Turkish Biochemical Society

GUIDELINE FOR VENOUS BLOOD COLLECTION (PHLEBOTOMY)

Prepared by the Preanalytic Phase Working Group of the Turkish Biochemical Society
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It is listed in alphabetical order by surnames.

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Abbreviations

CLSI: Clinical and Laboratory Standards Institute

COLABIOCLI: Confederación Latinoamericana de Bioquímica Clínica

EDTA: Ethylenediaminetetraacetic acid

EFLM: European Federation of Clinical Chemistry and Laboratory Medicine

ESR: Erythrocyte Sedimentation Rate

EU: Europian Union

G: Gauge-Needle size unit

HIMS: Hospital Information Management Sytem

LIMS: Laboratory Information Management Sytem

MRSA: Methicillin-resistant Staphylococcus aureus

WHO: World Health Organisation

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 (13), etc. 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.

Because it is not always known which specimen is infectious, all patients and laboratory specimens should be considered as infectious and handled according to "standard precautions". All personal protective equipment (including mask) must be used throughout all laboratory processes, including blood collection.

Health professionals who perform pre-laboratory procedures may have graduated from many different health/patient care disciplines and may be working with different titles. For example: nurse, laboratory technician, health care officer, etc. For the standardization of all of them, in this guide, they will all be called "phlebotomist" with a single title.

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. In addition, CLSI GP41-Ed7 and EFLM-COLABIOCLI Joint Recommendations for Venous Blood Collection were also considered in the 3rd edition updates. 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 and ventilated 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. Adequately soundproofed private sampling rooms should be arranged for pediatric patients.

Computer systems with easy access to HIMS and/or LIMS should be placed in the phlebotomy area. In accordance with the quality management systems and legal regulations, it is necessary to record the sampling time in terms of sample traceability.

RECOMMENDATION: In blood collection areas, there may be kept a sink with water and soap and paper towel in order to make phlebotomists wash and dry their hands (15). If there is no sink, 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 and vein imaging devices

Vein imaging devices should be used to identify sensitive and thin vessels. The tourniquet can be used on invisible veins if vein imaging devices are not available and only if necessary. However, the use of tourniquets is not recommended as it may cause analyte concentration alterations due to stasis in visible vessels. 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* (MRSA) (19). It is recommended to use a single-use tourniquet, especially in cases where the risk of infection is evident and, if possible, in all general applications. Alternatively, inpatients can be allocated a tourniquet for venous access during their stay. Follow your institution's infection control procedures.

NOTE: For patient comfort, the tourniquet should be applied over the clothing or a gauze pad should be placed so that the tourniquet does not compress the skin.

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	Tube with clot activator	None	
		Clot activator	
	Tube with gel/clot activator	Gel and clot activator	
	Trace element tube	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	
	Trace element tube	EDTA or heparin	
Whole blood	Tube with EDTA	K_2EDTA / K_3EDTA	
	ESR (Sedimentation) tube	Sodium citrate (4:1)	

(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 (17,25).

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, adrenocorticotropic hormone, free fatty acids, acetone, angiotensin converting enzyme (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).

Figure 1. Blood Collection Process Flow Chart

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 kept in written request forms and/or included in electronic media (HIMS and 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 15189:2022 (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.

RECOMMENDATION: It is recommended that the patient be informed about the intended test and sample collection, and that this information is in accordance with institution policy and legal requirements.
It is recommended that the phlebotomist obtain consent from the patient for sampling as defined in institution policy. Institutions should determine the means by which consent is obtained. It is recommended not to take blood without the consent of the patient or his or her guardian. If the patient withdraws consent during the procedure, the phlebotomist should immediately safely terminate the procedure. The phlebotomist should report the patient's objections to the physician or other healthcare professional.

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 two personal information should be asked from outpatients or conscious inpatients. In addition to the name and surname, the date of birth and/or TR ID number can also be queried. The patient's name and surname should be asked directly with an open question style (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) Authentication of sleeping, confused or comatose patients (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.

4) Assigning Identity to Unidentified Emergency Patients (15):

- A temporary but clear identifier should be created for the unidentified patients who apply to the Emergency Department.
- This ID may consist of numbers or alphanumeric identifiers according to hospital procedure.
- The identifier should be kept permanently attached to the patient's body.
- Patient's identity should be associated with test orders.
- As soon as the patient's true identity is established, it must be correctly associated with this identifier.

Extra care should be taken with authentication in the following high-risk, situations:

- Twins or siblings
- Newborns

- Commonly used names (eg Ahmet, Ayşe)
- Similarly written or pronounced names (eg Aslan Kılıç or Arslan Kılınç)
- Multiple patients sharing a room.

If more than one healthcare worker is performing the procedure (for example, one brings the patient from the waiting room to the blood collection room and the other draws blood), the patient's identity should be re-questioned.

Confirmation should not be expected by showing the forms to the patient. Forms can be used if conscious outpatients cannot speak

2.2.4. Examination of the Patient State Suitability for Blood Collection

In order to have correct test results, it is critically important to interrogate and prepare the patient before blood collection. The patient should be asked whether he has had any problems with blood collection before, and his history of latex allergy and syncope should be questioned. Depending on the material used (eg gloves, tourniquets, bandages, adhesive tapes) in hypersensitive patients with latex allergy, it may cause anaphylactic shock (15). From patients with a history of syncope, blood should be drawn in the supine position. Blood should not be drawn while sitting on chairs without backs or armrests or sitting on a stretcher.

The patient should be informed about the test and blood collection procedure, and the information given and the consent obtained should be compatible with hospital procedures and regulatory requirements. If the patient or his companion does not give consent for the procedure, the procedure should be terminated immediately and safely, and the clinician should be informed about the subject.

It may be needed that the patient should be in fasting or full, comply with particular treatment protocols, blood should be collected after the patient rests for a certain time, and observing what time of the day blood is drawn for some tests (circadian rhythm), may be required (Table 2).

While the blood is being drawn, the patient should not have food, drink or chewing gum in his mouth, except for necessary medications (eg neonates and ventilated patients).

NOTE: Against the risk of syncope, blood should not be taken from the patients while sitting on chairs without backrests and armrests or sitting on a stretcher.

NOTE: Patients should be questioned whether they have a latex allergy.

For routine blood tests, it is recommended to collect blood between 07:00 and 09:00 in the morning following a 12-hour fast (29). The things to be considered are, no caffeinated

drinks, cigarettes and chewing gum are consumed just before the sampling and no alcohol is consumed within 24 hours. This requirement is not required for emergencies and parameters that are not affected by fasting/satiety.

Some tests may require a blood draw at a certain time due to drug intake, treatment monitoring, and biological variations (circadian rhythm) without the need for fasting (15).

For example:

- Tolerance test (glucose tolerance test)
- Cortisol
- Prothrombin time (PT), activated partial thromboplastin time (APTT), digoxin and other drugs

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.	30-33
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.	34
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.	35

Query	Question	Test	Explanation	Reference
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 falsely elivated ferritin results.	34
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.	36
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.	37
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.	34
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.	38
Presence of tests related to circadian rhythm	You have tests that fluctuate daily within your test orders. Did your doctor inform you about this?	Cortisol, drug monitoring	For tests that vary with the circadian rhythm, samples should be taken within the appropriate time frame unless otherwise specified by the clinician.	15

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.

NOTE: Sampling equipment (needle and holder) should not be attached prior to patient authentication to maintain sterility.

CAUTION: In person from whom blood is collected frequently, blood collection may cause anemia (39).

- 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 (40).

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

2.2.6. Tube Labeling

After patient authentication and questioning of eligibility for blood collection, tubes should be labeled in the presence of the patient before or after blood collection according to institution procedures.

Patient barcode label should include at least the following information:

- Patient's name and surname,
- Patient number,
- Phlebotomist ID,
- 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.

The label should be large enough to contain all the necessary information. Labeling should be done in such a way that all the manufacturer's information (expiry date, additives, etc.) of the tube is visible. If a bar-code is used, labeling should be done at the correct angle and position so that automatic devices can read.

Blood collection date, time and blood recipient information should be defined in the LIMS. In cases where more than one sample will be taken, such as a glucose tolerance test, the time of blood collection should be indicated on the tube 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).

Postural changes occurring within 15 minutes prior to blood collection dramatically alter the results (29). For this reason, postural changes should be avoided for 15 minutes before blood is drawn. Blood should be drawn from the inpatient in the supine position, and from the outpatient after waiting in the waiting room for 15 minutes. After this period, it is unimportant for the patient to walk to the blood collection forehead shortly. Blood should be taken from the outpatient in a sitting position.

CAUTION: Patient specimens should not be collected while sitting upright on a barrier-free examination table, armchair, or patient bed.

2.2.8. Wearing Gloves

Phlebotomists must wear gloves. New gloves must be used for each patient. Gloves should be warn before applying tourniquet (15,16). Fingertips of the gloves must not be removed.

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).
- It is not preferred to collect blood from the arm on the side of the mastectomy, but when necessary, the patient should be evaluated first by the clinician for lymphostasis complications.
- 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 not 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. Collecting blood from IV cannulas is generally not recommended. The content of IV fluids can cause erroneous laboratory test results (41,42). It has also been shown that the risk of hemolysis is high in these samples (43). However, when necessary, blood should be drawn in accordance with the patient's condition and institutional procedures, in consultation with the clinician.

CAUTION: Phlebotomists who will draw blood from the veins should be trained in this regard.

- In some special cases, it may be necessary to collect blood from the scalp, ankles and lower extremities. Because of significant medical complications (eg phlebitis, thrombosis, tissue necrosis), no attempt should be made to draw blood from these sites without laboratory specialist's approval, physician's permission, and training.
- Blood should not be drawn from infected, inflamed and edematous areas.

2.2.10. Applying Tourniquet

In order to increase intravascular pressure, tourniquet must be applied before venous access. If the vein is sufficiently visible, the use of a tourniquet is not recommended. Vein imaging devices should be used to identify sensitive and thin vessels. The tourniquet can be used on veins that cannot be seen or felt if vein imaging devices are not available and only if necessary. However, long-term tourniquet use is not recommended, as it may cause analyte concentration differences due to stasis-related hemoconcentration in visible vessels (15).

Increasing intravascular pressure eases palpation (tactual perception) of the vein. Tourniquet should be applied 7.5-10.0 cm (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, 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 (44).

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 2.

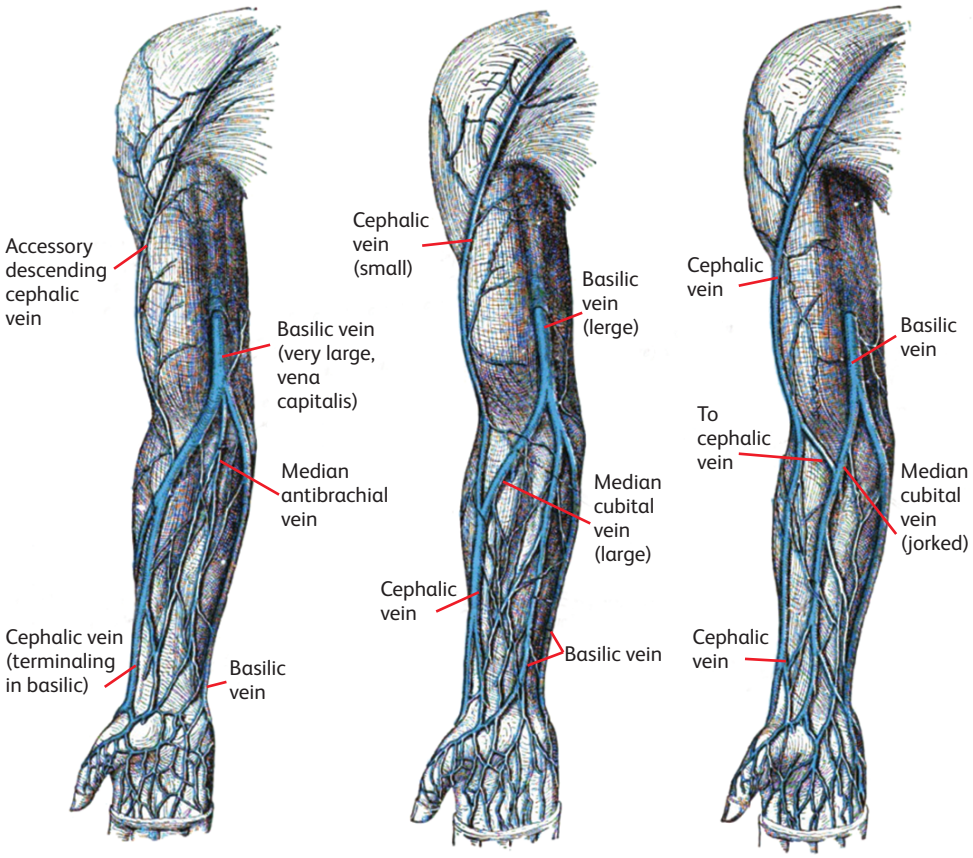

Figure 2. The most common vein patterns in front arm (45)

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 misdeterminetion (46).

The order of priority in the choice of vein to be drawn should be as follows:

1. Median cubital vein
2. Cephalic vein
3. Basilic vein (15)

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.

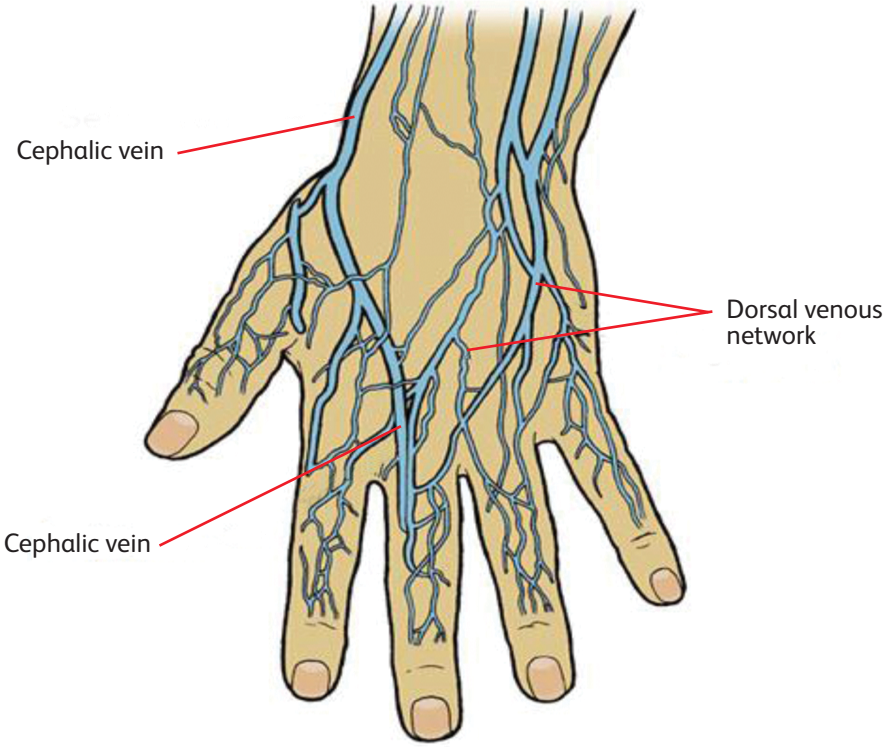

Figure 3. Veins of the dorsum of the hand suitable for blood collection (47).

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 from top to the bottom with a single movement at once (15,16). The disinfectant prescribed by your institution's infection control committee procedures should be used for blood culture sampling. Re-palpation should be avoided. The alcohol should be allowed to dry. May cause pain sensation or affect sample quality. It is not a source of hemolysis (48) .

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 ≤30° angle (Figure 4). Following venipuncture, the needle should be held stable as far as possible and not allow the needle to move within the vein. In order to prevent pain and perforation of the posterior wall of the vessel, an attempt should be made with the bevel of the needle pointing upwards.

If there is no blood flow, the needle should only be moved back and forth.

RECOMMENDATION: In order to understand if the needle is inserted in the vein, it is recommended to use needles with flash feature that show the first drop of blood in the chamber during the vein access.

CAUTION: The arm should be kept downwards as the tubes are filled to prevent backflow from the tube into the vein.

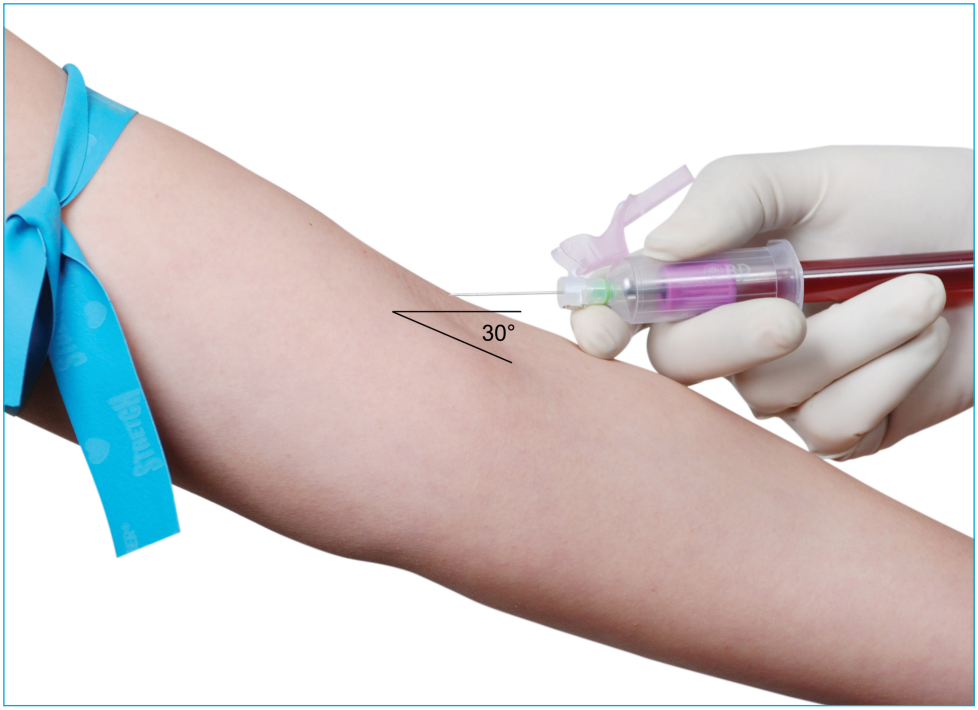

Figure 4. Proper angle for venipuncture

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 (49,50). Hemolysis and hemoconcentration cause false results for some analytes.

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

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 (51-53). The purpose of this order is to prevent chance of contamination among tubes containing additives.

It may take up to 15 seconds to mix each filled tube one by one while the blood collection is in progress. Therefore, each tube should be turned upside down at least once after filling and 4 more times after the process is completed (29).

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
	Blood Culture / Medium	Inverted in order to provide mixing of medium with blood*
	Coagulation tube/Citrated	3-4 times
	ESR tube/Citrated	3-4 times
	Serum tube/Non gel	5-6 times
	Serum tube/Gel	5-6 times
	Serum tube/Tube with thrombin clot activator/Gel or non-gel	5-6 times
	Plasma tube/ Heparin tube with or without gel or mechanical seperator	8-10 times
	Plasma tube/ EDTA tube with or without gel	8-10 times
	Plasma tube/ Sodium fluoride/ potassium oxalate; Sodium fluoride/ EDTA; Sodium fluoride / sodium heparin	8-10 times
	Special tubes for trace element testing can be EDTA or heparin added (there are also serum tube varieties; in this case, blood should be taken with serum tubes in the 5th row)	8-10 times

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

* *For best practices please see Blood Culture Application Guideline (54).*

CAUTION: If a butterfly blood collection set is used for the collection, a few drops of blood should be taken into a discard tube before the citrate tube in the blood collection sequence. Thus, the air in the butterfly set tubing should be discarded. This is important to ensure the correct blood to additive ratio, which is very important in citrate tubes. (For detailed information, see Turkish Biochemical Society Preanalytical Phase Guidelines for Coagulation Tests (55)).

CAUTION: Trace element analysis may require special handling or a change in blood collection sequence to avoid contamination during sampling. For this, laboratory procedures and manufacturer's recommendations should be followed.

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.

If more than one tube is to be drawn, the previous tube must be turned upside down at least once while waiting for next one to fill.

CAUTION: Tubes containing any additive should be mixed gently and by inverting (Figure 5) 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.

CAUTION: Tube caps must not be removed to fill tubes or to transfer blood from one tube to another (even if they are tubes with the same additive).

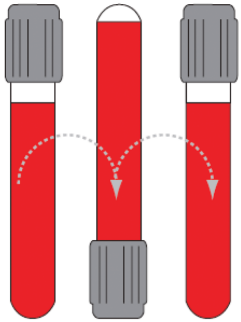

Figure 5. Way of inverting the tubes (56).

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: Cotton 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 hematom 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 shoul 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.

2.2.20. Transporting the Tubes to the Laboratory

The samples should be delivered to the laboratory as soon as possible (within 2 hours at the most). In this process, the tubes should be placed in the racks and kept upright with their caps up. Samples for analysis of some photosensitive analytes may be wrapped in aluminum foil.

All samples should be transferred to the laboratory where the analyzes will be carried out in a temperature and time controlled manner.

During the preparation of laboratory samples, tubes containing silica should be centrifuged after waiting for 30 minutes at room temperature, and tubes containing thrombin for 5 minutes. This period may be prolonged in patients with coagulation problems and in patients receiving anticoagulant therapy (15). (For detailed information, see the Turkish Biochemical Society Guideline for Centifuge Use in Medical Laboratories (57)).

2.2.21. Removal and Disposal of Gloves

Gloves should be changed after every process as they can be contaminated.
The first glove that is removed should be placed on the palm of the other hand and the other glove should be taken upside down on this glove. The outer surfaces of the gloves should not be touched and hands should be cleaned after being disposed of in the appropriate infectious waste bin.

2.2.22. Informing the Patient Following the Blood Draw

After the blood collection procedure, the patient should be instructed to press on the intervention site for 5 minutes and sit and rest.

It should be kept in mind that many patients may have syncope, anxiety or dizziness problems during the blood draw procedure or during relaxation after the fear has passed after the procedure. Therefore, before leaving the blood collection unit, the patient should be asked how he/she feels and be patient.

Finally, the patient should be informed that the results can be obtained as soon as possible, or they should be helped to find out where this information can be obtained.

3. INFORMATION ON IMPLEMENTATION OF THIS GUIDELINE

In order for this guide to be implemented successfully, first of all, existing institutional procedures should be reviewed and application deficiencies should be identified. In addition, a multidisciplinary team work is required during the determination of solutions after the correction areas are determined. Under the leadership of the medical laboratory specialist, all stakeholders should work together to prevent all errors that may arise from the preanalytical process, present their ideas and determine the most appropriate solution.

Based on this guide, there is a Preanalytical Good Practices Training consisting of 10 modules, created by the Turkish Biochemical Society Prenalytical Stage Working Group. You can direct your training requests from the Turkish Biochemical Society website contact addresses.

4. CONCLUSION

Venous blood collection is the most common medical interventional procedure in healthcare institutions. Compliance and standardization with this guideline is very important. For patient and healthcare worker safety, phlebotomist training in a repetitive structure is essential.

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