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GUIDELINE FOR VENOUS BLOOD COLLECTION (PHLEBOTOMY)



Prepared by the Preanalytic Phase Working Group of the Turkish Biochemical Society
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1. INTRODUCTION

Laboratory test results have a critical role in clinical decision for the patient. Two thirds of decisions to be taken about diagnosis and treatment follow-up, hospitalization and discharge of the patient and drug therapy initiation depends on laboratory test results.[1] However, laboratory test results is one of the important reasons of medical errors or malpractices that may influence patient outcomes. Laboratory test process is an extremely complex process and composed of three phases: preanalytical phase, analytical phase and postanalytical phase.[2-4] Preanalytical phase is the phase which is realised out of the laboratory but must be under the control of the laboratory and includes selecting and requesting tests which are applicable for patient's clinical status and collecting, transporting, processing, and preparing the sample in accordance with the analysis.[2] The process always begins and lasts with the patient. Preanalytical phase can be classified depending on the factors related to the patient and the sample or divided into periods such as before, during and after collecting the sample.[5]

With respect to the reliability of laboratory test results and malpractices, it is usually focused on analytical phase. In recent years, there is a consensus stating that most of the errors related to laboratory results root in staff practices and appear in the preanalytical phase, occurring before the sample reaches to the laboratory.[6-8] Venous blood collection is one of the critical steps of preanalytical phase and it is the most frequent interventional procedure in healthcare services. It is composed of steps distinguishing from each other and each step is receptive to potential errors with respect to patient safety. Among error sources: misspecification of the patient/sample, thus test results are not the results of the true patient;[9] alterations in analyte concentrations due to long-duration tourniquet application or contamination of the sample with intravenous fluids and or contrast media;[10] insufficient patient preparation, for instance not asking the patient his/her fasting or physical activity status;[11-12] false additive: blood ratio and thus insufficient sample volume causing effects on the results, etc.[13] Besides factors that can have effects on sample quality, some malpractices may threaten both patient's and healthcare worker's safety[14], for example,

insufficient disinfection of venipuncture site and breaking in the sterilisation of the site due to touching the site after disinfection. In addition, it may be inevitable to avoid exposure to blood borne pathogens in case the phlebotomist do not wear gloves or use proper methods during the disposal of sharps.

There are particular guidelines that are internationally applicable on proper venous blood collection procedures.[15-16] Foundation of these international guidelines are systematic reviews. National use of these reviews is limited because they are written in foreign languages and they are pretty well comprehensive, detailed and long texts. In addition, national regulations, complexity of blood collection and high number of patients from whom blood samples are collected make compliance with guidelines difficult. Therefore, it has been supposed that there is a need for venous blood collection guidelines that can be in accordance with our cultural and organizational structure (such as language, education and training, regulations and laws) on the basis of international guidelines for our country and, with this purpose, a guideline for venous blood collection has been prepared which is easy to be understood and accessed.

This guideline has been based on CLSI GP41-A6 and WHO blood collection guidelines. National regulations have also taken place in these guidelines. In addition, some user instructions related to blood collection products [BD-Becton Dickinson and Company (Franklin Lakes, NJ, ABD) and Greiner Bio-One (Kremsmünster, Avusturya)] that are widely used in our country are also included.

2. Best Practices in Venous Blood Collection

2.1. Equipments and Supplies

2.1.1. Properties of Blood Collection Area

Venous blood collection should be performed in a clean, silent, well-lit individual area which is reserved for this procedure, if applicable.[15-16] The area may be in the form of individual rooms for each patient or may be a hall. Regarding the areas constructed as a hall, blood collection site can be separated by a curtain or another separator in order to ensure patient privacy. For inpatients, bed curtains may be used.

Recommendation: In blood collection areas, there may be kept a lavabo with water and soap and paper towel in order to make phlebotomists wash and dry their hands.[15] If there is no lavabo, there should be kept hand antiseptics as stated in the Quality Standards of Healthcare Services in order to ensure hand hygiene.[17]

2.1.2. Venipuncture Chairs

Venipuncture chairs should be adjustable with its specifications.[17]

Recommendation: Venipuncture chairs is to ensure maximum comfort and safety for the patient. Phlebotomist should reach the patient easily and venipuncture chairs is to be reclined preferably in case the patient loses his/her consciousness in order to support patient and protect him/her against falling. For the chair, it is recommended to be with adjustable arms for the patient to place his/her arms.[15-16]

2.1.3. Specifications of Locker / Trolley / Tray in which Equipments for Blood / Collection are Kept

2.1.3.1. Locker / trolley

They are to be arranged in a manner that the phlebotomist can use it safely and the equipment should be seen clearly and reached easily. [15-16]

Recommendation: In case of using trolley, it is recommended that the trolley can move easily and silently on all kinds of surfaces.

2.1.3.2. Blood collection trays

They should be light in order to be carried easily and should have sufficient area on which the materials that will be used can be put with ease and should have a segment for the sharps container.

2.1.4. Supplies to be Used in Blood Collection

Prior to blood collection, working area should be prepared, necessary materials should be easily reached and be controlled with respect to their expiry dates. A well-arranged working area provides the continuity of all processes uninterruptedly. Every blood collection locker/trolley or tray should involve the following materials:

- Gloves
- Tourniquet
- Antiseptics with or without alcohol
- Cotton and/or gauze pads
- Needle, holders and winged blood collection sets
- Syringe systems
- Evacuated blood collection tubes
- Adhesive bandages
- Sharps container
- Test manual
- Other supplies (ice, aluminium foil, etc.)

2.1.4.1. Gloves

The gloves that will be used by phlebotomists should be for single use or disposable and fit the hand of the phlebotomist. It may be latex, vinyl, polyethylene or nitrile.[15]

Caution: Serious hypersensitivity reactions and anaphylactic shock cases have been reported in healthcare workers who have latex hypersensitivity. People who have such a hypersensitivity must avoid using latex gloves.[18]

Recommendation: It is beneficial to interrogate patients about their latex sensitivity.

2.1.4.2. Tourniquet

There should be tourniquet or a material to be used as tourniquet in order to increase intravascular pressure and stabilise the vein. False access into the veins that become evident or a potential damage to the nerves can be prevented by applying tourniquet.[15]

Recommendation: Tourniquets which are elastic, cloth-type and with a click provides ease of use.

Caution: Cleanliness of tourniquets is extremely important. There are studies demonstrating that tourniquets may be potential sources of methicillin-resistant *Staphylococcus aureus*. [19]

2.1.4.3. Antiseptics with or without alcohol

Antiseptic agents should be used in order to disinfect the area to be used. As antiseptic agent, 70% isopropyl or ethyl alcohol should be used. If blood culture sample will be collected, ready to use pads impregnated chlorhexidine is recommended to be used.

Caution: In blood samples which are contaminated with povidone iodine, test results of potassium, phosphorus and uric acid may result in falsely high concentrations.[16]

Caution: While collecting samples for alcohol measurements, non-alcohol based disinfectants (such as chlorhexidine) should be used. If alcohol-based disinfectant is absent, the site where the sample is collected should be allowed to dry for 30-60 seconds in order to minimise the risk of interference.[20]

2.1.4.4. Cotton and/or Gauze Pads

For cleaning the site where venous blood sample will be collected, gauzes which are previously folded (e.g. sizes 5 x 5 cm or 7.5 x 7.5 cm) or small pieces of cotton soaked with an antiseptic agent with or without alcohol should be used.

Caution: Fibres of cotton balls may remove platelet plugs which have been formed in venipuncture site accessing for venous blood collection. Therefore, it is not recommended to use these swabs following blood collection.[15]

2.1.4.5. Needles, Holders and Winged Blood Collection Sets

Needles are classified between 19G-23G according to their size numbers (gauge) and encoded with different colors. Size (gauge) number is inversely correlated with needle's diameter. Large size number corresponds to needles with narrow diameters and small size number corresponds to needles with wide diameters. Needle tips or winged sets with fit sizes in accordance with the site where blood collection is performed, its physical characteristics and blood volume to be collected should be used. Blood collection materials should involve single use, sterile needle tips with different sizes.

Recommendation: As per the EU Council directive (2010/32/EU), all medical devices produced for healthcare workers must be designed as safety engineered with the purpose of ensuring adequate safety and preventing occupational accidents.[21]

Caution: If needle size is greater than the size needed, it may tear the vein and cause hematoma. If needle size is small, blood cells may disrupt (hemolysis) during blood collection and cause false laboratory test results.[15]

Recommendation: With respect to ensure safety of workers, in order to prevent needle stick injuries, it is recommended to use needles which blocks or withdraws itself automatically during its removal from the skin after it is used.[22]

It is extremely important that the holders are completely compatible both with the needle and blood collection tubes used. Regarding the holders, being out of keeping with needles will cause air ingress into the tubes which is likely to result in foaming of samples. It is recommended to use single use or disposable holders, if appropriate.[16]

Caution: It should not be forgotten that holders which are not disposable may be contaminated with bacteria or blood. It should be kept in mind that this may create risks for phlebotomists.[16]

Recommendation: During pediatric phlebotomy or in cases that necessitate drawing blood from the dorsal of the hand, it is recommended to draw blood with winged phlebotomy sets.[16]

2.1.4.6. Syringe Systems

It should be avoided to collect venous blood with syringes unless it is necessary. It is not recommended to collect blood with syringes due to the following reasons:

1. The sample may hemolyse during blood collection with syringe and transferring sample into the tubes without removing the needle of the syringe.
2. During blood transfer with syringe into the tubes containing any additive, sample/additive ratio may be affected by less or more amount of blood sample transferred.

Recommendation: A safety engineered transfer device may be used in transferring blood into a proper tube in case of obligatory usage of syringe.[15]

Caution: However also in this case, during transferring blood into the tube, taking the need for removing the needle from the syringe into account, it should not be forgotten that healthcare workers are under a great risk of needle stick injuries.












2.1.4.7. Evacuated Blood Collection Tubes

Proper selection of evacuated tubes to be used in venous blood collection is among the particular issues related to the preanalytical phase in order to have reliable laboratory test results. These tubes are sterile and produced conveniently to blood collections in previously determined volumes.[15] With respect to providing proper blood/additive ratio, tubes should be kept under controlled temperature and humidity conditions suggested by the manufacturer and care must be taken not to exceed expiration date.

Caution: Tubes exceeding expiration date must not be used definitely.

Tubes that are commonly used in blood collection and their specifications are given in Table 1.

Table 1. Tubes commonly used in blood collection and their specifications

Sample type	Tube type	Additive	Cap color
Blood culture (Whole blood)	Blood culture bottle with variable content	None	Variable
Serum	No-additive tube (glass)	None	
	Tube with clot activator	Clot activator	 
	Tube with gel/clot activator	gel/clot activator	  
Plasma	Glucose tube	Sodium fluoride/potassium oxalate; Sodium fluoride/EDTA Sodium fluoride/Sodium heparin iodacetate/lithium heparin	
	Coagulation tube	Sodium citrate (9:1)	
	Heparin tube	Sodium heparin Lithium heparin	
Whole blood	Tube with EDTA	EDTA K2 EDTA K3	
	ESR (sedimentation) tube	Sodium citrate (4:1)	

EDTA; Ethylenediamine tetraacetic acid, ESR; Erythrocyte sedimentation rate, (9:1), (4:1); blood/additive ratio

Recommendation: Tubes are produced using glass or plastic material. It is recommended to use tubes made of plastic material with respect to healthcare worker's safety.[15]

2.1.4.8. Adhesive Bandages

Following blood collection, sterile adhesive bandages (must be hypoallergenic) and/or gauze should be present in order to provide bleeding to stop.[15]

Caution: In infants under 2 years of age, adhesive bandages may cause skin irritation. In addition, since infants have the risk of removing the tape from the skin and swallowing it, it is not recommended to use adhesive bandages.[23]

2.1.4.9. Sharps Container

Bins should be a box which is durable against perforating, tearing, crash and explosion, impermeable to water and leak proof, impossible to be opened and rummaged, made up of plastic laminated cartoon or made

up of a material such that and have an international biohazardous sign or emblem as well as the statement “Attention, Sharps Waste” on it. At least 3/4 of these containers should be filled. Containers must not be pressed, opened, emptied and recycled after filling.[24]

2.1.4.10. Test Manual

A test manual containing preanalytical qualifications required for various tests (preliminary, sample type, criteria of sample accessioning and rejection, sample transport requirements, etc.) is stipulated by the majority of regulatory authority.

2.1.4.11. Other Supplies

For some analytes, it is needed to transfer and centrifuge the sample under particular conditions.

Ice: Samples collected to test for analytes which lose their activities or degrade with temperature (ammonia, lactate, pyruvate, gastrin, renin, parathyroid hormone, catecholamines, adrenocorticotrophic hormone, free fatty acids, acetone, ACE) should be kept in chilled environment.[15-26] With this purpose, there should be ice or refrigerated cabinet system.

Caution: It is recommended to put the sample into ice-water mixture to keep it cold. It is not recommended to keep the sample directly on ice or dry ice in order to avoid hemolysis. In samples kept in cold for more than 2 hours, potassium should not be tested.

Aluminium foil: Samples collected to test for analytes which lose their activities or degrade with light (bilirubin, carotene, methotrexate, porphobilinogen, porphyrins, pyridoxal 5-phosphate, vitamin A, B1, B2, B3, C, E and K1) should be transferred to the laboratory in a manner that they are covered with aluminium foil and kept in dark until the analysis.[15-27]

2.2. Procedures of Blood Collection

2.2.1. Hand Hygiene

Phlebotomist must disinfect his/her hands with water, soap or alcohol-based solution or foam prior to the first contact with the patient. By this procedure, contamination of all surfaces touched by the phlebotomist during the contact with the patient is prevented.

If hands are washed with water and soap, soap should be rubbed covering all surfaces of the hands and fingers at least for 15 seconds and after rinsing with water they should be dried with a single use disposable towel.[28]

For decontaminating by scrubbing with alcohol-based solution, hands should be rubbed until they dry completely in a manner that the solution should contact all over the hands and fingers.

2.2.2. Review of the Tests Requested for the Patient and Preliminary Preparations

Tests requested by the clinician may be written and/or in electronic media. Necessary clinical pre-information and demographic information should be in written request forms and/or included in electronic media (hospital information management system; HIMS and laboratory information management system; LIMS).

Recommendation: It is recommended that the information needed to be included in test request forms and/or electronic media should be arranged according to the requirements of ISO 15189.[25] Test request forms and/or electronic media should include the following information:

- Name, surname, gender, date of birth, contact information (full address and phone number), TR identity number of the patient and the patient number;
- Clinician who requested the test and his/her contact information;
- Tests requested;
- Diagnosis, prediagnosis and other information that can be used in laboratory analysis and results interpretation (tests which require special preparation, drug treatments that the patient is receiving, etc.);
- Blood collection date and time.

Tubes that will be used according to the qualifications of the tests requested for the patient should be prepared after the request form is reviewed.

2.2.3. Patient Authentication

Patient authentication is a must for the phlebotomist in order to be able to be sure that the sample is collected from the right person. Dormant but conscious inpatients must be asked definitely, and must not rely on patient dossier or patient's record tags on/near the bed.[15]

- 1) Authentication of conscious and reachable patients.[15]
 - At least the name and surname should be asked patients who are outpatient or conscious inpatients (date of birth and/or TR identity number may also be asked). Name and surname of the patient should be asked directly (What is your name and surname?).
 - Accuracy of information gotten from outpatient should be matched with the information written on the request form, labels of sample container and/or electronic records and accuracy of information gotten from inpatient should be matched with the patient's wristband.
 - If inconsistency is observed between the two information, responsible person for phlebotomy unit or ward responsible nurse should be informed about the issue and blood sample is not to be collected definitely.
- 2) Authentication of the patients who is conscious and communication is not possible (children, foreign national or disabled persons).[15]
 - At least the name and surname of outpatient and inpatient should be asked (date of birth and/or TR identity number may also be asked) to patient's relative (legal nominee, translator).
 - Accuracy of information gotten from outpatient should be matched with the information written on the request form, labels of sample container and/or electronic records and accuracy of information gotten from inpatient should be matched with the patient's wristband.
 - If inconsistency is observed between the two information, responsible person for phlebotomy unit or ward responsible nurse should be informed about the issue and blood sample is not to be collected definitely.
- 3) Uyuyan, bilinci bulanık veya komadaki hastalara yönelik kimlik doğrulama işlemi.[15]
 - A patient who is sleeping must be awakened before blood collection. At least the name and surname should be asked to patients (date of birth and/or TR identity number may also be asked). Name and surname of the patient should be asked directly (What is your name and surname?).
 - In comatose or confused patients, authentication should be made by controlling wristband information.

- Accuracy of information gotten from the patient should be matched with the information written on the request form, labels of sample container and/or electronic records and accuracy of information gotten from inpatient should be matched with the patient's wristband.
- If inconsistency is observed between the two information, ward responsible nurse should be informed about the issue and blood sample is not to be collected definitely.

2.2.4. Examination of the Patient State Suitability for Blood Collection

In order to have true test results, it is critically important to interrogate and prepare the patient before blood collection. It may be needed that the patient should be in fasting or full, comply with particular treatment protocols, and blood should be collected after the patient rests for a certain time, etc. (Table 2).

Table 2. Query of the suitability of the patient prior to blood collection

Query	Question	Test	Explanation	Reference
Fasting (8-12 hours)	When was the last time you eat?	All Biochemistry laboratory tests	Many of the laboratory tests are affected by the nutrients taken within the diet. In addition, lipemia emerges in samples collected in the postprandial period may also cause false results in laboratory tests that are not related to fasting. Since most of the drinks include glucose as an ingredient, it may falsely elevate the glucose levels tested. Therefore, before collecting blood, the patient can be allowed only to drink water.	28, 29, 31
Patient's position	Resting for 15 minutes prior to blood collection or not?	All laboratory tests	Physical activity of the patient increases releasing of various hormones stimulating protein, lipid and carbohydrate synthesis (catecholamines and corticosteroids). In test requests including these hormones, special attention should be paid to patient's resting.	32

Query	Question	Test	Explanation	Reference
Treatment	Are you receiving any anticoagulant (blood thinner) drug?	Coagulation tests: PT, INR, thrombophilia screening tests (lupus, anticoagulant, protein S, C, activated protein C resistance)	If the patient has received any anticoagulant drug, blood should not be collected.	33
Treatment	Did you receive oral or IV ferritin drug within the last 10 days?	Serum ferritin	Using ferritin drug before giving blood or discontinued treatment a short while ago leads to get false high ferritin results.	32
Treatment	What is the name of your drug? When did you receive the last dose?	All drug levels (monitoring therapeutic drugs)	In order to monitor therapeutic drugs, blood should be collected after the drug reaches a stable level in blood. Hence, blood sample should be collected just before the next dose.	34
Treatment	When did you receive the last dose of your levothyroxine drug?	TSH, free T4, total T4	For levothyroxine dose received before giving blood affects TSH, free T4 and total T4 concentrations, it should not be taken.	35
Female hormones	Which day you are in your menstrual cycle?	LH, FSH, E2, progesterone, hCG	Concentrations of female reproductive hormones vary according to the day of menstrual cycle.	32
Treatment	What time did you eat your meal? Did you receive your treatment (insulin or oral antidiabetic agent)?	Glucose (postprandial)	When postprandial glucose concentration is measured, the patient should maintain his/her regular diet and regular drugs. Behaviours out of usual practices cause false glucose test results.	36

2.2.5. Preparation of the Equipment

According to the qualification of the test requested, all of the equipment and materials should be prepared before blood collection.

- Blood collection tubes with different volumes and containing different additives may be used according to the requested test qualifications. Tube volumes should be in accordance with the number of tests.

Caution: In person from whom blood is collected frequently, blood collection may cause anemia.[38]

- Needles with proper sizes (gauge) are used according to the physical characteristics and location of the vein as well as blood volume to be collected. Needles of different sizes should be carried.

Caution: Improper needle sizes may cause hemolysis of the sample.[39]

- Winged blood collection set for collecting blood sample from children and patients who have fragile and damaged veins
- Needle holder
- Tourniquet
- Cotton
- Disinfectant agent with (ethanol, isopropyl alcohol) or without alcohol (benzene)
- Adhesive bandages
- Sharps container

2.2.6. Tube Labeling

Tubes should be labeled after patient authentication and reviewing patient suitability for phlebotomy.

Patient barcode label should include at least the following information:

- Patient's name and surname,
- Gender,
- Patient number,
- Laboratory number.

In addition to the above items, there should be:

- Date of birth
- TR identity number
- Blood collection date and time
- Records of the phlebotomist who collects the sample should be included in process recordings, if not on the barcode label.

2.2.7. Positioning the Patient for Blood Collection

Patient's arm should place on the armlet of the chair in a stretching position. Arm should be supported very well by the armlet and not be bended from the elbow.

Regarding a patient who is laying down, it should be ensured that he/she is comfortable in decubitus position. If he/she needs an additional support, a pillow should be placed under the arm where venous access will be performed. The patient should be asked to stretch his/her arm from the shoulder to the wrist to create a straight line.[15]

2.2.8. Wearing Gloves

Phlebotomists must wear gloves. New gloves must be used for each patient. Gloves should be worn before applying tourniquet.[15-16]

2.2.9. Selection of Venipuncture Site

Anterior view of the elbow and interior part of the arm where there are large veins localizing just under the skin (antecubital fossa) are the preferred sites in blood collection. If these veins are not suitable, veins in the dorsum of the hand may be preferred for venous blood collection.

Caution: While selecting a venous blood collection site, it should be paid attention to the following considerations.[15]

- Avoiding areas recovered from burn (areas with large scars).
- Before collecting blood from the arm on the side of mastectomy, clinician should evaluate the patient with respect to complication of lymph stasis.

- Samples collected from a site with hematoma may result in error. Blood should not be collected from a site with hematoma whatever its size is. If other sites are not available, blood should be collected from the site where hematoma ends.
- Sample should be collected preferably from the arm with intravenous vascular access.
- From the arms with cannula, fistule, vascular grafting, blood should be collected after the assessment of the clinician.

2.2.10. Applying Tourniquet

In order to increase intravascular pressure, tourniquet must be applied before venous access. Increasing intravascular pressure eases palpation (tactual perception) of the vein. Tourniquet should be applied 7.5-10.0cm (3-4 fingers) above the site of vascular access.[15-16]

Caution: Tourniquet application should not exceed one minute because it can locally cease blood circulation (stasis) with hemoconcentration and infiltration of blood into the tissue. If it prolongs, all protein-based analytes, blood cell volumes and other cellular element levels result in higher values as false results.[15-16]

Recommendation: If the time for vein selection and cleaning and vascular access lasts for more than 1 minute, it is recommended to release tourniquet and reapply after two minutes in order to minimize hemoconcentration effect.[15]

2.2.11. Asking the Patient to Clench Fist

It is asked the patient to clench fist. Hence, the veins are provided to be more apparent and easier to be accessed by needle. The patient is not to be asked to open and clench the fist (pumping action). Pumping action of the hand causes increase in some blood analytes.[40]

2.2.12. Selection of the Proper Vein

Although the antecubital veins' location varies from person to person, the most common patterns in front arm can be seen in Figure 1.

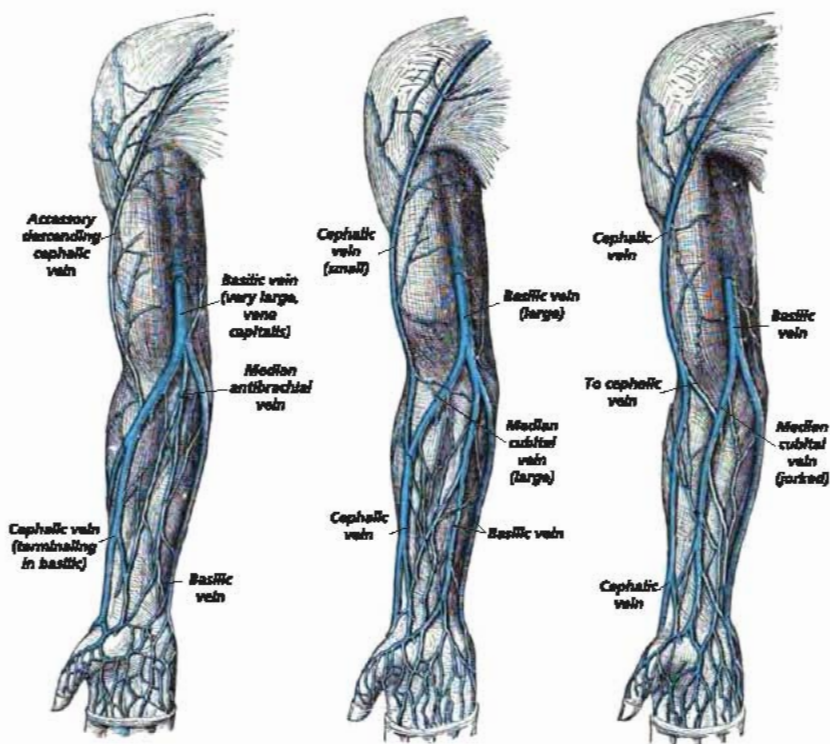


Figure 1. The most common vein patterns in front arm

(https://en.wikipedia.org/wiki/Median_cubital_vein#/media/File:Sobo_1909_597.png)

Vein used in blood collection should be selected with caution. Consistency of the vein that is to be used in blood collection should be determined by palpation. Index finger should be used in palpation, thumb should not be used because pulsation in the thumb will cause misdetermination.[41]

Caution: There pass brachial arteries and major nerves in the antecubital area. Perforation of the arteries and nerve damages are among the most common risks of venous blood collection.[15] If it is suspected that arterial access is happened (eg. fast hematoma forming or filling the tubes faster than expected), phlebotomy procedure must be interrupted immediately. The site should be applied direct pressure until bleeding stops for at least five minutes.

If the patient feels a sensation described as throbbing pain or tingling as electrification or pins-and-needles sensation, phlebotomy should be interrupted and another site should be selected.[15]

Venous blood collection from the dorsum of the hand

In cases when the antecubital region is not suitable for phlebotomy (newborns, children, patients in whom the vein cannot be seen, etc.), veins in the dorsal region of the hand can be used for blood collection.

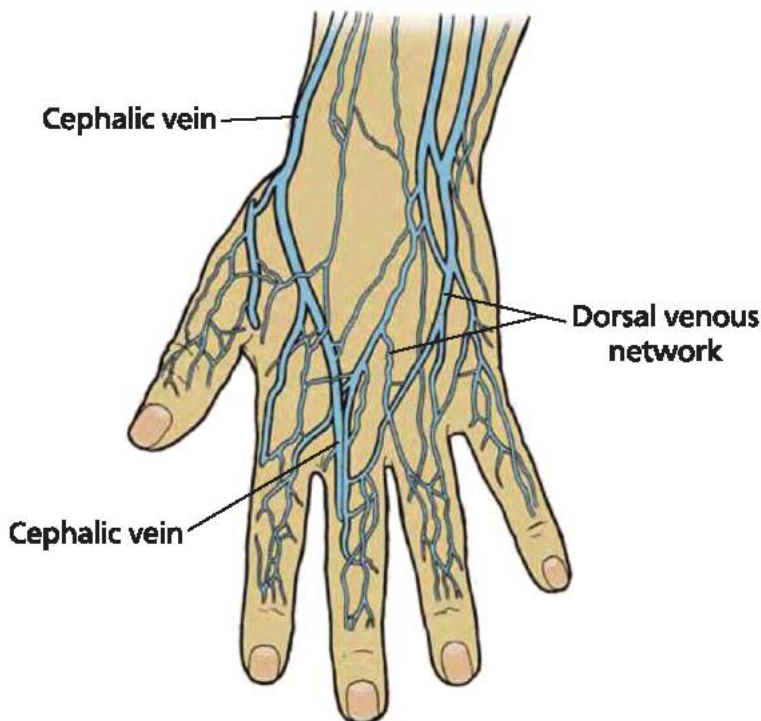


Figure 2. Veins of the dorsum of the hand suitable for blood collection

2.2.13. Cleaning Venipuncture Site

After determining the proper vein to be used in blood collection, venous access site has to be disinfected with the purpose of preventing microbial contamination of the patient and the sample. With this purpose, 70% isopropyl alcohol or sterile ethanol swab or gauze should be used. Skin should be swabbed with rotational movements from the center to the periphery.[15-16]

2.2.14. Anchoring the Vein

Vein is fixed by stretching the skin with the thumb, 2.5-5 cm beneath the site.

Caution: Due to the high risk of injury for the phlebotomist, stretching the skin above the site is not recommended.

2.2.15. Performing Venipuncture

After fixing the vein, the patient should be informed about the vein is just being accessed.

Caution: Phlebotomist should get ready for sudden and unexpected loss of consciousness that may develop in the patient.

After informing the patient, venipuncture should be done with $\leq 30^\circ$ angle (Figure 3). Following venipuncture, the needle should be held stable as far as possible and not allow the needle to move within the vein.



Figure 3. Proper angle for venipuncture

2.2.16. Observing Blood Flow, Releasing the Tourniquet and the Fist

Tourniquet is extremely important in making the veins explicit during venous blood collection. However, as soon as blood flow begins into the first sample tube, tourniquet must be released and the patient must release the fist.

Caution: Long term tourniquet application causes hemoconcentration and hemolysis in blood sample.[43-44] Hemolysis and hemoconcentration cause false results for some analytes.

2.2.17. Order of Draw by the Tube Specifications and Tube Filling

Samples should be collected into the tubes as following order in Table 3, in patients whose blood samples are to be tested for more than one analytes at a time.[45-46-47] The purpose of this order is to prevent chance of contamination among tubes containing additives.

Table 3. Blood collection order and number of inverting the tubes required to obey for sample tubes according to the specifications of the tests requested

Cap Color	Tube/Additive	Number of Inverting
Variable (1)	Blood Culture/medium	Inverted in order to provide mixing of medium with blood
(2)	Glass or plastic serum tube without additive	Not required
(3)	Coagulation tube/Citrated	3-4 times
(4)	ESR tube/Citrated	3-4 times
(5)	Serum tube/Non gel	5 times
(5)	Serum tube/Non gel	5 times
(5)	Serum tube/Non gel	5 times
(5)	Serum tube/tube with thrombin clot activator	5 times
(6)	Plasma tube/ Heparin tube with or without gel	8-10 times
(7)	Plasma tube/ EDTA tube with or without gel	8-10 times
(8)	Plasma tube/ Sodium fluoride/ potassium oxalate; Sodium fluoride/ EDTA; Sodium fluoride / Sodium heparin	8-10 times

EDTA; ethylenediaminetetraacetic, ESR; erythrocyte sedimentation rate

Caution: Tubes should be filled until the vacuum and blood flow exhausted. Tubes containing additives (EDTA, citrate, heparin, etc.) should be filled until the volumes stated by the manufacturer and being sure the accuracy of the blood/additive ratio.

2.2.18. Removing and Mixing the Tubes

Tubes should be removed from the needle holder following the cessation of blood flow. If continuing to blood collection, the same procedure should be applied to the next tube. After completing the last sample tube, first the tube should be removed from the setting and then the needle should be removed from the arm.

Caution: Tubes containing any additive should be mixed gently and by inverting (Table 4) in accordance with the recommendations of the manufacturer (Table 3) in order to provide sufficient mixture after collecting each sample. Tubes should not be shaken in order not to cause hemolysis in samples.

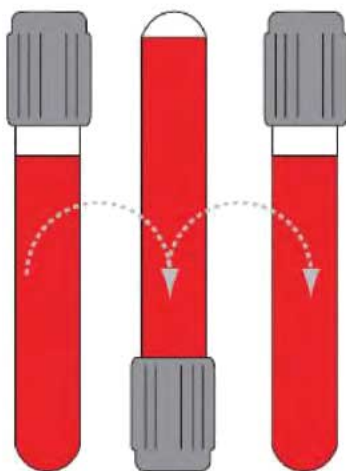


Figure 4. Way of inverting the tubes.[48]

2.2.19. Removing the Needle, Ensuring Safety and Applying Pressure on Venipuncture Site

After completing the venous blood collection procedure, the needle should be withdrawn by applying gentle pressure with dry gauze put on the needle tip.

Caution: Swab is not recommended because it removes clot stopper.

Patient should be told to apply strong pressure on the gauze and keep his/her arm straight and up and warned about not to bend his/her arm (because bending causes hematoma formation). Cessation of bleeding should be controlled and hypoallergenic adhesive bandage should be applied on the blood collection site after evaluating the patient for hematoma formation.

Caution: If hematoma occurs and bleeding lasts for more than 5 minutes, patient's related physician should be informed.

Recommendation: In cases when the patient cannot be followed, he/she is informed about the process and may make him/her to follow the process. The patient may be noticed to inform phlebotomy unit or his/her doctor if there is hemotoma formation and bleeding lasts for more than 5 minutes.

Needles should be eliminated in a perforation-durable sharps container after activating its safety mechanism in accordance with the manufacturer's recommendations

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