and microbiology or cytogenetics

Please Note:

Specimen should be transported to Cytogenetics Laboratory as soon as possible. If transport is delayed, specimen should be refrigerated, but NOT FROZEN.

Specimen Collection for Microbiology

A culture report is only as good as the specimen that is submitted. Collection is the first step of many to provide the physician with accurate data to treat infections. If collection is done improperly, the final report may be delayed, misleading and inaccurate, resulting in delay of proper treatment. Once collected, a specimen must be transported properly to the laboratory or the quality of the specimen may be compromised.

Labeling - Appropriate information is critical to proper processing of specimens. The following information should be provided on the lab requisition:

1. Patient Social Security number/medical record number
2. Patient name (written exactly as on specimen)
3. Sex
4. Race
5. Date of birth
6. Ordering physician's name
7. Collection date and time
8. Identification of collector
9. Source

Obtain specimen correctly

1. Explain completely to the patient or nurse collecting the specimen.
2. Use a sterile container.
3. Label correctly and send the specimen to the laboratory promptly.
4. Avoid contamination of the container.

Timing of collection

1. Sputum, urine, stool, etc. are best collected in the early morning and sent to the laboratory the same day.
2. Collection is best before treatment has started. Antimicrobial, antifungal, or antimycobacterial agents prior to collection can cause false negative results.

Procedures for Specific Microbiology Specimen Collection

Abscesses or Deep Closed Wounds (also see Wounds section if applicable)

1. The skin surface should be cleaned with alcohol and iodophor to prevent contamination of surface organisms.
2. The best method of collection is aspiration with needle and syringe.
3. All air should be expressed from the syringe, the needle removed and the syringe tightly capped. This is especially important for recovering anaerobes. The syringe should be taken as soon as possible to the laboratory.
4. If it is not practical to transport the syringe, express the aspirate into a sterile aerobic culture container and a proper anaerobic transport system if an anaerobic culture is desired.
5. If a needle aspirate cannot be performed, a culturette and an anaerobic swab collection system can be used for aerobic and anaerobic cultures from an opened abscess. Extend the swab into the depths of the abscess without touching the adjacent skin margins.

Anaerobic – See Alphabetical Test Listing “Culture, Anaerobic.”

Blood – Collection Guidelines

Direct inoculation into the culture bottle greatly reduces the chance of specimen contamination. **Please note that the use of SPS (sodium polyanestholesulfonate) tubes is not acceptable for extended transport of blood culture specimens.** Blood from SPS tubes should be at least 2/3 full and transferred within two to four hours to assure that suspected organisms are viable. Organisms quickly effected by this anticoagulant include Haemophilus influenza, Neisseria meningitis and anaerobic streptococci. Blood cultures should be drawn before initiation of antimicrobial therapy. List current antimicrobial therapy and/or any special organisms suspected.

1. When two separate blood cultures are ordered with no time interval specified, the time interval between cultures should be minimal and each culture should be drawn from a separate venipuncture site.
2. Set blood culture collection bottles upright and mark fill level on each bottle (10 ml for adults, 1-4 ml for pediatric patients).
3. Remove flip off cap from each bottle, wipe tops with alcohol and allow to dry.
4. Aseptically collect 20 ml blood with syringe or butterfly collection set following 2-set disinfection of skin with alcohol and an iodine cleansing product, working in a circular motion with friction. **Do not fan, blow or blot to speed drying. Do not touch or palpate area after cleansing.**
5. Draw and transfer 8-10 ml of blood into the aerobic bottle (green) and 8-10 ml into the anaerobic bottle (purple) for adults. For pediatric patients, transfer 1-4 ml to pediatric bottle (yellow). **Do not overfill. Do not force sample with pressure on syringe plunger, if used.**
6. Transfer at room temperature.

Body Fluids (Sterile) – Including but not limited to pleural, pericardial, peritoneal, amniotic, and synovial.

1. Because most fluids are collected as aspirates, it is important to properly clean the skin site with alcohol and an iodine compound prior to collection.
2. Cleansing should be vigorous and include 1-2 minutes of drying time for the iodine.
3. Usually 1-4 ml of fluid is required for bacteriological studies with an additional 2-4 ml for fungus and AFB studies.
4. All air should be expressed from the syringe, the needle removed and the syringe tightly capped. This is especially important for recovering anaerobes
5. If it is not practical to transport the syringe, express the aspirate into a sterile aerobic (i.e. urine) culture container and a proper anaerobic transport system if an anaerobic culture is desired.
6. All fluids should be transported to the laboratory as soon as possible.

Central Nervous System Specimens – These may include CSF, Ventricular fluid, subgaleal fluid, and shunt fluid.

1. Check all containers to be used for sterility and proper sealing to prevent leakage and contamination.
2. The lumbar puncture site must be cleansed with iodophor or 2% tincture of iodine. Allow this to dry.
3. The physician will collect the specimen aseptically.
4. At least 1-2 ml is suggested for bacteriological studies with an additional 2-4 ml for fungus and AFB studies. It is suggested that the patient not be on any antimicrobial, antifungal, or antimycobacterial agents prior to collection as these can cause false negative results.
5. These fluids should be transported to the laboratory as soon as possible.

Ear

1. Culture the canal wall with a saline moistened sterile swab prior to disinfection to compare to middle ear cultures.
2. Clean the canal wall with 70% alcohol, povidone iodine, or benzalkonium chloride. Allow solution to sit for one minute. Iodine solutions should be removed with sterile saline to prevent growth inhibition.
3. To obtain fluid from the middle ear, an 18 gauge spinal needle attached to a syringe may be inserted through the inferior portion of the tympanic membrane.
4. After collection, express all air from the syringe, remove the needle, tightly cap with a rubber stopper and deliver immediately to the laboratory.
5. Alternatively, a nasopharyngeal swab may be inserted into the myringotomy wound under direct vision through an alcohol-cleaned otoscope.

Eye

1. Swabs can be used to collect external specimens and drainage, but scrapings are the best specimens. Cotton or polyester swabs are recommended.
2. Scrapings are obtained using a Kimura spatula.
3. Aspirates may also be obtained by needle and syringe. Do not send syringe with needle still attached.

4. The physician often immediately inoculates ocular specimens to media at the bedside. If this is not possible, swabs and aspirates can be submitted as outlined above. All specimens should be transported to the laboratory as soon as possible.

Genital

1. Males
A thin sterile swab is inserted approximately 2 cm into the lumen of the urethra and gently rotated to obtain the specimen.
2. Females
 - a. Vaginal and urethral specimens may be useful in certain clinical situations, however, a cervical specimen is preferred for the recovery of *Neisseria gonorrhoeae*.
 - b. Cervical specimens should be collected by inserting a sterile bivalve speculum into the cervix.
 - c. Cervical mucus should be removed.
 - d. The cervix is compressed with the speculum blades to produce an exudate, which can be collected with a sterile swab.
 - e. If there is no exudate, the swab can be rotated to collect exudate from the endocervical glands.
3. Transportation of Genital Specimens
 - a. Insert the swab into transport media to prevent drying of the swab.

Do Not Refrigerate! Submit specimen swabs with transport media to lab within 12 hours.

Mycological (Fungal) Studies on Hair, Skin and Nail Scrapings

See separate Hair, Skin and Nail Collection procedures found in this section.

Nasopharyngeal specimen

For RSV testing, Rapid Flu A&B Antigen, *Bordetella pertussis* and other cultures, see separate Nasopharyngeal Specimen Collection procedures.

Throat

1. A cotton, Dacron, or alginate swab should be used to collect the specimen. **If specimen is for Rapid Strep A Screen, DO NOT use calcium alginate swabs or systems containing semi-solid transport media or charcoal.**
2. The tongue should be depressed and care taken not to touch the tongue or the uvula with the swab.
3. Vigorously swab the tonsillar fossae, posterior pharynx and any inflamed or ulcerated areas.
4. Insert the swab in a holding media for transportation.

Tissue

1. Tissue specimens should be as large as possible for the recovery of small numbers of organisms.
2. Place specimen in a sterile (i.e. urine) container. A small amount of sterile saline may be added to prevent drying.
3. A portion should be placed in a container that will maintain anaerobic conditions.

4. These specimens should be transported to the laboratory as soon as possible for processing to prevent drying of the tissue.

Sputum

1. The patient should be instructed to dry the mouth out with gauze and make every effort not to contaminate deep sputum with saliva. Alternatively, rinsing the mouth with saline or water (but not mouthwash) may reduce contamination with normal oropharyngeal flora.
2. Encourage deep cough with expectoration of the sputum into a sterile container.
3. If a fungus is suspected, request a fungal culture.
4. When the patient is unable to cough productively, the physician should be notified. An alternative method may be ordered, such as:
 - a. Induced sputum. This is done by a respiratory therapist on the orders of the physician. Involuntary deep coughing is induced by irritation.
 - b. Tracheal aspiration. The trachea is gently irritated with a small lumen suction catheter, which causes deep, productive coughing. Also, the specimen may be aspirated with a syringe.
 - c. Bronchial washings. The physician collects this specimen at the time of bronchoscopic examination.

Stool

1. Stool specimens should be collected during the acute stage of the disease as it will ensure the highest concentration of the infectious agent. A single stool sample cannot be used to rule out bacterial diarrhea and more than two samples should be submitted only if bacterial infection is highly suspected. Multiple specimens may be necessary to recover ova and parasites, therefore three specimens collected over a week are recommended. Consider *C. difficile* toxin testing for patients > 6 months of age with diarrhea and a history of antibiotics.
2. A clean container should be used for collection and transportation to the laboratory.
3. Stool specimen should be placed in the proper transport media.
 - a. For routine *Campylobacter* culture and Shigatoxin testing – place the specimen in liquid Cary-Blair media. **Please note a rectal swab is not acceptable. A minimum amount of 100 mg (walnut size stool) is required.**
 - b. For parasites – place the specimen in PVA and formalin transport vials within 1 hour. Additional stool to bring fluid to indicator line.
 - c. For Pinworm Paddle Collection), hold the paddle by the cap and remove it from the tube. Separate the buttocks and press the tacky surface against several areas of the perianal region. Replace the paddle in the tube for transport to the laboratory. Specimen should be refrigerated if exam is to be delayed for more than one day.
 - d. For *C. difficile* – place the specimen in a clean container and refrigerate. **Please note: A rectal swab is not acceptable. A minimum amount of 100 mg (walnut size) is required.**

Urine – Clean Catch

Urinalysis specimen should be sent in tubes, not in blue cap collection cup. Clients using the Vacutainer brand Urine Collection and Transport Kit Stock #364956 should send specimens to the lab in the 8 ml yellow top tube for urinalysis and the 5 ml gray top tube for culture and sensitivity. The blue screw cap specimen cup is for patient collection only, and should not be sent to Sentara Reference Laboratory.

1. The first morning specimen most accurately reflects the true number of organisms present.
2. Females

- a. The patient must wash hands well.
 - b. If patient is menstruating, instruct her to first insert a fresh tampon or use cotton to stop the flow.
 - c. Use one hand to separate the skin folds around the urinary opening and use the other hand to wash the area from front to back with liquid soap or gauze. Rinse well with sterile water. Commercially prepared collection kits contain antiseptic wipes to be used in place of the soap.
 - d. Keeping the skin folds apart with one hand, void a small amount of urine before collecting the specimen. Then the midstream urine is collected in a sterile container using the free hand. Instruct patient in keeping container sterile – not to touch the container to the genital area.
 - e. At least 5 ml of urine is best, however smaller amounts are acceptable.
 - f. If there will be a delay in transportation to the laboratory, refrigerate specimen or specimen may be placed in special tubes which contain chemicals to preserve the specimen.
3. Males
- a. The periurethral area is cleaned as above.
 - b. Void a small amount of urine before collecting the specimen. Then the midstream urine is collected in a sterile container. Instruct the patient in keeping container sterile – not to touch the container to the genital area.
 - c. At least 5 ml of urine is best, however smaller amounts are acceptable.
 - d. If there will be a delay in transportation to the laboratory, refrigerate specimen or specimen may be placed in special tubes which contain chemicals to preserve the specimen.

Wounds

1. A moistened swab should be used to collect a culture from a wound. If anaerobes are suspected, an anaerobic collection system should be used also.
2. Cleanse the surface with alcohol.
3. Collect the sample from within the wound and not on the wound surface. Extend the swab into the depths of the wound without touching the adjacent skin margins.
4. The swab should be in a transport media for transportation to the laboratory.

LIMITATIONS

- ◆ All specimens should be transported to the laboratory as soon as possible after collection.
- ◆ Transport or holding media should be used where indicated.
- ◆ Contact Client Services at (757) 388-3621 for additional questions concerning specimens.
- ◆ All culture containers must be tightly sealed and have no contamination on the exterior.
- ◆ All culture containers must be properly labeled with barcode or number sticker from requisition and patient name, patient Social Security number or patient identification number, date and time of collection and source of collection.

Hair, Skin and Nail Scrapings Collection

Collection of Hairs

1. The patient's scalp should be examined under a Wood's light to detect fluorescing hairs. It should be noted that not all fungi that invade hair would cause fluorescence. Hairs that are fluorescent, distorted, or fractured should be removed with forceps for culture. Hairs that

are infected are loose in the follicle and are easily removed. In instances where the patient is a young child who will not allow the epilation of hairs with forceps, the placing of a strip of transparent tape over the lesion and then removal of the tape will often serve as an excellent method for obtaining infected hairs. Culture of the basal portion of the infected hair is recommended. Place at least 10-12 hairs in a sterile container.

Collection of Skin Scrapings

1. Lesions should be untreated with topical antifungal agents for at least 1 week before culturing.
2. Lesions of glabrous skin due to dermatophytes are usually circinate, having a vesicular border with healing taking place in the center portion.
3. The lesion should be rubbed briskly with an alcohol gauze square (do not use cotton ball) and the entire periphery is then scraped with a sterile scalpel. Place scrapings in a sterile container.
4. If a lesion is markedly inflamed or contains fissures, the lesion may be cleaned with a gauze square moistened with sterile water rather than alcohol. The lesion must be cleaned in some manner in order to reduce the number of contaminating bacteria and fungus spores, which may be residing on the skin surface.
5. Although *Candida* species are not considered to be dermatophytes, they are often the etiological agents for skin lesions. Skin lesions due to *Candida* species are usually moist, erythematous, painful and may be covered with flakes of dried exudate. In many instances, it is not possible to clean these lesions without great discomfort to the patient. The periphery of the lesion should be scraped and dried exudate removed with forceps. As a last resort, a swab may be used to collect any moist exudate present. Do not use a swab to collect material from a lesion due to dermatophyte.
6. A skin biopsy specimen is also acceptable and should be transported in a sterile container moistened with sterile water or saline.

Collections of Nail Scrapings – Nail clippings are not acceptable.

1. Fingernail
 - a. Wipe the nail with an alcohol gauze. Have the patient rest the ball of the finger on the edge of a clean glass slide or on the inside surface of a clean container.
 - b. Shave the nail with a sterile scalpel, going from the proximal to the distal end. Discard the first few scrapings since contaminating bacteria or fungus spores may still be present. Scrape deeply enough to yield adequate infected nail tissue for cultures and microscopic studies. Debris under the nail may also be collected with the scrapings.
 - c. If more than one nail is to be scraped, USE A FRESH SCALPEL FOR EACH NAIL.
2. Toenail
 - a. Clean the nail as outlined above.
 - b. Sit in a chair facing the patient. Place a clean towel on the knee and request the patient to rest the heel of the foot on the clean towel.
 - c. Scrape the toenail as outlined above.

Transport of Specimens for Mycological Studies

1. Epilated hairs, skin and nail scrapings should be placed in a sterile container. Room temperature storage of these specimens is sufficient if there is any delay in transport.

2. Label the specimen with patient name, patient Social Security number or patient identification number, source of the specimen, date and test requested. Send all specimens to the laboratory as soon as possible.

Nasopharyngeal Specimen Collection

Labeling - Appropriate information is critical to proper processing of specimens. The following information should be provided on the lab requisition:

1. Patient Social Security number
2. Patient name (written exactly as on specimen)
3. Sex
4. Race (required for AFP testing)
5. Date of birth
6. Ordering physician's name
7. Collection date and time
8. Identification of collector

Obtain specimen correctly

1. Explain completely to the patient or nurse collecting the specimen.
2. Use a sterile container.
3. Label correctly and send the specimen to the laboratory promptly.
4. Avoid contamination of the container.

Timing of collection

Collection is best before treatment has started. It is suggested that the patient not be on any microbial, antifungal or antimycobacterial agents prior to collection as those can cause false positive results.

Procedures for specific specimen collection

For best sample quality, repeating procedure on each nostril will deliver the optimal combined sample. Optional: perform nasopharyngeal swab procedure prior to wash procedures. Samples may be combined.

Nasal Wash, Bulb Method

Materials:

Saline

1–2 oz. tapered rubber bulb (size of bulb as appropriate for infant, child, or adult)

Sterile specimen container

1. Suction 3–5 ml saline into a new sterile bulb.
2. Insert bulb into one nostril until nostril is occluded.
3. Instill saline into nostril with one squeeze on the bulb and immediately release bulb to collect recoverable nasal specimen.
4. Empty bulb into suitable dry, sterile specimen container.
5. Send specimen to the laboratory promptly.

2. Nasal Wash, Syringe Method

Materials:

Saline

3–5 ml syringe*
 2" 18–20 gauge tubing*
 Sterile specimen container

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

Patient Age	Catheter Size (French)**	Suction Pressure
Premature Infant	6	80-100 mmHg
Infant	8	80-100 mmHg
Toddler/Preschooler	10	100-120 mmHg
School Age	12	100-120 mmHg
Adolescent/Adult	14	120-150 mmHg

**To determine the length of catheter tubing, measure the distance from tip of nose to external opening of ear.

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. As an alternate in appropriate cases, patients may tilt head forward to allow specimen to drain into suitable sterile container.
4. If aspirated, inject aspirated specimen from syringe into suitable dry, sterile specimen container.
5. Send specimen to the laboratory promptly.

Nasopharyngeal Swab Method

Materials:

Nasopharyngeal swab with synthetic Dacron polyester fiber tip

1. Insert swab into one nostril.
2. Press swab tip on the mucosal surface of 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 and insert into the correct transport device.

Vacuum–assisted Nasal Aspirate Method

Materials:

Portable suction pump
 Sterile suction catheter
 Mucus trap (i.e., Luken's tube)

1. Attach mucus trap to suction pump and catheter, leaving wrapper on suction catheter; turn on suction and adjust to suggested pressure.
2. Without applying suction, insert catheter into the nose, directed posteriorly and toward the opening of the external ear. Note: Depth of insertion necessary to reach posterior pharynx is equivalent to distance between anterior naris and external opening of the ear.
3. Apply suction. Using a rotating movement, slowly withdraw catheter. Note: Catheter should remain in nasopharynx no longer than 10 seconds.
4. Hold trap upright to prevent secretions from going into pump.
5. Rinse catheter (if necessary) with 2.0 ml sterile saline; disconnect suction, connect tubing to arm of mucus trap to seal.
6. Send specimen to the laboratory promptly.

LIMITATIONS

- ◆ All specimens should be properly transported to the laboratory.
- ◆ Contact Client Services at (757) 388-3621 for additional questions concerning specimens.
- ◆ All culture containers must be tightly sealed and have no contamination on the exterior.
- ◆ All culture containers must be properly labeled with barcode or number sticker from requisition and patient name, patient Social Security number or patient identification number, date and time of collection and source of collection and initials of person who collected specimen.

Urine Collection

Collection kits are provided for the collection of urine samples for culture and/or routine testing.

NOTE: The blue screw cap specimen cup is for patient collection only, and should not be sent to the Sentara Reference Laboratory. The collection cup is prone to leak when used for transport and may render the sample unusable for analysis.

Processing Urine Samples with BD Vacutainer™ Collection Products

Plain UA Tube and Culture & Sensitivity (C&S) Preservative Tube, BD Ref #364956

Kit contains: One 4.5 oz. Specimen cup with sampling device; One 8 ml conical shaped Yellow-top tube for urinalysis; one 5 ml Grey-top tube for C&S; and one cleansing towelette.

Kit uses:

- ◆ for routine urinalysis collection only;
- ◆ for urine culture and sensitivity collection only;
- ◆ for collection of both routine urinalysis and C&S collection;
 1. Peel Back protective sticker to expose rubber-covered cannula (sampling device)
 2. Push C&S Preservative Tube (gray top) into the transfer port sampling device. Hold in position until flow stops, remove tube, shake tube vigorously.
 3. Push UA Preservative tube (Yellow top) into transfer port sampling device. Hold in position until flow stops, Remove tube, Invert UA Preservative tube 8-10 times to mix the sample.
 4. Place protective sticker back over the transfer port. Label both filled tubes with patient's name and the date & time of collection
 5. Remove lid from cup and dispose in sharps collector. Dispose of urine according to your facility's policy. Dispose of collection cup as a biohazard

Urine Transfer Straw Kit with C&S Preservative Tube – C&S only, BD Ref #364953

Kit Contains: One transfer Straw and one 5ml gray top tube for C&S

Used for collection of culture and sensitivity only

1. Submerge tip of transfer straw to bottom of urine container.
2. Place C&S preservative tube (gray top) in holder on transfer straw. Push tube all the way into holder.
3. Hold in position until flow stops.
4. Remove tube from transfer straw. Shake tube vigorously and label with patient name and the date & time of collection.
5. Lift transfer device and allow urine to draw from straw. Dispose of transfer straw in sharps container

24-Hour Urine Collection

For proper evaluation of tests on a 24-hour urine sample, it is important for patients to make a complete and accurate collection. Sentara Reference Laboratory recommends the following collection instructions for patients:

1. Empty your bladder when you get up in the morning and discard this urine. Record this start date and time on your collection jug.
2. Use the jug to collect all urine you pass during the day and night. You may collect each urine passing in a urine cup and pour it into the jug.
3. Make your final urine collection when you empty your bladder the next morning at the same hour. Record this end date and time on your collection jug.
4. Keep your urine refrigerated, if possible, and bring it to the laboratory or office as soon as possible after your 24-hour collection is complete.
5. Some tests require accompanying blood work. Please verify whether a blood specimen is needed when dropping off your urine specimen.
6. Note that medications may interfere with your test.

Although normal fluid intake is allowed during 24-hour urine collections, dietary restrictions are required for some procedures and are specified in the test listing. The laboratory technologist will initiate creatinine analysis if the validity of the specimen is questioned. If these findings are deemed critical, they will be reported. Since results are based on total volume, it is critical that volume is measured accurately and the information is included with the test requisition. Patient's height and weight must be indicated on the laboratory requisition whenever a clearance is requested.

Collection of Two Consecutive 24-hour Urine Samples

First 24-hour Urine - Collect in Jug with preservative

1. Empty your bladder when you get up in the morning and discard this urine. Record this start date and time on your collection jug.
2. Use the jug to collect all urine you pass during the day and night. You may collect each urine by passing into a urine cup, and carefully pouring it into the jug. Urine passed during bowel movements must be collected.
3. Make your final urine collection for this jug when you empty your bladder the next morning at the same hour. Record this end date and time on your collection jug.

Second 24-hour Urine - Collect in jug with no preservative or a second preservative

1. Record the date and time of the first urine of the day. This first urine is the same specimen included in the jug with preservative (See Step 3 listed above).
2. From now on, all urine passed for the next 24 hours is to be included in the second container. Do not mix urines from one day of collection to another.
3. You may collect each urine by passing into a urine cup and carefully pouring it into the jug. Urine passed during bowel movements must be collected.
4. Make your final urine collection when you empty your bladder the next morning at the same hour. Record this end date and time on your collection jug.

All urines should be stored in the refrigerator from the time collection begins until the containers are returned for testing. It is important not to allow the urine from one container to mix with urine from the other container.

Aliquots

If sending a 100 ml aliquot rather than an entire collection, please measure the total volume of the specimen (before removing aliquot) and write the volume and number of hours of the collection period on the test requisition. Also be sure to properly label the aliquot with the barcode or number sticker from the requisition along with patient name, social security number, volume of specimen and preservatives included, if applicable.

Note: For proper preservations and special collection requirements, see the **Alphabetical Test Listing** chapter of this manual.