Specimen Collection

Valid laboratory results are dependent upon proper specimen collection and handling prior to the arrival of the sample in the laboratory. Clinical Reference Laboratory (CRL) receives samples from four (4) different sources: insurance examiners, clinics, clinical trials, and in-house collections. The following is a basic phlebotomy procedure, followed by procedures specific for each type of collection.

Note: There are business specific and in-house requirements for filling out requisition/consent forms and for collections. Refer to appropriate business unit Appendix and/or procedures.

PHLEBOTOMY

1) Wash hands thoroughly before beginning any phlebotomy procedure. Be sure to check expiration dates on tubes before proceeding. DO NOT USE EXPIRED TUBES.

2) Confirm the identity of the patient by checking at least two identifiers before collecting the specimen(s). This can be done by asking the patient to state their full name and requesting to see the patient's driver's license to verify picture, name, date of birth and/or drivers license number and documenting the information on the consent/chain of custody form.

3) Explain the procedure, including small risk of hematoma, slight pain, and some light-headedness. Inquire whether the patient has a history of fainting or dizziness with phlebotomy procedures so that ammonia inhalants can be obtained if necessary. Explain that loss of vacuum or a collapsed vein may necessitate another draw.

4) On a table or desk, assemble all necessary equipment: cotton balls and/or gauze, tubes, safety needle, alcohol swab, tourniquet, gloves, and bandaid. Wearing safety gloves is MANDATORY. Wear additional protective equipment if contamination is expected. Safety needles should always be used; the only exception is if the patient is very hard to draw then a butterfly needle set may be used.

5) Position the patient so that they are seated comfortably in a chair with their arm extended on an armrest, desk, or table to form a straight line from the shoulder to the wrist. The patient’s arm and elbow should be firmly supported, and not bent at the elbow.

6) Check both arms to select the larger and fuller veins. Palpate and trace the path of the veins several times with your index finger. Tap the vein at the site of the draw with your index finger and second finger. This will cause the vein to dilate.
   The following factors should be considered in site selection:
   i) Extensive scarring. Healed burn areas or scar tissue should be avoided.
   ii) Specimens collected from an area with a hematoma may yield erroneous test results. If another vein site is not available, the specimen should be collected distal to the hematoma.
7) Apply the tourniquet.

8) Ask the patient to open and close his/her fist so their veins become prominent. Vigorous hand pumping is not necessary to activate blood flow and should be avoided.

9) Clean the venipuncture site with the alcohol swab in a circular motion from the center of the area to the outside. Allow the area to air-dry to prevent hemolysis and a burning sensation to the patient.

10) Insert the stopper of the first tube to be drawn into the adaptor. Do not push too far to avoid premature loss of vacuum via puncture of the needle.

   The recommended order of draw when drawing more than one tube is as follows:
   ♦ Non additive tube (red stopper)
   ♦ Coagulation tube (light blue stopper)
   ♦ Serum separator tube (SST) or serum tube
   ♦ Additive tube (lavender stopper, green stopper, etc)

11) Insert the needle into the vein with the bevel facing upward. Puncture the stopper on the tube by pushing it onto the end of the needle, and grasp the edge of the adaptor to provide stability once the blood flow has begun. Have the patient open his/her fist.

12) Fill the tube until the vacuum is exhausted. Remove the tube from the adaptor and insert subsequent tubes. Be sure that all tubes are completely filled to ensure sufficient blood sample for laboratory analysis.

13) Place a cotton ball or 2 x 2 square piece of gauze over the site. All used needles must be disposed of in a puncture proof biohazard receptacle. Never recap a needle. Recapping, purposeful bending, breaking, removing from disposable syringes, or other manual manipulations of needles is prohibited. Apply pressure to the site for 2-5 minutes. Place a bandaid over the puncture site.

14) Again verify that the information on the sample tubes match the consent/requisition form.

15) Remove gloves and dispose of in a properly identified biohazard bag or container. Wash hands thoroughly after phlebotomy.
ADDITIONAL VENIPUNCTURE CONSIDERATIONS

1) Prevention of Hematoma:
   a) Puncture only the uppermost wall of the vein
   b) Release the tourniquet before removing the needle from the vein.
   c) Use only major veins; not superficial veins
   d) Make sure that the needle fully penetrates the uppermost wall of the vein. Partial penetration may allow blood to leak into the soft tissue surrounding the vein by way of the needle bevel.
   e) Apply a small amount of pressure to the area with the cotton ball or gauze pad when bandaging the arm.

2) Prevention of Hemolysis:
   a) Mix anticoagulated specimens thoroughly by inverting each tube gently 8 to 10 times. Do not shake. Vigorous mixing may cause hemolysis.
   b) Avoid drawing blood from an area with a hematoma.
   c) Ascertain that the venipuncture site is dry without touching it.

3) If a Blood Sample is Unobtainable
   a) Change the position of the needle. If the needle has penetrated too far into the vein, pull it back slightly. If it has not penetrated far enough, advance it farther into the vein. Rotate the needle a half-turn.
   b) Try another tube; the tube may not have sufficient vacuum.
   c) Loosen the tourniquet. It may be applied too tightly, thereby stopping the blood flow. Reapply the tourniquet loosely. This procedure can be accomplished easily when using the velcro-type tourniquet by releasing it and quickly pressing it together again.
   d) Probing for the vein is NOT recommended as it is painful to the patient. In most cases, another puncture in a site below the first site is advised.
   e) Never attempt a venipuncture more than twice. Have another person attempt to draw the specimen.
**SPECIMEN HANDLING**

1) Gently invert SST tubes 5 times and all tubes with anticoagulant (EDTA, heparin, etc.) 8 to 10 times.

2) Ensure all tubes are labeled with identification number and second identifier (printed first and last name for Insurance applicants).

3) Let red-top and marbled tubes clot, preferably in an upright position, for 30 minutes, but not more than 45 minutes. Centrifuge the tube for 5-10 minutes at 2500-3500 rpm and transfer the serum into a properly labeled pour-off tube using a disposable pipette.

4) Some other factors that can affect the sample are:
   a) **Hemolysis.** Hemolysis is defined as the breaking down of red blood cells. This can be slight, moderate or severe. The three (3) causes of hemolysis are TIME, TEMPERATURE AND TRAUMA.
      i) **TIME:** Holding blood over two (2) hours before centrifuging can and usually does cause some hemolysis. Allow at least 10-20 minutes, but no more than 45 minutes for the blood to clot prior to centrifuging.
      ii) **TEMPERATURE:** Never store blood in too warm an area; hot cars, hot sun, etc. Allowing blood to freeze in cold weather will also produce hemolysis.
      iii) **TRAUMA:** Going through the vein, accessing a collapsed vein, or using a needle that is too small can all cause hemolysis. The needles provided by the labs are usually 21 or 22 gauge. If a 23 gauge is used, it is very possible that hemolysis may occur. Only use a smaller needle when absolutely necessary. When doing fingersticks, DBS, etc., squeezing the finger is the main cause of hemolysis.
   b) **Lipemia:** Lipemia is defined as an abnormal amount of fat in the blood. This is usually caused by the patient not fasting.
**URINE COLLECTION - Routine UA**

To collect a routine urinalysis a clean mid-stream specimen should be obtained.

1) Explain carefully to patients the mechanics of midstream collection and the importance of collecting an uncontaminated specimen. Teach them how to handle the specimen container to keep it free of contamination.

2) A clean-catch specimen is necessary to confirm the presence or absence of infecting organisms in urine. The specimen must be free of any contaminating matter that might be present on the genital organs; therefore, patients should be urged to follow the steps below.

**Instructions for the Female Patient:**

1) If you are menstruating, first insert a fresh tampon or use cotton to stop the flow.
2) Separate the skin folds around the urinary opening.
3) Wash the urinary opening and its surroundings from front to back with a sterile antiseptic pad.
4) Begin urinating into the toilet, making sure you keep the skin fold apart with the fingers of one hand.
5) Wait until the urine stream is well established before moving the container into the path of the stream to catch the rest of the urine. Do not touch the container to the genital area.
6) After collecting urine specimen in the cup provided, document urine temperature on the consent, if required, and pour off into the urine transport tubes provided.
7) Seal the top of the non-barcoded urine tube(s) with security seal signed by donor.

**Instructions for the Male Patient:**

1) Wash the end of the penis well with soapy water. Let it dry.
2) Begin urinating into the toilet. Wait until the urine stream is well established before moving the container into the path of the stream to catch the rest of the urine. Do not touch the container to the genital area.
3) After collecting urine specimen in the cup provided, document urine temperature on the consent, if required, and pour off into the urine transport tubes provided.
4) Seal the top of the non-barcoded urine tube(s) with security seal signed by donor.
DRIED BLOOD SPOT COLLECTION
Please complete ALL information on the consent/requisition before performing collection. For Insurance testing, make sure the applicant has signed the Notice and Consent & Chain of Custody Statement appropriately AND has signed the security seal at the bottom of the consent. Read directions for specimen handling carefully.

Fingerstick Collection Instructions
1) Have the applicant wash his/her hands in warm, soapy water. Rinse and dry completely.

2) Thoroughly clean site of skin puncture with alcohol swab and allow finger to air dry. Remove collection card from envelope.

3) Puncture finger firmly with lancet near the tip but slightly to the side. It is important to obtain a free flow of blood without excessive squeezing, as this may dilute the blood with tissue fluid.

4) With the applicant’s/donor’s arm slanting downward, palm upright, add drops of blood to the small rectangular section of the card until the strip turns red at the first (dark) line. Do not touch the collection strip in the middle of the card. Lay the card on a clean, dry surface while placing blood on the card.

5) When the card is filled per above, apply pressure to fingertip with alcohol swab until bleeding stops.

6) Place adhesive bandage over puncture site.

7) Do not attempt to re-cap lancet. Place used lancet in lancet disposal tube, dispose of lancet disposal tube per your company’s protocol. NEVER RETURN A USED LANCET!

8) Allow blood on card to air dry for at least 20-30 minutes. Place card into Blood Sample Return Bag and seal. Place the signed security seal from the consent over sealed end of Sample Return Bag.

9) PLEASE BE SURE TO INCLUDE THE SIGNED AUTHORIZATION WHEN SENDING SPECIMENS TO THE LAB.
ORAL FLUID COLLECTION
Each kit contains:
♦ Oral collection devise
♦ Authorization Form with Tamper Evident Tape (to be signed by applicant)
♦ Subject Information Pamphlet
♦ Absorbent pad for shipping
♦ Pre-addressed & pre-paid shipping box

An Oral collection device, such as Epitope's OraSure, consists of a Collection Pad (looks like a flat toothbrush) and a Specimen Vial. The vial contains a blue, non-toxic liquid which preserves the specimen during shipment to the laboratory.

Documenting Your Collection
1) Carefully explain each step of the procedure to the proposed insured prior to collecting the specimen.
2) Complete the Authorization Form (be sure to indicate the insurance company).
3) Have the proposed read, sign, and date the Consent section of the Authorization Form.
4) When collecting specimens for HIV testing, provide the insured with the pamphlet entitled Subject Information.

Collect the Specimen
1) Open the outer package to access the Collection Pad.
2) Carefully open the individually packaged Collection Pad. Be sure to peel open the packaging far enough to sufficiently expose the pad to allow for easy removal.
3) Offer the Collection Pad, handle end first, to the proposed insured, instructing them to remove the Collection Pad by grasping the handle.
4) Instruct the proposed insured to place the pad portion between their lower cheek and gum, and to gently rub back and forth until the pad is moist.
5) Instruct the proposed to leave the pad in their mouth. Keep track of the time the pad is in their mouth.
6) The pad must stay in the proposed insured's mouth at least two minutes and no longer than five minutes.
7) While timing the two minutes, remove the Specimen Vial from the outer pouch. Write the date, proposed insured's name, and their social security or driver license number on the specimen vial.
Prepare Specimen for Shipping
1) Holding the vial upright, with the tip pointed down, carefully remove the cap from the vial using a rocking motion.
2) After two minutes, ask the proposed insured to remove the Collection Pad from their mouth.
3) Insert the Collection Pad into the vial while holding the handle.
4) Push the Pad all the way to the bottom of the vial.
5) Ask the proposed insured to bend the handle against the inner lip of the vial until the upper half of the nylon handle breaks off.
6) Replace the cap, making sure you hear the snap that means the cap is properly sealed in the vial.
7) Secure the Sample
8) Have the proposed insured initial and date the Tamper-Evident Tape.
9) Initial and date the tape yourself.
10) Carefully place the Tamper-Evident Tape over the vial cap and avoid covering the identification section of the Specimen Vial
11) Be sure to protect the vial from excessive temperatures. ORAL FLUID collections should be protected from temperatures over 98 degrees F.

Ship the Sample to the Laboratory
1) Wrap the absorbent pad around the specimen vial to absorb any leakage.
2) Place the specimen vial and absorbent pad in the return envelope that has been provided.
3) Verify all information is complete on the Authorization Form AND that the proposed insured has signed the Chain-of-Custody section of the Authorization Form.
4) Sign and date the collector portion of the Chain-of-Custody section of the Authorization Form. Place the top copy (original) of the Authorization Form in the return envelope with the oral fluid collection.
5) Seal the return box and send to the laboratory by US Mail within 24 hours. Sample must be received within 20 days of collection.
6) Distribute the remaining copies of the Authorization Form as noted at the bottom of the form.
BACTERIOLOGY SPECIMEN COLLECTION
Collection of Specimens for Culture: General Information
1) Labeling. Appropriate information is critical to proper processing of test requests. Although pertinent clinical information is highly desirable, if it is not available, please provide at least the following information
   a) Patient’s name and second unique patient identifier
   b) Source of specimen or collection site
   c) Date
   d) Specimen
   e) Test desired

2) Obtain specimen correctly.
   a) Explain completely to the patient.
   b) Use a sterile container
   c) Label correctly and send the specimen to the laboratory promptly
   d) Avoid contamination of the container

3) Timing of collection
   a) Sputum, urine, stool, etc. are best collected in early morning and sent to the laboratory the same day.
   b) Blood
      i) A blood culture requires two bottles of blood — one for aerobic and one for anaerobic culture. Each blood culture should be collected from a separate venipuncture.
      ii) Collect blood specimens before treatment is initiated, if possible.
      iii) Collect two or three sets early in the illness; repeat if they are negative after 48 hours of growth.
      iv) Organisms are continuously shed during intravascular infections, such as endocarditis, but they are intermittently shed during occult infections. In some instances of occult infection, there is a predictable fever pattern. If this is the case, the blood for culture is best collected 30 minutes prior to the fever spike.
      v) The yield beyond three or four cultures is minimal in most circumstances, and collection of more than this is discouraged.

Procedure for Specific Specimen Collection
Upper Respiratory Tract. This section describes procedures for obtaining culture specimens from the nasopharyngeal area and the throat.
   1) A nasopharyngeal culture is obtained by inserting a thin sterile swab gently through the nose to touch the pharynx; gently rotate and remove.
   2) A throat culture is obtained by introducing a sterile swab into the mouth. Use a tongue blade to avoid contaminating the specimen with oral secretions. Firmly swab both tonsilar fossae, posterior pharynx, and any inflamed or ulcerated areas.
**Lower Respiratory Tract Sputum.** This section discusses sputum cultures, including such alternatives as induced sputum, tracheal aspiration, and bronchial washings.

1) 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 specimen collection cup that is labeled with the patient's name and second patient identifier.
3) Do not send saliva (spit) for culture.
4) When the patient is unable to cough productively, notify the physician. 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. These are done by the physician in the operating room at the time of bronchoscopic examination.
5) A small amount of sputum is all that is required, but it must be sputum and not oral secretions.

**Specimens of Wound Exudate.** Follow these steps for using a sterile transport swab in collecting wound exudate specimens.

1) Gently cleanse the area, using dry, sterile gauze to remove any contaminants.
2) Using a sterile red-stopper swab culture collection system, introduce deeply enough to obtain a moist specimen; replace the swab in the container. Do not break the container.
3) Store at room temperature.

**Stool for Culture.** When collecting stool specimens, follow these guidelines.

1) A small amount is all that is required, about the size of a walnut. If several different types of cultures are requested, submit a walnut-sized sample for each. Place the specimen in transport medium or in a sterile leakproof container.
2) When stool cultures are not readily obtainable, rectal swabs are acceptable; however, it must be indicated whether the specimen is a stool or a rectal swab.

**Use of Sterile Swab (Red-Stopper) Collection Kit**

The swab system is guaranteed sterile until the seal is broken. Directions for use:

1) Peel open and remove the swab from the package.
2) Remove the cap/swab stick from the tube.
3) Collect the appropriate specimen and put the cap/swab into the tube. Push the cap to bring the swab into contact with the transport medium.
4) Print the patient's name, second patient identifier and the culture site on the specimen tube.
5) Place the specimen in a zip-lock bag and put the completed test request form in the side pouch.
6) Store it at room temperature.
7) Send specimen to the laboratory.
**APPENDIX A - INSURANCE COLLECTION**

Note: Eight (8) hour fast is recommended prior to blood collection.

1) The following must be filled out on the requisition form found in the collection kit.
   - insurance company name
   - type of insurance (take from exam order)
   - agency name taken from exam slip (if not available, leave blank)
   - agent name / number
   - applicant name (verify correct spelling of name with applicant)
   - applicant date of birth
   - social security number (optional-per carrier instructions)
   - gender
   - applicant's driver's license number
   - picture verified
   - applicant address, city and state are essential
   - last food and drink (date and time)
   - date and time specimen was obtained
   - urine temperature
   - smoking questions must be answered
   - exam company name, city, state and phone number
   - applicant's signature and date
   - examiner's name and examiner's signature and date

2) The applicant must read and sign the authorization slip. Do not draw specimen if applicant declines to sign or alters the slip.

3) Confirm the identity of the applicant by checking at least two identifiers before collecting the specimen(s). This can be done by asking the applicant to state their full name and requesting to see the patient's driver's license to verify picture, name, date of birth and/or drivers license number and documenting the information on the consent/chain of custody form.

4) **VERY IMPORTANT** - Make sure that no blood drawing equipment is left at the applicant's home or place of business. Discard needle and gloves in approved biohazard containers.

5) A urine sample is required to be sent with all blood profile kits.

6) Have applicant collect sample in cup provided. See Urine Collection procedure below.

7) Record temperature of urine on requisition slip.

8) Pour urine into appropriately labeled urine pour-off tubes.

9) Tamper-evident tape must have: applicant's signature and date. Affix tape to tube that does not have a barcode label.
10) If applicant is unable to void, arrange for a second appointment for the entire kit.

11) After following all specimen handling procedures, repackage the blood kit. Make sure all tubes are placed in the proper position for packing and ship kit to lab via overnight courier service or other shipping service (e.g., U.S. Postal Service) as directed. Specimens should be shipped within 24 hours, or on the next available shipping day. If the specimen collection cannot be shipped on the day of the collection, refrigerate until the specimen collection is shipped.

12) Send one (1) copy of the authorization slip to the lab with the kit and return remaining copies to the paramed office. Leave one (1) copy with the client.

13) Notify local paramed Manager immediately if any problems of any kind occur.

APPENDIX B – CLINICS:

1) Fill out the requisition with the following information: patient name or identifying number, sex, birthdate, date drawn and authorized person ordering the test. (Include any additional clinical information that may be available; i.e., fasting status, time of draw if applicable, diagnosis, etc.).

2) Mark tests ordered by physician or write in under other requests.

3) Place specimens and corresponding paperwork in shipping container for transport to the laboratory.

4) Refer to the section on Clinical Profiles & Tests for descriptions of specimen requirements and preservatives if needed. All clients are supplied with customized specimen collection kits, shipping containers and courier transport. If the tests ordered are not present in the catalog, or there are any further questions concerning collection, please contact Customer Service or your Account Executive at 800/445-6917.

5) If additional tests are ordered by an authorized person via the telephone, the laboratory subsequently attempts to obtain a written or electronic requisition within 30 days from the client and/or physician.
COLLECTION TUBES
The following is a list of the most commonly used specimen collection tubes listed in our catalog:

♦ Mottled Red Top - contains clot activator with gel separator in the bottom, for collection of serum samples
♦ Red Top - contains no anticoagulant, for collection of serum samples
♦ Purple Top - contains EDTA (ethylenediaminetetra-acetate) for collection of hematology and hemoglobin analysis samples
♦ Green Top - contains sodium heparin for hematology and chemistry samples
♦ Gray Top - contains sodium fluoride and potassium oxalate, which are glycolysis inhibitors
♦ Light Blue Top - contains sodium citrate for coagulation samples
♦ Royal Blue Top - may contain sodium heparin for trace metal studies

APPENDIX C - IN-HOUSE COLLECTION
1) Fill out the CRL requisition as completely as possible (i.e. SSN, name or unique identifier). The individual or family physician's name must be filled in to comply with state regulations. The sex and birth date are also necessary. Designate with N/C if it is a no-charge draw. The phlebotomist must initial and record time drawn in the upper right hand corner of requisition.

2) The employee or family member must read and sign the Specimen Collection Informed Consent Form. Attach this form to the requisition. This form must be completed in order for results to be released.

3) Mark tests ordered by physician or write in under other requests.
   a) Note 1: All employees and their family members are entitled to a CBC, Chem 20 and CHD (coronary risk panel) at no charge. Males may also receive a PSA. Employees should use their employee identification number to ensure confidentiality. This is extremely important if an HIV is to be run on an employee. Any special tests such as tumor markers must be approved by the Laboratory Manager or Vice President before being ordered.
   b) Note 2: Tests ordered on an employee that must be sent to another lab will be paid for by the employee.
   c) Note 3: A list of specimen requirements for tests routinely performed at CRL is included below. If there is any question about the appropriate specimen, consult with the department BEFORE collection. For send-out specimens, consult the appropriate reference laboratory's manual.

4) Collect the specimen. (refer to – Internal Venipuncture Procedure).

5) Refer to Internal Venipuncture Procedure for adverse reactions which include fainting, seizures and injuries.
6) After the blood has been collected, the specimens and the paperwork are transported in a closed container to the Tox Clinical Set-Up Department to be processed. The container must be closed to prevent exposure of employee / family member information or personal health information (PHI).

**Specimen Requirements for Commonly Performed Tests at CRL**

**Red Top/Serum:**
- Chem: all chemistry panels
- Thyroid: T7, T4, T-Uptake, TSH
- Coronary Risk (CHD): Chol, HDL, LDL
- Tumor Markers: PSA, CEA, B-HCG, AFP
- Hepatitis: HBsAg, HBsAb, IgM anti-HBc, Hepatitis C, Hepatitis A (HAVAB, HAVAB-M)
- Infectious disease molecular tests for RNA or DNA.
- AIDS Panel: HIV-1 EIA, Western Blot
- Misc: RPR (syphilis), RA screen, nicotine, alcohol, beta-2 microglobulin, CDT

**Lavender/Purple Top/EDTA tube:**
- CBC
- Molecular test for RNA or DNA
- Glycohemoglobin (glycoHgB, glycoA1C, Hgb A1C)
- Sedimentation Rate (ESR)
- Reticulocyte Count

**Blue Top/Sodium Citrate:**
- PT
- APPT
- Fibrinogen

**Gray Top/Sodium Fluoride:**
- Glucose, Blood Alcohol

**Urine:**
- Urinalysis
- Drug Screen