EFLM Recommendation for venous blood sampling
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on behalf of the Working Group for Preanalytical Phase (WG-PRE), of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM)

THIS IS THE FINAL DRAFT VERSION

we invite EFLM National Society Members to take part in the final stage of the development of the first official EFLM Recommendations for venous blood sampling prepared by the WG-PRE.

If you have any comment please contact your EFLM National Representative who is in charge of collecting comments on behalf of your National Society. Deadline for comments to EFLM is: December 10, 2017

All comments will be taken into account during the revision of the document. After this public consultation and revision, final version of this Recommendation will be sent for final voting to all EFLM National Societies.
Title: EFLM Recommendation for venous blood sampling

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Abstract

This document provides the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE) recommendation for venous blood sampling and practical guidance on how to successfully overcome potential barriers and obstacles to its widespread implementation. It offers guidance on the requirements for ensuring that blood collection is a safe and patient-centred procedure. Target audience for this recommendation are healthcare staff members directly involved in blood collection. This recommendation applies to the use of a closed blood collection system and does not provide guidance for the blood collection with an open needle and syringe and catheter collections. Moreover, this document neither addresses patient consent, test ordering, sample handling and transport nor collection from children and unconscious patients.

The recommended procedure is based on the best available evidence. Consensus opinion about each step and requirement throughout the procedure was reached after detailed discussions and involving a mixture of stakeholders from over 16 EFLM member countries, including nurses, phlebotomists, specialists in laboratory medicine and representatives of venous blood collection products manufacturers. Each step was graded using a system that scores the quality of the evidence and the strength of the recommendation. The process of grading was done at several face-to-face meetings involving the same mixture of stakeholders stated previously. The main parts of this recommendation are: 1) Pre-sampling procedures, 2) Sampling procedure, 3) Post-sampling procedures and 4) Implementation. We encourage professionals throughout Europe to endorse, adopt and implement this recommendation to improve the quality of blood collection practices and increase patient safety.
Introduction

The aim of this document is to provide a simple, condensed, risk and evidence-based recommendation for venous blood sampling. Although several documents of same or similar aim and scope already exist, we believe that this document is necessary to encourage and catalyse standardization of blood collection practices across Europe. There are several reasons behind this. A study published by our WG, in 2013 showed that out of the 28 European countries questioned, only 7 had their own written nationally accepted protocols (guidelines, recommendations) for venous blood sampling (1). Furthermore, the existing international guidelines and recommendations do not provide clear and unambiguous guidance for all steps during blood collection and some important details may not be considered. Moreover, as not all steps are equally important from the safety perspective, we believe that guidelines and recommendations should offer some level of critical assessment of potential risk associated with non-compliance. This is important to assist laboratories in prioritizing and focusing their corrective and preventive activities. Finally, the evidence behind some recommendations is not well defined or is even absent, or the quality of the evidence is not appraised or weighted.

One important aspect that has not been considered in the existing documents is how to successfully implement the recommended procedure. The current document provides a comprehensive overview of the most critical steps for a standardized blood collection procedure and practical guidance on how to successfully overcome potential barriers and obstacles to its widespread implementation.

This document is a result of the effort of the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) Working Group for Preanalytical Phase (WG-PRE) to address all the above-mentioned issues. Besides specialists in laboratory medicine, the authors of this document are representatives from national nursing associations, hospital nurses, phlebotomists and representatives from manufacturers of blood collection systems. Their input has been invaluable and we wish to thank them for their contribution. We encourage professionals throughout Europe to endorse, adopt and implement this recommendation to improve the quality of blood collection practices and increase patient safety.
Scope of the guidance

This document covers all steps of the venous blood collection procedure for in- and outpatients. The outpatient blood collection differs mostly in the patient preparation, patient position and physical activity prior to blood sampling. These issues are covered in the respective parts of the manuscript. The rest of the document applies equally for in- and outpatients.

This document only applies to the use of a closed blood collection system (i.e. blood collection systems where the tube cap is not removed throughout the blood sampling process) and does not provide guidance for the blood collection with an open needle and syringe. Also, it is restricted to blood collection using needles and therefore does not cover collection from a catheter. EFLM WG-PRE discourages blood sampling from an intravenous catheter, as it has been shown by many studies that catheter blood collection increases the risk of hemolysis (2, 3, 4). In cases where catheter blood collection is the only option, care must be taken to minimize the risk of hemolysis and contamination of the sample caused by admixing of intravenous (i.v.) fluids or flushing solution (these steps are outside the scope of this document).

This document is directed to healthcare staff directly involved in blood collection (hitherto referred to in the text as a phlebotomist) as the primary target group and is limited to the venous blood collection procedure. It offers guidance on the requirements for ensuring that blood collection is a safe and patient-centred procedure. It should however be noted that all national rules and recommendations take precedent over this document if they are different in any way.

This document does not address how to obtain the consent of a patient, as this may depend on the institutional policy. Test ordering, sample handling and transport as well as collection from an unconscious patient and children are also outside the scope of this document.

Disclaimer

Different manufacturers offer different products for venous blood collection. This document applies equally to all of them. All authors of this recommendation wish to disclose here that they do not have any preferences for the use of any particular product or any manufacturer.
Methodology

This document has been produced by EFLM WG-PRE following the identification of the critical preanalytical procedures involved in venous blood sampling (5) and is, wherever possible, consistent with Clinical and Laboratory Standards Institute (CLSI) and World Health Organization (WHO) guidelines (6, 7). The steps in the procedure are based on the best available evidence and a consensus opinion was reached following detailed discussions and involving a mixture of stakeholders including medical and scientific laboratory specialists from over 15 EFLM member countries, nurses, phlebotomists, specialists in laboratory medicine and representatives of venous blood collection product manufacturers (Becton Dickinson, Greiner and Sarstedt).

Once all the steps in the venous sampling procedure were agreed, each was graded based on a system that scores both quality of the evidence and the strength of the recommendation (8, 9). A grading system was used as it allows a gold standard process to be established, but still leaves room for arbitrary adaptation to local requirements for the less strongly graded steps. Grading spans from 1A being the strongest and best evidenced to 2C which is very weak in both evidence and recommendation strength. The grading system is provided in Table 1. Steps and respective grades for the quality of the evidence and the strength of the recommendation are provided in Table 2. The process of grading was performed as above via discussion at a face to face meeting involving the same mixture of stakeholders stated previously. Main parts of this recommendation are: 1) Pre-sampling procedures, 2) Sampling procedure, 3) Post-sampling procedures and 4) Implementation.

Pre-Sampling

General considerations on appropriate mode of communication with the patient

Patient communication is a key to a successful patient encounter (10, 11). During the entire blood collection process, an empathetic and confident communication with the patient is important and should always include the following basic steps:
1. Introduce yourself, maybe also with your first name for a more personal note, and explain your role within the particular health care setting.

2. After you have identified the patient correctly (see Step 1), explain what you will be doing, why you want to do it and what the patient has to do. Act confidently and calmly. This way the patient feels more comfortable, knowing that you are a professional and competent person.

3. Tell the patient that you have come to collect her/his blood and ask if a patient agrees to have her/his blood collected.

4. If asked, give a reasonable time expectation for the venous blood collection procedure itself and for the laboratory results to be returned. Be precise in your explanations.

5. Ask the patient if they feel they have been properly informed about the procedure and if there are any further questions. Be mindful and listen to the patient’s concerns. Often you will get some helpful comment on which of her/his veins are better for blood collection.

6. Ask the patient if he/she is afraid of blood collection. The evidence shows that this simple question may help identify individuals who are at increased risk of experiencing vasovagal reaction (syncope) (12). If a patient is afraid, he/she should be closely monitored during and after the blood collection, in order to prevent injuries from fall during fainting. If you feel that the patient is nervous about the forthcoming blood collection, you can give her/him a simple task to perform, such as counting upwards or taking a deep breath before the puncture.

Patient position

It has been shown that change of a body position from supine to upright and vice versa can dramatically affect the concentration of many laboratory parameters (13, 14, 15, 16). Therefore, the patient should not change his/her position within 15 min prior to blood sampling. If the patient was lying, blood sampling should be done in the lying state (this is mostly the case for hospitalized patients). Outpatients should ideally sit for 15 min prior to blood sampling. If a change in posture is unavoidable within this time period, it should be documented to allow correct interpretation of test results (17).
Step 1. Patient identification (1C)

1.1 We recommend the use of identification bracelets/bands for all inpatients.

1.2 All patients must be positively identified, in an active and engaging manner, by asking a patient a question: “What is your name?” and “What is your date of birth?” (18).

1.3 For adequate identification, at least two (patient name and date of birth) and preferably one additional identifier should be used. Additional identifiers which may be used for patient identification include:

- address,
- health insurance number,
- patient identification number,
- ID card details or any other personal identifier.

Understandably, the more data used to identify the patient, the smaller is the chance of patient identification errors (10).

1.4 The patient identity must be compared with those of the blood test request. If tubes are labelled before the blood sampling, the phlebotomist should also make sure to compare patient identity with the tube label and ensure this way the traceability of the patient identity with the test tube label. If data obtained from the patient do not match with the data on the request form or on the tube label, blood sampling procedure must be postponed until the identification issue has been resolved.

Recommendations 1.1-1.4 are grade 1C recommendations. They must be applied to all patients and on every occasion, without exception. Although we strongly recommend that this step is executed exactly as described above, there is unfortunately a paucity of evidence for exposing a patient to harm in the case of non-compliance. However, we believe that benefits of following this procedure clearly outweigh the amount of time and effort invested to ensure compliance.
Step 2. Verify patient is fasting and properly prepared (1B)

2.1 In accordance with our previously published recommendation, blood for all blood tests should be drawn in the morning (between 7-9 am) in a fasting state, 12 hours after the last meal. Water consumption is allowed during the fasting period, but patients should refrain from alcohol for 24 h prior to blood sampling. In the morning, prior to blood sampling, patients should not drink caffeine-containing beverages (coffee, energy drinks and tea). Cigarette smoking is also not permitted in the morning before the blood sampling (19).

2.2 We recognise that fasting requirement might pose certain logistical difficulties and find it acceptable to collect blood during the day for non-fasting patients only for: a) emergencies and b) tests which do not have circadian variations and for which there is evidence that fasting is not required.

2.3 Patient fasting status should be verified before blood is drawn. Whenever possible, blood should not be drawn if the patient is not properly prepared (emergencies are exceptions to this rule). If blood collection is done in the non fasting state, or a patient has not been properly prepared, this fact should be documented to allow correct interpretation of test results.

2.4 Intense physical activity should be avoided 24 hours before the blood sampling.

2.5 Time of collection of blood for therapeutic drug monitoring (TDM) will depend on the drug and indication for testing (optimizing the drug dosage, monitoring drug adherence, adverse effects, drug intoxication, etc.). Specific recommendations for the exact time of blood sampling from the ordering physician should be followed for TDM.

2.6 Laboratory staff should be aware of all other potential factors such as regular or/and recent physical activity, food intake and intake of drugs, over-the-counter medicines, food supplements and herbal preparations, etc. which are known to affect the concentration of certain analytes and make sure to check whether the patient has followed necessary instructions before blood sampling (20, 21, 22). If some of the above issues have been identified and blood sampling can not be postponed, the laboratory should document it to allow a correct interpretation of test results.

The postprandial response to food and drink depends on various non-modifiable (age, gender, genetic background, blood group, etc.) and modifiable factors. Modifiable
factors are diet (23, 24, 25, 26), intake of drugs, over-the-counter medicines, food supplements and herbal preparations (27), lifestyle, physical activity, such as diving, marathon, strenuous exercise, and some other activities (28, 29, 30), body weight, smoking, alcohol consumption, etc. To limit the variation in postprandial response as a consequence of inter-individual heterogeneity the EFLM WG-PRE has in 2014 published a recommendation on how to standardize the definition of fasting requirements. The above requirements are fully in line with this recommendation.

Physical activity is one very important modifiable factor which is known to exert both acute and chronic effects on human metabolism and blood composition. Whereas chronic effects of sport may be considered as adaptation of human organism, the acute effects may be obviated by avoiding intense physical activity 24 hours prior to blood collection.

Step 3. Obtain supplies required for venous blood collection (2C)

3.1 Venous blood collection should be performed in a clean, quiet and private environment. The blood collection area may contain pictures with relaxing landscapes on the walls, to make the space more comfortable.

3.2 Dedicated venous blood collection chairs and/or bed should be in place as well as a chair for the phlebotomist. The armrests of the chair should be adjustable to enable the optimum position for blood collection to be obtained. If a dedicated venous blood collection chair is not available the chair must have arm rests to prevent patients from falling if they feel faint (6, 7, 31).

3.3 There should be hand washing facilities with soap, running water and paper towels in the room.

3.4 Patient sample collection facilities shall have separate reception/waiting and collection areas to ensure patient privacy (32). Patient privacy should be ensured throughout the entire blood sampling procedure. We do recognize that conditions may differ in outpatient and inpatient settings and for inpatients with different clinical conditions. However, care should be taken to ensure that blood sampling is always done with respect to patient privacy.
3.5 Equipment and supplies should be available in sufficient quantities and appropriate for their intended use in the venous blood collection process. Available equipment may include:

- utility cart
- blood collection trays
- gloves
- blood collection system with safety features (needles and holders, or needles with integrated holders)
- blood collection tubes (a full range of tubes with different volume, within the expiry date)
- tourniquet (preferably single use)
- antiseptics to clean the puncture site
- bandages
- sharps bin
- sample mixer
- leak proof transportation bags

3.6 All required materials must be assembled prior to venous blood collection and per requested tests. The workplace should be arranged so that a phlebotomist can reach all necessary supplies without leaving their place.

3.7 Equipment should be properly maintained and kept clean.

3.8 A stock management system should be in place to ensure supplies are used before expiration.

3.9 Needle, holder and the blood tube make together an integral blood collection system. Only individual components of the same manufacturer should be used as a part of the blood collection system. Whereas manufacturers ensure the full compatibility between the components of their system, individual components from different manufacturers should never be used together, since their combinations are not validated for the intended use and may compromise patient and healthcare worker safety (33).

Storing tubes under conditions not consistent with the manufacturer’s recommendations can affect the draw volume, as well as the stability of gels and additives. Environmental factors such as temperature, humidity, altitude and light exposure can have a significant
impact on the quality of the blood collection equipment. Pre-evacuated blood collection tubes which are beyond the expiry date have a decreased vacuum which may lead to drawing a less than optimal volume of blood and lead to an improper blood to additive ratio (34, 35). Moreover, expired tubes may suffer from some chemical deterioration of the tube additive. To ensure sample quality, blood collection tubes should be discarded after their expiration date.

Recommendations listed under 3.1–3.8 are grade 2C recommendations (weak recommendation, low quality evidence). We were unable to find any firm evidence besides manufacturer's recommendations, one study in humans and one veterinary study (34, 35) to support the above listed recommendation.

Step 4. Labeling and/or identifying tubes (1C)

4.1 Tube labeling or tube identification must be done in the presence of the patient. Otherwise, there is a risk that the tube will be left unlabeled and possibly incorrectly identified. Labelling or identifying before or after blood collection should be based on a prospective risk analysis of the venous blood collection process in each institution.

4.2 Each institution should have a standard written procedure to which all personnel should adhere.

4.3 Essential information about the sample and the patient must be registered within the laboratory in such a manner that the tube is traceable and unambiguously linked to the patient, collected sample, test request, requestor and phlebotomist. These data include but are not limited to:

- identification of a requesting physician,
- patient’s full name,
- patient’s date of birth,
- patient’s address (home address or hospital department for inpatients)
- unique sample identification number,
- date and time of sampling,
- identification of phlebotomist
4.4 A minimum of two independent identifiers (patient’s full name and date of birth) and preferably three (unique sample identification number) should be used to identify the tube. It is not essential that all the above listed data are recorded on the blood tube. If not on the tube, this information must be documented in paper records or linked to the laboratory information system and easily retrievable.

**Sampling**

Step 5. Put on gloves (1C)

5.1 Gloves should always be worn to protect the patient and the staff performing the venous blood sampling.

5.2 Hands should be cleaned in front of the patient, before putting on gloves. Cleaning of hands (washing or sanitizing) in front of patients is important not only to minimize the risk of transmitting the infection during glove removal, but also to reassure the patient.

Unfortunately, although we consider this a strong recommendation, we were unable to find high quality evidence to support it. A recent Cochrane Database Systematic Review has showed that the role and level of protection of personal protective equipment is still unclear (36). Nevertheless, given the potential associated risk, until proven otherwise, we recommend that gloves are used to protect the patient and the healthcare worker. In the event of a needle-stick injury, gloves act as a barrier or protection to minimize the amount of blood that might be transmitted during the needlestick injury (37, 38). Given the fact that a substantial proportion of healthcare staff directly involved with blood collection has at some time point been exposed to a needle-stick injury during their working time, wearing gloves sounds like a reasonable infection prevention measure (39, 40). The evidence also shows that the use of sterile gloves during blood collection for blood culture reduces the risk of contamination of the sample (41, 42). In addition, apart from being exposed during the needlestick injuries, venous blood sampling is always associated with a risk for blood contact and contamination during the procedure. There is evidence showing that this risk is reduced using gloves (43, 44). It has been shown that hand cleansing is the key to the reducing the risk of the infection of the
healthcare staff and cross-transmission of antimicrobial resistant pathogens. Moreover, proper hand cleaning and wearing gloves protects the patient against infections (45). Unfortunately, the evidence shows that gloves are not widely used among healthcare workers (46).

CLSI GP41-A7 guidelines recommend putting gloves on after applying tourniquet. However, there is evidence that the time of tourniquet application may then be > 1 min if this CLSI recommended procedure is followed (47). Therefore, to reduce prolonged blood stasis we suggest the gloves are put on prior to tourniquet application.

5.3. a) Assemble the needle and the holder (if not already pre-assembled) or b) assemble the needle with an integrated holder with the blood collection tube (for users which are using the blood collection systems with aspiration technique).

Step 6. Apply tourniquet (1A)

The tourniquet is conventionally defined as a constricting or compressing (elastic) device, which can be used to limit venous circulation to an extremity (usually an upper arm) for a limited period of time. In the absence of some other device which may be used to make veins visible, the use of the tourniquet may be helpful, especially in those patients with small or scarcely visible veins. However, we recommend that blood collection is done preferably without tourniquets (especially in patients with prominent veins) and that tourniquets are used only when necessary.

6.1 The tourniquet should be applied approximately one hand width (7.5 cm) above the anticipated puncture site and should be tight enough to stop venous but not arterial blood flow.

6.2. We recommend that disposable tourniquets are used to minimize the risk of infection and cross-contamination of patient and healthcare staff.

Evidence shows that reusable tourniquets can be colonised with multiresistant microorganisms and may thus serve as a reservoir and source of transmission of various pathogens to hospitalised patients (48, 49). Reusable tourniquets may even be contaminated with Methicillin-resistant Staphylococcus aureus (MRSA) and thus pose a
great risk for patients and healthcare staff. Given the risk associated with the use of reusable tourniquets and the quality of available evidence, we have graded this recommendation as 1A. Unfortunately, disposable tourniquets are not widely used, especially in some developing or non-developed countries in Europe (50). Hospital management should be made aware of the risk associated with the use of reusable tourniquets and potential benefit of the use of disposable tourniquets for the safety of the patients and healthcare staff.

6.2. To minimize the risk of venous stasis, especially if multiple tubes are to be drawn, instead of tourniquets, vein illumination devices may be used to locate the veins. This is especially useful in patients with difficult veins. It has been shown that vein illumination devices may serve as a useful alternative for tourniquets to avoid venous stasis and subsequent alterations of the concentration of various biochemistry, hematology and coagulation parameters in the blood (51, 52, 53). The use of vein illumination devices may be a valuable perspective for the future, although more clinical evidence is necessary before widespread implementation can be recommended.

6.3 Warn the patient not to clench or pump the fist. Fist clenching and pumping may case pseudohyperkalemia and alterations of some other biochemistry and hematology parameters (54, 55, 56, 57, 58, 59).

Step 7. Select venepuncture site (1A)

7.1 Selecting the best vein and recognizing the most appropriate site to insert the needle for venous blood collection is important for sample quality, patient satisfaction, to avoid nerve damage, to avoid arterial puncture, for the ease and speed of collection and ultimately for a successful blood collection procedure (60).

7.2 The main veins in cubital fossa are cephalic, basilic, median cubital and median antebrahcial veins (Figure 1). If available, these most prominent veins should be the first choice. The cubital vein is the most preferable choice, as it is usually the most prominent, does not roll under the skin and can be found on the same place in most patients. Only if these veins are unavailable should dorsal hand veins be used as an alternative. Blood collection from the veins in the wrist is discouraged.
Figure 1. The most frequent variations of the veins of the forearm. Reprinted from (61) with kind permission of the Elsevier GmbH

Cross sectional graphic presentation of the cubital fossa is depicted in Figure 2. Understanding the anatomy of this region helps reducing the risk of injuries during the blood collection procedure.
Figure 2. Topographic anatomy of the cubital fossa (cross-section at the elbow); vessels: CV) cephalic vein; RA) radial artery; BV) basilic vein; tendons: α) biceps brachii tendon; β) triceps brachii tendon); nerves: a) lateral antebrachial cutaneous nerve; b) medial antebrachial cutaneous nerve; c) median nerve; d) ulnar nerve; e) posterior lateral antebrachial nerve; f) radial nerve; muscles and bones: 1) brachioradialis; 2) brachialis; 3) pronator tenes; 4) trochlea (humerus); 5) olecranon (ulna); 6) anconeus. Reprinted from (56) with kind permission of the Croatian Society of Medical Biochemistry and Laboratory Medicine.

7.3 Do not collect blood from previously placed peripheral venous catheters, indurated veins, paretic arms or arms with lymphatic drain disorders.

Recommendations 7.1-7.3 are grade 1A recommendations. They must be applied to all patients and on every occasion, with no exception. There is ample evidence demonstrating that blood collection procedure may cause some serious injuries if in the case of failure to find appropriate vein for performing the venous blood collection (62, 63).

Step 8. Clean sampling site (1A)

8.1 Selected site should be cleaned with 70% ethyl alcohol prior to blood sampling to prevent contamination with skin pathogens. Cleaning should be performed with one wipe and the selected site should be left to dry. Do not wipe the sampling site with the same gauze twice.

8.2 For blood culture collection, we recommend to adhere to the instructions provided by the Hospital Department of Microbiology and/or to the information provided by the disinfectant manufacturer. Cleaning the sampling site by disinfecting twice using separate gauze pads seems advisable. Let disinfectant dry for at least 60 seconds (64, 65).

8.3 Do not touch the disinfected site after the cleaning.
Contamination of blood, by the normal flora of skin, during the blood collection procedure has been demonstrated to occur if the venepuncture site has not been properly cleaned (66, 67). Cleaning is therefore of utmost importance, especially if blood is collected for blood culture.

Alcohol evaporates quickly and already within 10 sec the amount of alcohol is reduced by half of the initial amount (68). Although the failure to let the alcohol dry may indeed cause an itchy sensation in some patients, it will not compromise the blood collection procedure and the quality of the sample. It has been shown that the presence of alcohol (in case the venepuncture site was not let to dry) on the collection site is not a source of spurious hemolysis (69). Moreover, under ideal blood collection conditions, the use of ethanol before venous blood collection does not interfere with blood alcohol measurement (70). Nevertheless, to avoid a risk of false-positive alcohol results, we suggest that in collections of blood samples for forensic alcohol testing the alcohol should be left to dry before performing a venous blood collection. Alternatively non-alcoholic antiseptic cleanser approved for use by the institution may be used to avoid the risk of contamination.

Step 9. Puncture the vein (Figure 3) (1A)

9.1. Puncture the vein with the bevel up, as it minimizes the pain and reduce the risk for perforation of the back wall on the vein.

9.2 Prevent the rolling veins by extending the patient's skin.

9.3 Insert the needle longitudinally into the vessel with determination and prudence at an approximately 5-30 degree angle depending on the vein’s depth so that at least one-fourth of the needle is inserted into the vessel.
Figure 3. Needle should be inserted into the vessel at an approximately 5-30 degree angle, depending on the vein's depth. (a) inserting the needle for the users of pre-evacuated tubes and b) inserting the needle for the users of blood collection systems using the aspiration technique)

9.4 Hold the tube holder steady and by supporting your hand against the patient's arm. Ensure that the patient's fist is open and not clenched when blood comes (6, 7, 71).

9.5 If a vein cannot be located, a repositioning of the needle may help to find the vein.

9.6 The use of sharps device with flash visualisation may be helpful, especially with non-experienced staff, or in children and patients with difficult veins. These devices provide a visible venous flash when the needle is connected to the vein (Figure 4).
Step 10. Drawing blood into the first tube (1A)

10.1. a) Insert the tube in the holder so that the cap is perforated and the blood is drawn (vacuum technique) or b) withdraw the plunger slowly (aspiration technique). Follow the EFLM recommended order of draw (72). Since blood collection technique may differ with respect to the manufacturer, specific recommendations of the manufacturer should always be followed, along with the recommendations in this document, during blood collection.

The recommended order of draw is as follows:

1. Blood culture tube
2. Citrate tube
3. Plain tube or tube with clot activator
4. Heparin tube
5. EDTA tube
6. Glycolysis inhibitor tube
7. Other tubes (e.g. tube with no additives for trace elements)
10.2. When coagulation tube is collected as the first or the only tube

- and a straight needle is used for blood collection, no discard tube is needed (73, 74).
- and a winged blood collection set (butterfly devices) is used, a discard tube must be collected to prevent underfilling of the tube with subsequent bias in test results (6).

10.3. Ensure that tubes are fully filled (e.g. up to the indicated level on the tube). Underfilling of the tubes (tubes filled with less than 90% of draw volume) is strongly discouraged and should be avoided.

Although some would argue that incorrect order of draw when using closed blood collection systems is not the source of contamination (75, 76), there is firm evidence showing that contamination still occurs more commonly than might be expected and can be difficult to identify (77, 78, 79, 80). This is probably because venepuncture is not always performed under ideal conditions. There are still clinical settings such as emergency departments, where blood sampling is performed in less than ideal conditions and where only a minor proportion of blood collections is performed using the conventional manufacturer prescribed closed collection technique (81). Given the reasons explained above, and because there is no disadvantage in following the order of draw, we recommend that the order of draw is followed without exceptions during every blood collection.

Step 11. Release the tourniquet (1A)

11.1 The tourniquet should be removed as soon as the blood flows into the first tube.
11.2 If the blood collection is unsuccessful, the tourniquet should be released and blood collection should be done on an alternative site.

Tourniquets cause a temporary occlusion of veins and temporary venous stasis. If applied for a long period of time (over 1 min) a tourniquet induces a substantial variation of blood composition, due to extravasation of water and small molecules such as ions.
from the vessel into the subendothelial space. During that process, large molecules such as lipoprotein particles, proteins and protein-bound substances, cells and coagulation factors remain within the vessel, so that their concentration progressively increases. Most of these changes are negligible within 1-min of the application of the tourniquet, but can become clinically significant afterwards (82, 83, 84).

Step 12. Gently invert the tubes once immediately after collection (1B)

12.1 Mix all tubes once immediately after the blood has been drawn. Any delay may affect the quality of the sample.

12.2. Mix tubes gently by inverting it once, before collecting the next tube. One inversion involves turning the tube vertically for 180° and putting it back to the starting position (Figure 5).

Figure 5. One mixing cycle. One inversion involves turning the tube vertically for 180° and putting it back to the starting position. Reprinted from (22) with kind permission of the Croatian Society of Medical Biochemistry and Laboratory Medicine

12.3 The dominant hand should be used to hold the needle and a holder in place throughout the collection to maintain control. Also, the hand should not be changed during the drawing of the additional tubes (Figure 6).
Figure 6. Gently invert the tube once immediately after collection. Hold the needle with a dominant hand. Do not change hands during mixing and drawing of the additional tubes (a) mixing the tube for the user of pre-evacuated tubes and b) mixing the tube for the users of blood collection systems using the aspiration technique.

12.4 Avoid vigorous mixing of the specimens (e.g., shaking) to prevent blood cell injury, hemolysis, platelet activation or blood clotting (85).
12.5 The use of automated mixing tables/devices is considered to be a gold standard and is highly recommended as it enables immediate mixing of samples without engaging a phlebotomist.

Appropriate mixing of the blood tube after the blood has been drawn is an important step which ensures that tube additive (anticoagulant, clot activator, etc.) is adequately mixed, blood samples are homogenous, and sample quality and integrity are maintained. We are aware that manufacturers are providing their specific recommendations on the number of inversions for a particular tube, i.e. that tubes should be gently inverted at least five to ten times, depending on the tube type (6, 86, 87).

Over the past few years there has been a debate about whether mixing does or does not affect the quality of the sample. Some studies have shown that failure to mix the primary blood tube most probably will not introduce a bias in many test results. The explanation for these observations could be that blood turbulence that is caused by standard vacuum pressure inside the primary tubes is sufficient, in itself, to provide both solubilisation, mixing and stabilization of additives and blood during venepuncture (88, 89, 90). It could certainly be that under optimal conditions mixing the tube after venous blood collection might not be mandatory (91, 92, 93). However, in some borderline conditions and circumstances, the failure to mix the tube may affect the quality of the sample and, for example, lead to sample hemolysis or clotting. Given the reasons explained above we strongly recommend that tube mixing is done always without exception.

In cases when more than one tube needs to be collected, mixing the first tube and putting the next tube into the holder at the same time is practically impossible, if a phlebotomist holds a holder with one hand and is mixing the tube with another hand. If a phlebotomist chooses to first mix one tube (for example for 10 times) and only after that to leave this tube, take the next one and insert it into the holder, the average time necessary to complete the mixing and put on the next tube would be at least 15 sec (unpublished observations). If multiple tubes need to be drawn, the total time during which a patient has a needle in his/her vein might be substantially prolonged. To overcome this and ease the patient discomfort, while not significantly compromising the quality of the samples, we herein recommend that if multiple tubes are to be drawn, each tube is mixed only for one full inversion and only when all tubes are collected and needle
is removed from the patient vein, all tubes are mixed for additional 4 times (see Step 18).

Step 13. Draw additional tubes following the recommended order of draw (1B)

13.1 Draw all subsequent tubes and gently mix each tube once (1 full inversion), as explained in the previous step (see Step 12).

13.2. Draw tubes in the recommended order of draw (See Step 10)

Step 14. Remove the needle from the vein and ensure the safety mechanism is activated (1A)

After disconnecting the last tube put a gauze pad on the venous blood collection area, without applying the pressure. Gently remove the needle trying not to cause any injury and press the puncture site with the gauze pad to avoid bleeding.

There are safety engineered blood collection devices on the market that may differ in the way they are activated (e.g. while the needle is still inside the vein, or after the needle has been removed from the vein). In accordance with the European Directive 2010/32 EU we recommend that only safety engineered blood collection devices are used to prevent exposure of healthcare workers and patients to a contaminated needle (94). Manufacturers’ recommendations should be followed depending on the device used.

Step 15. Dispose of the needle (1A)

15.1 Immediately after the safety mechanism has been activated, the used blood collection device should be disposed into a puncture-resistant sharps container.

15.2 Walking to sharps container is not an acceptable practice.
Step 16. Bandage the puncture site (1C)

16.1 Check that the bleeding has stopped. Treat the wound by applying a patch or a bandage by placing an adhesive tape tight over a dry pad/gauze square.

Step 17. Tell the patient to apply pressure and do not bend the arm (1C)

17.1 Patient should be advised to apply pressure and not to bend the arm, in order to minimize the risk of hematoma or prolonged bleeding.

17.2. Elevating the arm may be useful to stop bleeding from the puncture site.

A pressure should be applied until the bleeding has stopped, which is usually a period of up to 2 minutes for routine draws and up to 10 minutes for patients on anticoagulation. If the cubital vein was punctured, the patient’s arm should be straight. Although one study in Denmark found no difference in the risk of bruising irrespective to whether the arm was bent or not (95), many studies have shown that bending the arm can cause a hematoma (96, 97).

Step 18. Invert all tubes at least 4 times (1B)

18.1 After removing the needle from vein and activating the safety mechanism in place, invert all tubes at least 4 times (the number of full rotations should ideally correspond to manufacturers’ instruction). For information about the proper mixing procedure please refer to Step 12.

18.2 If only one tube is collected invert it 5 times directly after collection.

Step 19. Remove gloves (1A)

19.1. Since used gloves might be contaminated with body fluids and/or microorganisms, we recommend that gloves are changed after every venous blood collection.
19.2 We recommend that following procedure is used for glove removal: remove one glove and turn it inside out (Figure 7, left), enclosing the first glove by rolling the second glove over it (Figure 7, right).

19.3 Discard the gloves and clean your hands (98).

![Figure 7. Removing the gloves: remove one glove and turn it inside out (left), enclosing the first glove by rolling the second glove over it (right).](image)

**Post sampling**

Step 20. Advise the patient to rest for 5 min (1B)

20.1 Advise the patient to rest for 5 min or wait until the bleeding has stopped (if longer than 5 min) before leaving the blood collection facility.

20.2 Be empathetic and ask a patient how he/she feels before leaving the blood collection facility. This may help identify patients who are at risk of experiencing dizziness or even syncope.

20.3 Thank the patient and leave her/him with the assurance that she/he will obtain his laboratory results as soon as possible.

With this step, we want to draw attention to the period after the blood sampling, during which patients may feel dizzy, or even faint, due to a vasovagal syncope. There are patients who are afraid of needles or feel discomfort when seeing blood. Such patients,
especially young ones may in some circumstances even experience syncope during or immediately after the blood collection (99, 100). Syncope during or after the blood collection may occur as a result of either anxiety, or a sudden relief from anxiety, when a patient no longer feels threatened (101). Therefore, to make sure that patient is well and that no acute complications have occurred, we suggest that a patient is monitored in the blood collection area for at least 5 min, or longer until the bleeding has stopped.

As already explained earlier (under heading: Patient Communication), empathetic and confident communication with a patient is very important. Assessing the degree of fear of blood collection may help identify patients who are at increased risk of experiencing syncope during or after the blood sampling (12, 102). In these patients comfort or distraction may enhance patient response to stress from blood sampling and reduce the risk of syncope.
Implementation of the guidelines

Potential barriers and challenges

Successful implementation of the guidelines depends on overcoming any potential barriers or challenges. In order to make a good and feasible implementation plan, one has to first identify all barriers and challenges. Potential barriers and challenges at the individual level which might compromise successful implementation of this recommendation are the resistance of an individual to change, language barrier, the lack of knowledge, awareness and understanding. Finally, even if there is a positive attitude towards a change, such change could be difficult if there is nobody who is responsible to manage the change or said responsible individual has some other priorities.

Barriers and challenges at the level of the hospital could be of financial nature. There could also be issues such as the lack of staff who could take over the responsibility to manage the change. Certainly, a change would be difficult if it is deemed as low priority to hospital management.

There are also several possible barriers which could arise at the national level. As is the case at the level of the individual hospital, possible barriers at the national level could be the lack of awareness and understanding about the necessity for implementing the recommendation as well as the lack of a professional entity who could take over the responsibility to manage the change. Also, in some countries there is more than one professional group whose members are involved in the blood sampling process. The existence of such groups might be an obstacle towards the successful implementation of the recommendations, if they do not agree to work together. In some countries, recommendations are supported only if they come from a regulatory body. Finally, if the existing national legislation is in conflict with this document, this could pose a considerable difficulty to the implementation of this recommendation.

As of the potential barriers at the European level, it could be that some countries and national associations would find it difficult to implement the recommendation if it is not officially endorsed or even included in some internationally recognised regulatory document (such as CLSI, ISO, etc.). Also, due to the difficulty in finding appropriate communication channels or targeting responsible entities in each European country, it
can indeed be a great challenge to ensure all European countries accept and implement this recommendation.

Framework for a successful implementation of this recommendation

There are many ways to deal with the resistance of an individual to a change (103). We believe that majority of medical staff are highly concerned with patient safety and well-being. Therefore, their resistance to learn and adopt new blood sampling procedure is basically caused by their lack of understanding of the potential harm to the patient or themselves which may arise as a consequence of non-adherence to the recommended procedure. By educating staff about potential risks to the patient posed by poor blood sampling procedure, awareness is raised about the necessity to adhere to the recommended procedure (104, 105, 106). Education increases the level of confidence and improves quality of procedures (107). Nevertheless, the effects are usually short-term and this is why education should be continuously repeated (108).

There is a low level of knowledge and understanding of some basic preanalytical issues among students in biomedicine (medical school, pharmacy, veterinary medicine) (1, 109). The education about blood sampling procedure should therefore be available to medical staff during their formal education to become qualified (theoretical and practical). As different professions are involved in blood sampling in different European countries, the professions who would need to receive such education vary from country to country (110).

Education about blood sampling procedure should also be available to all newly employed medical staff involved in blood sampling. Also, besides education, which is mostly theoretical, newly employed staff should undergo a practical training of the blood collection procedure. Practical training should preferably be offered in the laboratory outpatient unit, during the period of 1 week during which a new staff member should perform at least 100 blood collections, under the supervision of the responsible staff. An observational audit should be done during the first five and last five collections, to assess the level of compliance with the recommended procedure and identify potential deviations.

We recommend that each institution establishes its own system of certification of staff involved in the blood sampling procedure. Certification should be granted to all new
members of staff only after successful completion of initial education and training. Knowledge testing and observational audit are suggested as requirement for a certificate. To obtain a certificate, a member of the staff should successfully pass the knowledge test (80% of the correct replies). Furthermore, all 4 and 5 category steps during observation should be done in accordance with the recommended procedure.

We also recommend that each healthcare institution has a system of continuous auditing and re-training for all staff members. We recommend that auditing is done in the form of observational audit using the standardized structured auditing checklist. Observational audit should be done periodically in each clinical department at least once per year. During each observational audit at least 20 blood collections, performed by at least three different phlebotomists (at least three per each phlebotomist) should be observed.

Periodical education (theoretical and practical) should be made available to all staff members after every three years at minimum. This education could even be organized as e-learning, if resources are available. As education and training could be time demanding and in settings where human resources are limited, we recommend that a system is established to “train the trainers”, meaning that at each department there is a member of the medical staff (chief nurse of the department) responsible for education, training and auditing of the staff.

We recommend that a knowledge test is used to assess the level of knowledge and understanding as well as to raise awareness of the staff prior to education. Also, we recommend that a knowledge test is used to assess the level of knowledge and awareness of the staff after the education. The knowledge test should assess the understanding about the below listed issues and facts:

- most frequent errors in preanalytical phase
- the impact of preanalytical errors on the quality of the sample and patient outcome
- how to properly prepare a patient for blood sampling?
- how is fasting defined and why is it important?
- proper patient ID and tube labeling procedure
- tube types, additives
- the order of draw
- the use of tourniquet
- adequate mixing procedure
- why blood-to-additive ratio matters?
- hemolysis – causes and consequences
- clotting – causes and consequences
- patient and healthcare worker safety

Quality indicators are efficient tool for obtaining information about the risk of errors, error frequencies and their distribution throughout the total testing process (111). We recommend that quality indicators are used to monitor the quality of samples received in the laboratory (112, 113, 114). Laboratories are recommended to monitor the frequency of under-filled tubes, clotted samples, sample hemolysis, ID errors, etc.. as they are a good tool to detect certain “spikes” and point to some specific problems during blood collection procedure. The choice of the quality indicators to be used will depend on the local requirements and particular problems and issues at the level of each hospital. Quality indicators should be used to act upon them and correct the issues.

To overcome the language barrier, the recommendation should be translated to the local language and made available to all involved in the blood sampling process.

As of the ways to overcome barriers at the level of the hospital, one has to be able to present the benefits of the implementation of this recommendation, such as the cost of poor sample quality, potential savings, reduction of patient harm or improvement of patient safety and satisfaction (115, 116). Furthermore, it has been demonstrated that adherence to the recommended blood collection procedure minimizes the risk of patient harm and frequency of unsuitable samples (117). This important safety aspect needs to be demonstrated to the hospital management. Finally, hospital management is likely to be interested in any intervention which could potentially be regarded as a matter of prestige among similar institutions.

For successful implementation of the recommendation, there should be a member of the staff who should be responsible to manage the change at the level of the hospital (a so called: “ambassador”). This person should have time dedicated for this task.
Also, this person should have a team consisting of several key stakeholders in the hospital, such as the chief nurse and possibly representatives from the:

- laboratory,
- clinical staff (medical doctors),
- laboratory technicians,
- epidemiologists,
- department for hospital infections and worker safety,
- quality department,
- top hospital management.

This team should meet on a regular basis and discuss and plan strategy for successful implementation and continuous improvement.

At the national level, there should also be an “ambassador” who will take the lead in the process of implementation of this recommendation. To facilitate the implementation there should be a working group for preanalytical phase or some other entity which will be responsible for educational interventions and raising the awareness among all stakeholders and professions (of same or different background and level of education) involved in blood sampling about the necessity for the implementation of the recommendation. National journals and their Editors are also encouraged to raise awareness about preanalytical phase and venous blood sampling in particular, by offering their journal as a efficient and powerful vehicle for sharing knowledge and information (118, 119, 120). The implementation process should be done as a joint effort in close multidisciplinary collaboration of all stakeholders at the national level. National “ambassadors” are responsible to identify and recruit key stakeholders such as national nursing associations, professional societies in laboratory medicine and preferably even patients.

It is highly advisable to involve regulatory bodies, such as professional chambers, associations, national regulatory entities and even governmental bodies like Ministry of Health to support and endorse the implementation activities.
If some national rules are in conflict with this document, there should be a mechanism to agree on the modification of the document at the national level and accept the revised version for implementation.

The EFLM WG-PRE as the leading professional entity involved in preanalytical phase feels responsible to provide a framework for a successful implementation of this document at the European level (121, 122). It is our aim to encourage European Association for Accreditation to endorse this document as a standard and encourage its use at the national level in each European country during accreditation assessments.

To facilitate the implementation EFLM has prepared following tools:

1. a power point presentation describing some basic issues related to venous blood sampling and the entire procedure (to be used during education of staff)
2. video describing the entire procedure (to be used during education of staff)
3. a knowledge test to assess the level of knowledge and raise awareness of the staff prior and after the education
4. a checklist to be used for auditing the blood sampling procedure during periodical observational audits
5. posters with a cartoon describing the entire procedure (to be used at blood collection facilities)

These tools are freely available at the EFLM website (www.eflm.eu/index.php/wg-preanalytical-phase.html) and professionals are encouraged to download and use these tools to implement the recommended procedure for venous blood collection and establish a quality system in place to maintain and continuously improve the quality of the procedure.
Table 1. Grading recommendations used in the evaluation of available evidence.

(http://www.uptodate.com/home/grading-guide#GradingRecommendations)

<table>
<thead>
<tr>
<th>Grade of Recommendation</th>
<th>Clarity of risk/benefit</th>
<th>Quality of supporting evidence</th>
<th>Implications</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1A.</strong> Strong recommendation, high quality evidence</td>
<td>Benefits clearly outweigh risk and burdens, or vice versa.</td>
<td>Consistent evidence from well performed randomized, controlled trials or overwhelming evidence of some other form. Further research is unlikely to change our confidence in the estimate of benefit and risk.</td>
<td>Strong recommendations, can apply to most patients in most circumstances without reservation. Clinicians should follow a strong recommendation unless a clear and compelling rationale for an alternative approach is present.</td>
</tr>
<tr>
<td><strong>1B.</strong> Strong recommendation, moderate quality evidence</td>
<td>Benefits clearly outweigh risk and burdens, or vice versa.</td>
<td>Evidence from randomized, controlled trials with important limitations (inconsistent results, methodologic flaws, indirect or imprecise), or very strong evidence of some other research design. Further research (if performed) is likely to have an impact on our</td>
<td>Strong recommendation and applies to most patients. Clinicians should follow a strong recommendation unless a clear and compelling rationale for an alternative approach is present.</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Strength</th>
<th>Recommendation</th>
<th>Evidence</th>
<th>Confidence in the estimate of benefit and risk and may change the estimate.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1C.</strong></td>
<td>Strong recommendation, low quality evidence</td>
<td>Benefits appear to outweigh risk and burdens, or vice versa.</td>
<td>Evidence from observational studies, unsystematic clinical experience, or from randomized, controlled trials with serious flaws. Any estimate of effect is uncertain.</td>
</tr>
<tr>
<td><strong>2A.</strong></td>
<td>Weak recommendation, high quality evidence</td>
<td>Benefits closely balanced with risks and burdens.</td>
<td>Consistent evidence from well performed randomized, controlled trials or overwhelming evidence of some other form. Further research is unlikely to change our confidence in the estimate of benefit and risk.</td>
</tr>
<tr>
<td><strong>2B.</strong></td>
<td>Weak recommendation, moderate quality evidence</td>
<td>Benefits closely balanced with risks and burdens.</td>
<td>Evidence from randomized, controlled trials with important limitations (inconsistent results, methodologic flaws, indirect or</td>
</tr>
<tr>
<td>2C.</td>
<td>Weak recommendation, low quality evidence</td>
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<tr>
<td>2C.</td>
<td>Uncertainty in the estimates of benefits, risks, and burdens; benefits may be closely balanced with risks and burdens.</td>
<td></td>
<td></td>
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<tr>
<td>2C.</td>
<td>Evidence from observational studies, unsystematic clinical experience, or from randomized, controlled trials with serious flaws. Any estimate of effect is uncertain.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2C.</td>
<td>Very weak recommendation; other alternatives may be equally reasonable.</td>
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</tbody>
</table>

Some uncertainty in the estimates of benefits, risks, and burdens. Imprecise, or very strong evidence of some other research design. Further research (if performed) is likely to have an impact on our confidence in the estimate of benefit and risk and may change the estimate. Patients under some circumstances.
Table 2. Venous blood sampling - the order of steps

<table>
<thead>
<tr>
<th>Step</th>
<th>Strength of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Identify a patient</td>
<td>1C</td>
</tr>
<tr>
<td>2. Verify patient is fasting and properly prepared</td>
<td>1B</td>
</tr>
<tr>
<td>3. Obtain supplies required for blood collection</td>
<td>2C</td>
</tr>
<tr>
<td>4. Label/identify tubes</td>
<td>1C</td>
</tr>
<tr>
<td>5. Put on gloves</td>
<td>1C</td>
</tr>
<tr>
<td>6. Apply tourniquet</td>
<td>1A</td>
</tr>
<tr>
<td>7. Select venepuncture site</td>
<td>1A</td>
</tr>
<tr>
<td>8. Clean sampling site</td>
<td>1A</td>
</tr>
<tr>
<td>9. Puncture the vein</td>
<td>1A</td>
</tr>
<tr>
<td>10. Draw first tube</td>
<td>1A</td>
</tr>
<tr>
<td>11. Release the tourniquet</td>
<td>1A</td>
</tr>
<tr>
<td>12. Gently invert the tube once (one full inversion)</td>
<td>1B</td>
</tr>
<tr>
<td>13. Draw additional tubes following order of draw</td>
<td>1B</td>
</tr>
<tr>
<td>14. Remove needle from the vein and activate safety feature</td>
<td>1A</td>
</tr>
<tr>
<td>15. Dispose of the needle</td>
<td>1A</td>
</tr>
<tr>
<td>16. Bandage the puncture site</td>
<td>1C</td>
</tr>
<tr>
<td>17. Tell a patient to apply a pressure for 5-10 minutes and not to bend the arm</td>
<td>1C</td>
</tr>
<tr>
<td></td>
<td>Task</td>
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<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>18.</td>
<td>Invert all tubes 4 times</td>
</tr>
<tr>
<td>19.</td>
<td>Remove gloves</td>
</tr>
<tr>
<td>20.</td>
<td>Advise patient to rest for 5 minutes and ensure bleeding has stopped before leaving the site of venous blood collection</td>
</tr>
</tbody>
</table>
References:


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