PHLEBOTOMY
Module #5 of the CLIA Lab Director Certification Course
Course Director: Matthew D. Krasowski, MD PhD
Edits: James E. Ross, MBA BS (June 2018)

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Educational Objectives
Upon completion of this module, the learner should be able to:

1. Identify the pre-analytical factors that impact validity of patient laboratory results.
2. Identify causes of hemolysis when performing a venipuncture using an evacuated tube system, syringe, or IV infusion set.
3. State the correct collection order (direct draw) or transfer order (syringe draw) when obtaining a specimen for multiple tubes, e.g., blood culture, EDTA, Na heparin, gel separator, Na Citrate, plain, and other anticoagulants.
4. State the correct order of collection of samples from a skin puncture.
5. Describe the appropriate sites for skin puncture on an adult, child, or infant.
6. Explain techniques to help prevent hemolysis when performing a skin puncture.
7. Identify the minimum amount of blood required to fill an Na citrate tube (blue stoppered) and to fill a micro-capillary tube for a hematocrit.

I. Introduction
Quality patient care can be provided only if decisions are based on valid data. The validity of laboratory data is dependent on pre-analytical factors just as it is on analytical parameters. These pre-analytical factors will be discussed in the following text. Clinical and Laboratory Standards Institute (CLSI) has established standards for the pre-analytical phase.
II. Patient Education and Preparation
The first step is to educate and properly prepare the patient. If the test requires the patient to be fasting, does the patient understand the requirements? For example, the patient may ask: "Can I have my morning coffee and/or cigarette if I don't eat anything?" Next, consider the pre-existing patient conditions or habits that might influence test results; exercise, medications, and disease states should be considered. Additionally, the importance of the time of day (diurnal variation), the timing (tolerance tests, half-life of drug), and the patient's posture (supine or upright) when drawing a blood sample should be considered.

The laboratory performing the test analysis often can provide information regarding pre-analytical requirements as they pertain to test results and reference ranges. Be sure to check with them regarding any questions.

III. Laboratory Requisitions (test orders)
Complete and legible laboratory requisitions must contain the following:
- Patient's full name and age
- Patient's identification number (e.g., social security or hospital number)
- Date of collection
- Time of collection
- Collector's initials
- Physician's name and code
- Patient's room number (hospital patient only)
- Accession number
- The department for which the work is being done
- Test(s) ordered
- Other information as needed, e.g., special comments

Incomplete or illegible requisitions may result in delay of patient's test results, an incorrect test being performed, or a test being performed on the wrong patient.
IV. Patient Identification
Identification of the patient is crucial. Briefly greet the patient, identify yourself, and establish a comfortable rapport with her/him. This is to help put the patient at ease. The patient being drawn must be identified as the person designated on the requisition.

Ask the ambulatory patient to state her/his full name, address, and identification number (e.g., hospital number or social security number) or birth date. (Do NOT ask, "Are you Ms./Mr. Smith?" A nervous patient might answer “yes,” even if she/he did not clearly hear the question.) Compare the stated information to that on the requisition. Correct and verify any discrepancies. If a patient does not have identification and cannot verify the information, a parent or close relative should confirm the identification and birth date. Again, the stated information must exactly match that on the requisition. Correct and verify any discrepancies.

V. The Blood Sample
The integrity of the sample is dependent upon good venipuncture or skin puncture technique, drawing from an appropriate site, and avoiding hemolysis or contamination of a sample. When performing a venipuncture, do not draw above an IV site, from a vein that is sclerosed, from an area with a hematoma, from an arm with a fistula or shunt, or from the same side where a patient has had a mastectomy. When performing a skin puncture, do not use a finger or heel that is bruised, cold, swollen or cyanotic. Each procedure will include information on the appropriate site to select.

The following procedures for obtaining a blood sample, whether performing a venipuncture, fingerstick or heelstick, assume that the phlebotomist has 1) greeted the patient, 2) verified the patient's identity, 3) interviewed the patient to verify adherence to any pre-testing requirements, and 4) explained the procedure to the patient and/or guardian.

Note: All patient blood specimens are to be treated with "Standard Precautions," as it is frequently impossible to know which specimens might be infectious. Gloves are to be worn when performing a venipuncture or skin puncture procedure.
Assemble all the equipment you might need in an organized manner.

- **Needles**: 18 gauge to 23 gauge, single and multi-draw
- Evacuated tube hub: Many are now available with safety release or retract features.
- **Evacuated tubes**: SST, EDTA, Na Citrate, Na Heparin, Plain, and etc.
- Syringes: 1 ml, 3 ml, 5 ml, 6 ml, 10 ml or 12 ml
- IV Infusion sets: 21 and 23 gauge, possibly 25 gauge

- Evacuated tubes and micro-container or "bullet" tubes

- Luer adapters: Luer adapters are available that come pre-assembled to a tube hub.
- Lancets or semi-automatic skin puncture devices: The devices typically have safety features where the needle or blade irreversibly retracts.

- Heparinized micro-capillary tubes: If not using self-sealing tubes, you will also need tube sealing clay.
- Tourniquets: 3/4 in. or 1 in.(adults) and 1/8 in. (children) by 18 and 12 inches, respectively
- Gloves (Latex allergy protocol)
- Clean cotton or rayon balls
- 2X2 gauze pads (sterile is not required)
- 70% isopropyl alcohol (v/v)
- Betadine (TM) (Used for cleansing venipuncture site for blood cultures.) This procedure is not addressed.
- Sharps disposal container
- Band-aids
- Biohazard waste container
- Tube rack or container to hold tubes upright, or a safety syringe shielded transfer device
Latex-free products are available. Examples of latex-free supplies include:

- **Band-aids:**
  - Nexcare™ Active™ Bandages (Caution: packaging contains latex)
  - CURAD® Flex-Fabric™ Bandages (Caution: packaging contains latex)
  - Readi-Bandages™ (Check with manufacturer regarding packaging)

- **Gloves:**
  - Nitrile, neoprene, thermoplastic elastomers, vinyl, etc. *(Caution: vinyl gloves do not provide adequate barrier to viruses)*

- **Syringes:**
  - Air-Tite Products Co., Inc. Norm-Ject® plastic syringes
  - TERUMO® plastic syringes

- **Tourniquets:**
  - Avcor Health x-tourn™
  - Kent Elastomer Products Free-Band®

Venipuncture using evacuated tube/hub system (to be performed on adults or older children)

1. Check the test ordered and organize the required tubes in the proper **order of draw**.
2. Inspect the site. Determine the type of needle and drawing system to be used. Whether to use a hub/vacuum tube, needle/syringe or IV infusion set is determined by the site and **vein** quality.
3. Assemble the equipment. It is helpful to have an extra tube of each type within reach or in your pocket as a tube might be lacking sufficient vacuum or in case you lose tube vacuum on a difficult stick.
4. Tie the tourniquet. It should be placed approximately 4 inches above the venipuncture site, with the ends pointing upwards, away from the site. Oftentimes a tourniquet must be applied before a person is able to determine the venipuncture site and the type of drawing system to use. The total time a tourniquet may be tied before it affects results by hemoconcentration, becomes uncomfortable for the patient, or potentially could cause petechiae is 1 minute. Release the tourniquet while you assemble the equipment and then re-tie immediately prior to the actual stick for best results.
5. **Palpate** the vein.

6. Clean the site with 70% isopropyl alcohol. Allow the site to air dry. "Wet" alcohol left on the skin can cause hemolysis and an unpleasant stinging sensation for the patient.

7. Anchor the vein. Using gentle pressure, place your index finger or thumb just below the insertion point. (Some phlebotomists prefer to hold the vein both above and below the insertion point. This practice has added safety risks should the patient move suddenly.)

8. Insert the needle with the bevel facing up. The needle should be at a 15 to 20 degree angle, and be placed in the same direction as the vein. If you need to palpate the vein one more time before inserting the needle, you must clean your gloved finger just as you did the venipuncture site.

9. Engage the evacuated tube. Be sure to hold the needle/hub steady or you may lose the vein or cause pain or injury to the patient. The hand holding the needle/hub may be steadied by lightly resting your hand on the patient's arm. With your other hand, hold onto the hub flange and gently engage the tube. If you do not see blood being pulled into the tube as soon as you engage the tube, you may need to adjust the position of the needle slightly. However, do not probe with the needle. A second puncture is less painful for the patient and will likely provide a better sample. If multiple tubes are to be drawn, mix each tube when you remove it from the needle/hub assembly; engage the next tube, etc., until you have drawn all the required tubes. You must follow the correct tube draw order to prevent a risk of contamination between tube types.

10. Release the tourniquet.

11. Remove the needle/hub assembly and apply pressure to the venipuncture site. Ask the patient to hold the gauze in place for you.

12. Discard the needle in a puncture resistant biohazard sharps container.

13. Again gently invert the tubes and label them.

14. Check the patient's arm and apply a bandage as necessary.

15. Ensure the patient is feeling fine before allowing the patient to stand and/or leave the area.

*Note: If the patient is feeling queasy or faint, follow your lab's protocol for a fainting patient.*
Venipuncture using a syringe

Syringes typically are used when the patient's veins are small or fragile and the evacuated tube suction could cause the vein to collapse. Using a syringe allows the phlebotomist to control the amount of suction applied to the vein.

The venipuncture procedure using a syringe follows the same steps as the evacuated tube system procedure. It differs slightly in equipment preparation and assembly, pulling the blood into the syringe, and transferring blood into evacuated tubes.

The entry into the skin and the vein is exactly the same as with the evacuated tube system.

Once you feel that the needle is in the vein, pull back gently on the syringe plunger while holding the syringe barrel securely to keep the needle in place in the vein. Use the syringe flange to brace against as you pull back on the plunger, just as you do when changing tubes using a needle/hub system. Fill the syringe with the desired amount of blood, release the tourniquet and complete the procedure exactly as you would using an evacuated tube system. (See needle redirecting when using a syringe.)

To transfer the blood from the syringe to the appropriate tubes, remove the needle from the syringe and replace it with an 18 or 19 gauge needle. Using a large bore needle will help prevent hemolysis of the blood and maintain the integrity of the sample. Do not apply pressure to the plunger, allow the tubes to fill by the negative pressure of the vacuum tube. Since there is the possibility of the formation of micro clots, the blood should be transferred in the appropriate order as quickly as possible into the tubes containing anticoagulant and mixed immediately. Again, label the tubes and check the status of the patient before allowing the patient to stand or leave.
Venipuncture Using an IV Infusion Set

An IV infusion set (butterfly) is used for venipuncture when you draw from a hand or foot vein or from a very small or fragile vein; when the angle of needle entry is awkward, e.g., when a patient is in bed and repositioning of her/his arm is difficult or painful; or when the patient's vein is difficult to find or draw. If a small child must have blood drawn using the venipuncture procedure, a 23 gauge IV infusion set attached to a 1 ml or 3 ml syringe is typically used. This allows better control and helps prevent excessive suction from the syringe if the blood is drawn slowly and carefully.

The butterfly's needle and plastic "wings" are attached to a length of flexible tubing which is, in turn, attached to either a syringe or luer adapter/hub assembly. The butterfly is lighter and less cumbersome than either of the other two assemblies; thus, it allows better control and "feel" when drawing a patient. Additionally, as soon as the needle is in the vein, blood is visible in the tubing rather than having to wait and see as when using either of the other two methods.

The venipuncture procedure using an IV infusion set follows the same steps stated previously in the evacuated tube system procedure. The IV infusion set differs slightly in a lower angle of needle entry and equipment preparation and assembly. A set attached to a syringe will require transfer of blood to tubes, while use of a hub/tube assembly will result in blood drawn directly into tubes.

When you are ready to perform the venipuncture, grasp the wings between your thumb and index or middle finger, hold the skin and vein taut with your other hand, and enter the skin with the needle.

As soon as you see blood in the tubing, you may pull back on the syringe plunger or engage the vacuum tube. If you do not see blood in the tubing you will need to redirect the needle. When the
needle is well anchored in the vein, you may release the butterfly "wings"; otherwise continue to gently hold the "wings" during the procedure. If using a syringe, fill it with the desired amount of blood, release the tourniquet, remove the needle and complete the procedure exactly as you would using a syringe. If using a needle/hub assembly, fill the tubes and complete the procedure as you would drawing directly into evacuated tubes. Again, be sure to check the status of the patient before allowing the patient to stand or to leave.

*Note:* If you are using a safety infusion set, be sure to immediately slide the safety cover over the needle and discard the set. Alternatively, to prevent accidental re-stick with the infusion set needle, hold the base of the needle or the wings as you remove the needle and do NOT let go of the needle base until it is being placed in the biohazard sharps container.

**Skin Punctures**

Blood obtained from a skin puncture comes from a mixture of arterioles, venules and capillaries, and contains interstitial and intracellular fluids. There is more of the arterial blood than venous blood due to pressure differences in the capillaries. Also, the venous blood in the skin more closely resembles arterial blood than in other parts of the body. This is especially true when the puncture site has been warmed. [Warming the skin](#) primarily increases the arterial blood flow. Because of the differences in the blood concentration of certain analytes in capillary versus venous or arterial blood, the blood collection technique and site both should be noted on the reports form. This allows the physician to consider the collection technique used when interpreting the results.

The key to obtaining a good skin puncture sample (finger or heel) is performing a puncture that results in free flowing blood. This is dependent upon accessing the capillaries, venules, and arterioles of the dermis and subcutaneous tissues. Manufacturers have developed varied types and sizes of skin puncture devices to safely access this juncture. The devices are usually designed specifically for:

1. heelstick on a pre-term baby
2. heelstick on a full-term baby
3. finger puncture on a pediatric patient
4. Finger puncture on an adult patient

These devices vary in the depth and width of the cut or puncture.

Preventing hemolysis is also often technique dependent. Be sure the alcohol has completely air dried before performing the puncture. Residual alcohol may cause red blood cell lysis. Also, if blood flow is inadequate or begins to decrease, do not excessively squeeze the adjacent tissue; rather, perform a second skin puncture using all new equipment. Never re-stick the same site or re-use a skin puncture device. Most safety devices will lock after use and prevent re-use. Allow the tube to fill by capillary action. Do not scrape the tube against the site as this may cause mechanical lysis of the RBCs. Technique is even more important when performing a skin puncture on an infant. Infants often have high packed cell volumes and increased red blood cell fragility.

The Finger Puncture Procedure

A finger puncture procedure is performed instead of the venipuncture or heelstick when the patient is a small child older than six months, or the specimen was unattainable by venipuncture. The finger puncture procedure is not to be performed on infants as the distance from the skin surface to the bone at the thickest portion of the distal phalanx of a newborn is between 1.2 to 2.2 mm. The currently available skin puncture depth range is 0.85 to 4.5 mm and thus could easily cause injury to the bone.

The finger puncture is typically used for lower sample volume tests that can be placed into special micro-sized tubes.
These tests include CBC, white blood cell differential, hemoglobin, hematocrit, and limited chemistry tests, e.g., Na, K, Cl, CO2, BUN, creatinine, and glucose. Fingersticks are also frequently used in public health screening events (e.g., cholesterol, HDL), and glucose self-monitoring at home.

Again, this procedure assumes that patient education and preparation, test request verification, patient identification, and procedure explanation have occurred.

The steps for the finger puncture are:

1. Select skin puncture site. You may use either hand, but the less dominant hand is usually not as callused. Use the palmar surface of the distal phalanx of the middle or ring finger (3rd or 4th digit). The only finger that should not be used is the last finger (5th digit).
2. Warm the site if necessary.
3. Organize equipment.
4. Clean the site with 70% isopropyl alcohol. Allow the site to dry completely.
5. Puncture the site with a disposable lancet. Hold the finger and hand firmly to immobilize the finger as some patients’ response is to pull away as you perform the skin puncture. This often necessitates repeating the procedure in order to obtain the sample. There are two basic types of lancets. One type has an exposed blade that will penetrate the skin to a pre-determined depth. When using this type of lancet, perform a quick puncture to the pre-determined depth of the lancet, perpendicular to the fingerprints and at a 90 degree angle. The second type is a semi-automatic lancet. This type has either a plunger or button to push to perform a puncture or an incision. To use these, immobilize the finger and hold the lancet at a 90 degree angle to the finger, perpendicular to the lines of the fingerprint. Using moderate pressure, depress the plunger completely, then release the plunger and remove the lancet.
6. Wipe away the first drop of blood using clean gauze. This drop of blood contains the highest concentration of tissue fluids.
7. Collect the specimen by allowing the drops of blood to fill the collection tube by capillary action. When drawing hematology and chemistry tests, always draw the hematology tests first. Immediately mix the hematology micro-collection tubes at least 8-10 times to ensure adequate mixing of the small volume. One manufacturer suggests mixing their micro-collection EDTA tube 20 times. If the blood flow becomes inadequate or stops, repeat the skin puncture using a different site and a new lancet.

8. Apply pressure to the puncture site using sterile gauze.

9. Discard the lancet in a puncture resistant biohazard sharps container.

10. Label the tubes.

11. Apply a band-aid as necessary. Do not apply a band-aid on a small child as she/he may swallow the band-aid. Colorful stickers may bring a smile to an older child after the procedure is done.

12. Check the status of the patient before allowing the patient to stand or leave.

**The Heelstick Procedure**

Skin puncture of the heel is frequently the least problematic method for obtaining a blood sample from an infant. The puncture is performed on the most medial or lateral portion of the plantar surface of the heel. Do not perform a puncture on the central area of the foot, the arch of the foot, nor the posterior curvature of the heel. See the shaded portion on diagram.

Also, do not puncture a previously used site as it may be infected. The National Committee for Clinical Laboratory Standards states that the puncture depth should be no more than 2.4 mm. Studies indicate that for certain infants, including premature infants, even this depth may be excessive.

The heel puncture procedure follows the same steps as the finger puncture procedure. Some of the disposable lancets are specifically designed for heelsticks.

1. Select the heel puncture site.
2. Warm the site for three to ten minutes, if necessary.
3. Organize your equipment.
4. Clean the site with 70% alcohol and allow to air dry.
5. Perform heel puncture. Firmly hold the heel, place the lancet perpendicular to the heel, and quickly perform puncture.

If for any reason you are using a lancet that will enter at a depth greater than 2.4 mm, do NOT enter the skin at a perpendicular angle. The risk of hitting a bone is too great. Place the lancet almost parallel to the heel, facing toward the toes, and enter at a shallow angle. You need only to access the capillary bed. **It is never recommended to use a lancet that will puncture the heel at a depth of greater than 2.4 mm.** Good technique and the appropriate device should negate any reason for exceeding the recommended maximum depth of 2.4 mm.

6. Collect specimens. The order of draw is the same as for finger punctures.
7. Apply pressure to the site. Apply a band aid only if there is no danger of the infant getting it into her/his mouth.
8. Discard the lancet into a biohazard sharps container.
9. Label the tubes. Tubes too small to be labeled, such as hematocrit tubes, may be placed in a plain (red top) tube and the tube can be labeled.
10. Check the site and the patient again before allowing the patient to leave.

**VI. Specimen Handling and Transportation**

Once a sample is drawn properly, it must be processed, stored, assayed and/or transported correctly or the results may be invalid. Certain blood samples must be placed on ice, others kept at body temperature, and some centrifuged and frozen immediately. If you are sending samples drawn in your office to an outside laboratory for analysis, make sure your specimen collection, handling, and transportation procedures meet the guidelines set by the reference laboratory.
The following is a list of pertinent hyperlinks.

- [http://www-medlib.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html](http://www-medlib.med.utah.edu/WebPath/TUTORIAL/PHLEB/PHLEB.html)
  - This site is a part of "WebPath: The Internet Pathology Laboratory for Medical Education." One of the mini-tutorials is phlebotomy. It provides succinct information, including nice graphics and photographs. Check this site for varied medical information and education. The main "WebPath" URL is: [http://www-medlib.med.utah.edu/WebPath/webpath.html](http://www-medlib.med.utah.edu/WebPath/webpath.html).

- [https://www.osha.gov/SLTC/latexallergy/](https://www.osha.gov/SLTC/latexallergy/)
  - This Occupational Safety and Health website addresses latex allergies and preventing latex allergy, and it includes patient handouts, current articles and many resource links.

- [http://www.aspt.org](http://www.aspt.org)
  - This is the website of The American Society of Phlebotomy Technicians.

**Bibliography**

**CLSI: Reference**

Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

The Clinical and Laboratory Standards Institute (CLSI) issues national guidelines and ultimately standards for laboratory procedures. These documents are considered the gold standard for laboratories and industries. The most recent revision of the CLSI standard for venipuncture is CLSI document GP41, "Collection of Diagnostic Venous Blood Specimens, 7th Edition" (2017). This document addresses safety (gloves, standard precautions, isolation, etc.), facilities (outpatient and hospital settings), the venipuncture procedure (adults and children), additional considerations (hemolysis, patient questions, etc.), and special considerations (timed specimens, fistulas, IVs, etc.).
The CLSI standard approved September 2008 for skin punctures is Document GP42 "Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens, 6th Edition." This document addresses safety precautions, the sites, devices, and techniques for skin puncture and sample collection on infant through adult patients, and the analyte concentration in the skin puncture specimens.

Other CLSI documents pertinent to sample collection include the following (check the CLSI website for the latest version):

2. C52: "Toxicology and Drug Testing in the Medical Laboratory, 3rd Edition" (2017) This document includes chain of custody information.

CLSI Address:
Clinical and Laboratory Standards Institute
950 West Valley Road, Suite 2500
Wayne, PA 19087
Phone: (610) 688-0100
FAX: (610) 688-0700
http://www.clsi.org/
Needles

Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

1. Needles, both single and multidraw, are packaged color coded relative to their gauge size. A large gauge number (e.g. 23) indicates a small needle bore, while a small gauge number (e.g. 18) indicates a large needle bore. The most commonly used sizes are 19-23 gauge needles. Larger gauge needles (small bore), such as 25 gauge, are more likely to be a cause of hemolysis. An 18 gauge needle is slightly more painful for the patient and is not needed for most venipuncture procedures.

2. When you assemble the equipment, always leave the needle cover on until just before you are ready to insert the needle. This will help maintain sterility and help prevent unintended needlesticks.

3. Always inspect the needle before performing the venipuncture. Do not use the needle if the tip is bent, curved or you can see spurs. This rarely occurs but should always be checked.

Evacuated Tubes

Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

Evacuated tubes are manufactured to withdraw a predetermined volume of blood and are typically sterile. They have an expiration date beyond which they should not be used, as the vacuum may not be accurate or the anticoagulant may not be effective. The expiration date and the tube type are printed on each individual tube. Tubes selected for general use should be displayed on a wall chart in one or more locations in the venipuncture area to assure proper information for various types of collections. The most common use of the tubes are listed below.

1. Speckled stopper, serum separator tube (SST) or plain tube (red stopper): general chemistry tests such as electrolytes, BUN, creatinine, total protein and albumin, etc.

2. Red stopper (no additive): therapeutic drugs or many of the general chemistry tests
3. Purple stopper, ethylenediaminetetraacetic acid (EDTA): complete blood count (CBC), white blood cell (WBC) differential

4. Blue top (Na citrate): coagulation tests such as prothrombin time (PT), partial thromboplastin time (PTT), fibrinogen (NCCLS standards require the blue top tube to be filled at least 90% full for the correct blood-anticoagulant ratio.)

Note: Be sure to check with the laboratory performing the test for their specific tube requirements. (return to page 4)

IV Infusion Sets

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Top: Safety female adapter, used to safely transfer blood drawn into a syringe into an evacuated tube.

Left to Right: Needle/hub/evacuated tube assembly; IV infusion set/luer adapter/hub/evacuated tube assembly; IV infusion set/syringe assembly. (return to page 4)
Evacuated Tubes and Micro-container or "Bullet" Tubes

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Evacuated tubes and micro-collection tubes. (Left to right: EDTA, Na Citrate, SST, Plain, Na Citrate, Na Heparin, EDTA, Acid Solution A, EDTA, and SST) The sample volumes of these tubes range from 250 microliters to 10 milliliters. (return to page 4)
Lancets or Semi-automatic Skin Puncture Devices

*Finger*

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Top row: Skin puncture devices for fingersticks.
Bottom row: Skin incision devices for fingersticks.
Lancets or Semi-automatic Skin Puncture Devices

Heel

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Commercial heel warmer and skin puncture devices for heelsticks.

Suggested Order of Draw Using an Evacuated Tube System and Order of Transfer From a Syringe to Sample Tubes

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1. Blood culture tube
2. Plain tube, non-additive (e.g., red stopper)
3. Coagulation tube (e.g., blue stopper)
4. Additive tubes:
1. Gel separator tube (e.g., black/red speckled stopper)
2. Heparin (e.g., green stopper)
3. EDTA (e.g., lavender stopper)
4. Other additive tubes (e.g., oxalate/fluoride gray stopper)

Note:
1. When transferring blood from a syringe to evacuated tubes, place the tube(s) in a tube holder or rack. Do NOT handhold a tube while you are pushing the needle through the stopper. As soon as the needle is penetrated the stopper, it is fine to hold the tube in your hand. Alternatively, use a safety-syringe shielded transfer device.
2. Do NOT place any pressure on the syringe plunger when transferring blood from a syringe to evacuated tubes. The tube's vacuum will provide the negative pressure to pull the blood into the tube. Excess pressure may cause hemolysis.
3. The most current NCCLS standard states that if a coagulation tube is for APTT or PT only, the first tube drawn may be used for testing. However if special coagulation testing (e.g., Factor VIII) is being drawn, the second or third tube should be used. (A plain tube may be drawn and discarded if the special coagulation tests are the only tests being drawn.)

Veins

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Choosing the Site:
1. Choosing an appropriate site is crucial for a successful venipuncture. The location, size, and feel of the vein are important in selecting which vein to use. The widest, deepest part of the vein is usually the easiest from which to draw. However, do not draw at the point where two veins join as there is a valve at these junctures.
2. The veins most often used for venipuncture are located in the antecubital area. Typically the order of choice in vein selection is as follows:
i. Cubital vein: This vein is usually the largest and fullest vein and is best anchored by the surrounding musculature of the arm.

ii. Cephalic vein: This vein is next largest and next better anchored by the surrounding musculature of the arm.

iii. Basilic vein: This vein is typically smaller than the two above and is not anchored as well by the surrounding musculature. Therefore, if this vein is used, the phlebotomist must ensure he anchors the vein well by holding the vein and the skin taut just below the needle insertion point. The basilic vein is close to the brachial artery, so there is more risk of hitting an artery. Exercise caution when drawing from this area. Additionally, this area is often more sensitive, thus a stick is slightly more painful for the patient.

Note: If you do enter an artery, complete the draw and hold the site for a minimum of 5 minutes. Ensure all bleeding has stopped before allowing the patient to leave.

3. The actual feel of the vein is key in selecting the vein which will most likely result in a successful venipuncture. With the tourniquet appropriately tied, a good vein should feel resilient or slightly bouncy when palpated slowly. If the vein rolls away when you palpate it, be sure to anchor it well if you choose to draw from that vein. A vein that feels hard is often sclerosed and should not be used. Also, do not attempt to perform a venipuncture in an area that has a hematoma. (return to page 5)

Palpate

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Palpating a Vein:
To palpate a vein, gently and firmly push down on the skin with your index finger, then slowly release the pressure. If you are palpating a vein, you will feel the vein bounce back as you release the pressure. If you are palpating a tendon, it will feel like a rope or thread that is pulled tightly. If you are in doubt, release the tourniquet and palpate the area again. If the "tight rope" is still there, you were palpating a tendon. If you feel a pulse, you were palpating an artery. (return to page 5)
Venipuncture Using Evacuated Tube/Hub System

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Photographs by Joel Carl, M.A.

Venipuncture using a multidraw needle/hub/evacuated tube assembly.
Venipuncture Using Evacuated Tube/Hub System
Risks Involved with Anchoring the Vein

Julie C. Paulson Happel, M.T. (ASCP), M.A.
Photographs by Joel Carl, M.A.

Venipuncture using a multidraw needle/hub/evacuated tube assembly. Anchoring the vein above and below the insertion point poses a greater risk of an accidental needlestick to the phlebotomist.

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Adjusting Needle Position when Using Evacuated Tubes (Needle/Hub Assembly)
Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

Adjusting Needle Position:
Sometimes when performing a venipuncture, the needle isn't initially positioned correctly in the vein for good blood flow. If the needle is close to the correct place, re-directing the needle is better than immediately withdrawing the needle. Slightly re-adjusting the needle may eliminate a second venipuncture. Do not attempt to stick the patient more than twice; ask another trained person to help.

To redirect the needle, first feel for the vein to determine if the needle is beside the vein, is not in quite deep enough, or is in too deep. If the needle has been pushed through the vein you will see a brief spurt of blood when you engage the tube, pull back slowly until you see blood entering the tube. If the needle is on top of the vein or not in quite deep enough to obtain normal flow into the evacuated tube, anchor the vein, increase the degree of angle of the needle slightly, and move forward in the same direction as the vein. If the needle is beside the vein, anchor the vein, pull the needle back slightly, re-adjust the needle and move forward into the vein. If you are sure the needle is in the vein but no blood is flowing into the tube, change to a new tube of the same tube type. (If, while re-directing the needle, you hear a hiss, you have lost the vacuum and will need to replace the tube with a new one.) (return to page 6)

Label
Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

The blood collection tube(s) must be immediately positively identified at the time of collection. Samples obtained in tubes must be identified with a label firmly attached to the tube. The label should have at least the following information:

- Patient first and last name
- Identification number (e.g. social security or hospital number)
• Date and time of specimen collection
• Initial or name of person collecting the specimen

Alternatively, a tube may be identified by the requisition label attached to the tube. The requisition and its label are preprinted with a number. The requisition must contain the information as stated previously.

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**Equipment Assembly when Using a Syringe**

Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

*Equipment Preparation:*

When preparing the needle/syringe needle assembly, pull back on the syringe plunger before attempting to draw with a syringe. It often takes a significant amount of pull to release the plunger from the base of the syringe barrel the first time. After the plunger has been pulled back once, it can be pulled back fairly easily and smoothly and is ready for use.

Be sure to attach the needle to the syringe tightly enough so that no frothing of blood occurs at the connection when you apply the suction. This pooling and frothing will cause hemolysis.

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Venipuncture Using a Syringe

Julie C. Paulson Happel, M.T. (ASCP), M.A.
Photographs by Joel Carl, M.A.

Venipuncture using a needle/syringe assembly.
Venipuncture Using a Syringe
Risks Involved with Anchoring the Vein

Julie C. Paulson Happel, M.T. (ASCP), M.A.
Photographs by Joel Carl, M.A.

Venipuncture using a needle/syringe assembly. Anchoring the vein above and below the insertion point poses a greater risk of an accidental needlestick to the phlebotomist.

Needle: Re-directing when Using a Syringe

Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

Re-directing the needle when using a needle/syringe assembly is very similar to the procedure when using a needle/hub assembly. First feel for the vein to determine if the needle is beside the vein, is not in quite deep enough, or is in too deep. If the needle has been pushed through the...
vein, pull back slowly, while still pulling gently on the plunger, until you see blood entering the syringe. (The distance is often only about the length of the tip bevel.) If the needle is on top of the vein or not in quite deep enough to obtain normal flow into the syringe, anchor the vein, increase the degree of angle of the needle slightly, and move forward in the direction of the vein. Then proceed to pull back on the plunger for blood flow. If the needle is beside the vein, anchor the vein, pull back slightly, re-direct the needle, and move forward into the vein.

*Note: If there is significant resistance to pulling the plunger back, even with some blood flow, the needle is not positioned correctly, and continuing to forcibly pull the plunger back may cause hemolysis of the sample.* (return to page 7)

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**Suggested Order of Draw Using an Evacuated Tube System and Order of Transfer from a Syringe to Sample Tubes**

*Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.*

1. Blood culture tube
2. Plain tube, non-additive (e.g., red stopper)
3. Coagulation tube (e.g., blue stopper)
4. Additive tubes:
   a. Gel separator tube (e.g., black/red speckled stopper)
   b. Heparin (e.g., green stopper)
   c. EDTA (e.g., lavender stopper)
   d. Other additive tubes (e.g., oxalate/fluoride gray stopper)

*Note:*

1. When transferring blood from a syringe to evacuated tubes, place the tube(s) in a tube holder or a rack. Do NOT hand-hold a tube while you are pushing the needle through the stopper. As soon as the needle has penetrated the stopper, it is fine to hold the tube in your hand. Alternatively, use a safety-syringe shielded transfer device.
2. Do NOT place any pressure on the syringe plunger when transferring blood from a syringe to evacuated tubes. The tube’s vacuum will provide the negative pressure to pull the blood into the tube. Excess pressure may cause hemolysis.
3. The most current NCCLS standard states that if a coagulation tube is for APTT or PT only, the first tube drawn may be used for testing. However if special coagulation testing (e.g., Factor VIII) is being drawn, the second or third tube should be used. (A plain tube may be drawn and discarded if the special coagulation tests are the only tests being drawn.)

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**Equipment Assembly when Using an IV Infusion Set (Butterfly)**

*Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.*

The integrity of the tubing should be checked before using an infusion set, as on rare occasions there may be a defective tube or connection between the tubing and the needle. It is very quick to do, and it is also a good time to straighten the tubing and prepare the syringe as stated above. Attach the butterfly to a syringe, pull back on the plunger and expel the air through the infusion set tubing. You should hear air rush out of the end of the needle. If not, check the connection to the syringe and try again. If you do not hear the appropriate sound, discard the butterfly and attach a new one.
Venipuncture Using an IV Infusion Set

Julie C. Paulson Happel, M.T. (ASCP), M.A.
Photographs by Joel Carl, M.A.

Venipuncture using a safety IV infusion /syringe assembly. (return to page 8)

Site No. 1: This is typically the better or easier-to-draw site as the vein is straight, longer, and is likely better anchored. Also the vein is usually easier to anchor on the flat area of the back of the hand rather than on the edge of the hand.

Site No. 2: This is an acceptable or good vein but it is more difficult to anchor and maintain the needle during the drawing process.
Re-directing the Needle when Using a "Butterfly" (IV Infusion Set)

Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

When you have not entered the vein, redirecting of the needle into the vein is basically the same as it is for either the needle/hub or needle/syringe assembly. However once you are in a small vein with a 23 gauge/butterfly assembly, very slight movement will stop or start blood flow. Frequently the cause of this is that the small bevel of the needle touches the vein wall. Raising the back of the butterfly needle slightly often restarts blood flow. If not, next try repositioning the needle by slightly moving the needle sideways, and finally forward or backward. Again feeling for the vein and holding the skin and vein taut helps. (return to page 8)

Warming the Skin Puncture Site

Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

Warming the skin puncture site with a warm, moist towel at a temperature no more than 42 degrees C or using a commercially available heel warmer for at least three minutes is recommended. This can increase the blood flow through the site seven-fold. When collecting blood for pH or blood gas determination, warming the site is essential. (return to page 9)

Device Reference (Heelstick)

Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

Heelstick:
Burns, Edward, MD: "Development and Evaluation of a New Instrument for Safe Heelstick Sampling of Neonates". Laboratory Medicine, Vol. 20, No. 7, July 1989. This article compares puncture versus incision types of semi-automatic skin puncture devices, including differences in healing of the wound. (return to page 9)
Skin Incision Devices for Heel and Finger Stick

Julie C. Paulson Happel, M.T. (ASCP), M.A.
Photographs by Joel Carl, M.A.

A. Lasette: Laser finger perforator for capillary blood sampling. In July 1998, the FDA cleared it for home use in glucose and hematocrit blood collection for ages 5 years old to adult. Developed by Cell Robotics and marketed by Chronimed. Image from Cell Robotics, copywrite for use by Media Page

(return to page 9)
B. Varied sizes of Accriva Diagnostics skin incision devices for heelsticks.

C. Varied sizes of Becton Dickinson lancets.
Technique: Preventing Hemolysis (Skin Puncture)

Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

Hemolysis may occur due to:
1. Residual alcohol on the skin puncture site. Alcohol lyases red blood cells. Ensure the site is dry before performing puncture.
2. Increased red blood cell fragility and/or high packed cell volume. This is often found in newborn infants.
3. Excessive squeezing due to poor or decreasing blood flow. It is better to perform a second skin puncture. Never re-stick the same site or re-use a lancet.
4. Scraping the collection tube against the skin. This will mechanically lyse the red blood cells. Always allow the drop of blood to fill the collection tube by capillary action.
5. Shaking the micro-collection tube too vigorously.

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Micro-collection Tubes

Julie C. Paulson Happel, M.T. (ASCP), M.A.
Photographs by Joel Carl, M.A.

Micro-collection tubes. Sample volumes range from 250 microliters to 1.8 ml.

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Proper Location for Performing a Fingerstick

Julie C. Paulson Happel, M.T. (ASCP), M.A.

Photograph by Julie C. Paulson Happel, M.T. (ASCP), M.A.

Perform finger or heel puncture across the fingerprints as the blood will more likely bead rather than run down the "channels" of the fingerprints. Also, the puncture devices (the depth) are designed to be used in this manner.
Fingerstick Procedure

Julie C. Paulson Happel, M.T. (ASCP), M.A.
Photograph by Julie C. Paulson Happel, M.T. (ASCP), M.A.

Fingerstick procedure using a skin puncture lancet.

Collection of Blood by Skin Puncture

Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

Blood Collection:
It is acceptable to apply gentle pressure from the base of the finger progressing to the tip, then ease the pressure and repeat. Do NOT squeeze the finger hard or excessively. You will obtain a much better sample by performing a second skin puncture.
(Tests First) Order of Draw for Skin Puncture

Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.

When collecting blood samples from a skin puncture, the correct order of collection is:

1. Slide for differential or stain.*
2. Blood gas or pH specimen. (Warming the site is essential)
3. Hematology specimens.**
4. Chemistry or other specimens.

* If blood for cell morphology cannot be collected directly from the skin puncture site onto a clean slide, blood smears from blood collected into EDTA should be made within one hour.

** Micro-capillary tubes for spun hematocrit (packed cell volume) should be filled at least 2/3 full and be run in duplicate. (return to page 12)
Infant Skin Puncture Sites

Julie C. Paulson Happel, M.T. (ASCP), M.A.
Photographs by Joel Carl, M.A.

Shaded areas represent acceptable skin puncture sites on an infant's heel. (return to page 12)
Heelstick

Julie C. Paulson Happel, M.T. (ASCP), M.A.
Photographs by Joel Carl, M.A.

Heelstick using an incision device.  (return to page 13)
Bibliography

Julie C. Paulson Happel, M.T. (ASCP), M.A., Photographs by Joel Carl, M.A.