

Capillary blood sampling: national recommendations on behalf of the Croatian Society of Medical Biochemistry and Laboratory Medicine

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Abstract

Capillary blood sampling is a medical procedure aimed at assisting in patient diagnosis, management and treatment, and is increasingly used worldwide, in part because of the increasing availability of point-of-care testing. It is also frequently used to obtain small blood volumes for laboratory testing because it minimizes pain. The capillary blood sampling procedure can influence the quality of the sample as well as the accuracy of test results, highlighting the need for immediate, widespread standardization. A recent nationwide survey of policies and practices related to capillary blood sampling in medical laboratories in Croatia has shown that capillary sampling procedures are not standardized and that only a small proportion of Croatian laboratories comply with guidelines from the Clinical Laboratory Standards Institute (CLSI) or the World Health Organization (WHO). The aim of this document is to provide recommendations for capillary blood sampling. This document has been produced by the Working Group for Capillary Blood Sampling within the Croatian Society of Medical Biochemistry and Laboratory Medicine. Our recommendations are based on existing available standards and recommendations (WHO Best Practices in Phlebotomy, CLSI GP42-A6 and CLSI C46-A2), which have been modified based on local logistical, cultural, legal and regulatory requirements. We hope that these recommendations will be a useful contribution to the standardization of capillary blood sampling in Croatia.

Key words: recommendations; capillary blood; blood specimen collection; standardization; preanalytical phase

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Introduction

Capillary blood sampling, which refers to sampling blood from a puncture on the finger, heel or an earlobe, is increasingly common in medicine. It enjoys several advantages over venous blood sampling: it is less invasive, it requires smaller amounts of blood volume and it can be performed quickly and easily. This technique has become more and more popular, especially with the widespread use of point-of-care testing (POCT), which has become the fastest growing area in laboratory medicine (1).

Obtaining blood by skin puncture instead of venipuncture can be especially important in pediatric

patients in order to avoid the effects of blood volume reduction (2) and reduce the risk of anemia (3). Thus, 56% of all procedures in the neonatal unit are performed using capillary blood samples, making it the most frequent invasive procedure performed during the neonatal period (4,5). Skin puncture blood sampling is also recommended for adult patients with severe burns, those who are obese or older or anxious about sampling, those with a tendency toward thrombosis, those whose surface veins need to be spared for intravenous therapy, those with fragile or inaccessible veins, and those who self-test their blood, such as for glucose (3).

If carried out incorrectly, capillary blood sampling can cause inaccurate test results, pain and tissue damage (6). In addition, the small volumes involved and the variability in sample quality based on puncture site and technique make capillary sampling particularly susceptible to errors during the pre-preanalytical phase, which are beyond the control of clinical laboratory personnel (7-9). This highlights the need for standardized procedures, yet capillary blood sampling procedures are not standardized in Croatia (10,11).

In this country, capillary sampling is performed mainly by nurses, laboratory technicians, and individuals with undergraduate degrees in laboratory medicine (11). While these individuals have theoretical and practical qualifications for performing blood sampling procedures (12), most are not systematically taught Clinical Laboratory Standards Institute (CLSI) or the World Health Organization (WHO) standardized procedures for capillary sampling. A recent survey of medical laboratories in Croatia showed that 22% of laboratories do not provide their staff with written procedures and instructions for capillary blood sampling, and only 30% of laboratories provide written instructions for the order of draw for cases when multiple tubes with different additives are sampled (11). This argues for the need to develop and promote standardized recommendations for medical staff across the country. These recommendations should be carefully elaborated based on medical evidence and provided in written form at every workstation.

As a first step towards the development and promotion of standardized capillary sampling procedures, the Working Group for Capillary Blood Sampling within the Croatian Society of Medical Biochemistry and Laboratory Medicine has generated the following recommendations, based on thorough review of the relevant literature, particularly WHO Guidelines on Drawing Blood (13) and CLSI document GP42-A6 (formerly H04-A6) (3), from which some material was adapted by permission of the CLSI. These recommendations are intended primarily for laboratory staff who sample capillary blood, but they may also be useful for educating nurses and other medical professionals.

Recommendations

Recommendations on where to perform heel prick sampling were published by Blumenfeld in 1979 (14) and continue to form part of the guidelines issued by the CLSI and WHO. Since then, various national and international professional and regulatory bodies have published standards and recommendations. The most relevant CLSI documents for capillary blood sampling are 'Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens' (GP42-A6) (3), 'Blood Gas and pH Analysis and Related Measurements' (C46-A2) (15) and 'WHO Guidelines on Drawing Blood: Best Practices in Phlebotomy' (13). Other standards and recommendations have been published as part of ISO standards (16) and in CLSI document POCT07-P (17). The American National Academy of Clinical Biochemistry has issued Laboratory Medicine Practice Guidelines for POCT and POCT-based diagnostics (18). Croatian standards for capillary blood sampling have been published by the Croatian Chamber of Medical Biochemists (19). Despite the existence of these various sets of recommendations, a recent nationwide survey of policies and practices related to capillary blood sampling in medical laboratories in Croatia has indicated low compliance with CLSI and WHO guidelines (11). Therefore the Working Group for Capillary Blood Sampling felt the need to prepare the present recommendations. The aim of this document is to provide step-by-step recommendations for capillary blood sampling, as a first step towards developing and promoting standardized capillary sampling procedures in Croatia. The document is intended primarily for laboratory staff performing capillary blood sampling within medical biochemistry laboratories, but it may also help other medical and non-medical professionals who perform capillary sampling.

These recommendations have been issued after a review of relevant literature on capillary sampling procedures, and they are based primarily on WHO Best Practices in Phlebotomy (2010) (13) and the following CLSI guidelines: 'Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens' (GP42-A6), 6th Edition of Approved

Standards (2008) (3), 'Blood Gas and pH Analysis and Related Measurements' (C46-A2), and the 2nd Edition of Approved Standards (2009) (15).

This document includes recommendations for each step in the skin puncture technique for taking all types of capillary blood sample. It also includes recommendations, based on recent literature, for minimizing the influence of the limitations of capillary blood sampling. However, it does not contain specific recommendations that may be appropriate for certain clinical applications where capillary sampling is often used, which include analysis of blood gases and acid-base balance, newborn screening, point-of-care testing, and glucose self-monitoring. Readers are referred to the specialized literature for more detailed discussions of capillary sampling in specific contexts.

The recommendations are presented with the steps of the sampling procedure first, followed by explanation and discussion of relevant literature. Sampling steps are summarized schematically in Figure 7. Item 24 contains recommendations about the limitations of capillary sampling.

The draft of this document was sent to numerous national and international experts for their comments and document was corrected, following their valuable suggestions. The list of their comments and corresponding changes in the Annex is an integral part of this document.

Recommendation 1: Preparation of supplies for capillary blood sampling

Before performing capillary blood sampling, every workstation should be fully equipped with the following materials (3,20):

- a written procedure for capillary blood sampling;
- alcohol disinfectant (ethyl or isopropyl alcohol);
- non-alcohol disinfectant (benzen)
- lukewarm tap water;
- test request form;
- capillaries and microcontainers with various additives;
- capillary blood sampling device (retractable incision device) with different blade lengths for different incision depths;

- cotton pads;
- gauze;
- adhesive bandages or tape;
- single-use gloves; and
- container for disposal of used sampling devices after skin puncture.

The workstation for capillary blood collection should preferably also include an automatic mixing device.

For the analysis of gases in the blood samples of blood capillaries, additional materials are needed and will be prescribed by Croatian national recommendations on blood gases and acid-base balance.

All supplies should be confirmed to be within the expiry date, and the blood sampling specialist should enjoy unhindered access to all necessary supplies (3). According to European Council Directive 2010/32/EU, all blood sampling devices must be engineered to adequate safety standards in order to minimize the risk of professional injury (21).

Recommendation 2: Hand disinfection

To minimize risk of infection, all patients and all samples must be treated and handled using standard safety precautions. To avoid infection, Croatian national guidelines on hand hygiene in healthcare facilities should be followed (22). These guidelines recommend that workers sanitize their hands using warm water and soap or disinfection gels or foams immediately before their first contact with the patient. Similar recommendations can be found in CLSI and WHO guidelines on capillary sampling (3,13).

Recommendation 3: Approaching the patient

The healthcare worker performing the skin puncture should identify him- or herself to the patient, establish communication, gain the patient's confidence and explain the procedure. The skin puncture procedure must not be conducted without the consent of the patient or accompanying person. In that case, the attending physician should be notified and this must be recorded according to facility policy. If the patient is a legal minor or is unable to communicate, the worker should obtain

consent from the parent or accompanying person and explain the procedure to him or her (3).

Recommendation 4: Inspecting the test request form

The test request form should be inspected as described in the Croatian national recommendations for venous blood sampling (20). These national guidelines are in accordance with ISO 15189 standards on quality and competence (23).

The request form should include the following information:

- patient name, surname, gender, date of birth, contact details (address, telephone number) and unique identifier (health insurance number or personal identification number);
- requesting physician's name, professional identifier code and contact details (address of primary healthcare provider or full name of hospital ward);
- the specific tests requested; and
- all clinically relevant information about the patient and his or her condition that may influence how the sampling is performed or how the results will be interpreted, such as whether the patient is scheduled for certain tests or therapies.

Recommendation 5: Identifying patients

Failure to correctly identify the patient may lead to some serious diagnostic errors and affect patient management. Accurate patient identification is therefore a crucial step during blood sampling. International standards emphasize the use of at least two patient identifiers, which do not include the patient's room number or physical location, whenever "administering medications, blood, or blood components; when collecting blood samples and other specimens for clinical testing; and when providing treatments or procedures" (24,25).

Patient identification should be performed according to the following guidelines:

- For accurate patient identification, at least two and preferably three patient identifiers are necessary.

- The following patient data are recommended as appropriate patient identifiers: full patient name, date of birth, address or health insurance number in the case of outpatients.
- Identification should be done by engaging the patient and asking open-ended questions such as: "Please state your name." and "Please state your date of birth."
- The information obtained should be compared with the information on the request form.
- Any discrepancies should be reported, recorded and resolved before sample collection.

Barcode wristbands should be used if available because this type of identification significantly reduces misidentifications (26,27).

The options for correct patient identification can be limited in some cases, such as in unconscious or semi-conscious patients, young children, deaf or cognitively impaired patients or non-native speakers. In fact, capillary blood sampling often involves such patients because it is the recommended sampling method in pediatrics and for follow-up blood oxygenation testing of intensive care patients, many of whom are unconscious. In such cases, the patient should be identified with the assistance of the ward nurse, legal guardian, parent or accompanying person. The question should be phrased in an open-ended way, such as: „Please state the child's (or patient's) name" and "Please state the child's (or patient's) date of birth". The healthcare worker must not rely on a bed tag, crib card or charts placed on the bed, nearby tables or equipment. All data must match the data on the sampling request form, and the name of the person who helped verify the patient's identity must also be documented (3,24).

Recommendation 6: Verifying patient preparation for skin puncture

Croatian national recommendations for venous blood sampling stipulate that laboratory staff should verify that the patient has been properly prepared for blood collection. The necessary preparations may depend on the specific tests requested (20). The healthcare worker about to perform capillary blood sampling should verify whether

the patient is undergoing any therapy or has any dietary restrictions or latex allergies (3).

In certain situations, capillary sampling requires different patient preparation than venous sampling. For example, when capillary samples will be used to assess the effects of ventilatory changes or to assess pulmonary function, a ventilation 'steady state' is recommended. Detailed sampling recommendations in such cases will be a part of Croatian national recommendations on blood gases and acid-base balance.

Recommendation 7: Labeling the microcollection device for capillary blood collection

Capillaries and microcontainers should be labelled with appropriate small labels (Figure 1a-c). Whether the labelling is performed before or after sampling depends on the policy of the healthcare institution. It is recommended that microcontainers be labelled immediately after patient identification and verification of patient preparation for laboratory testing, but before skin puncture. If microcontainers are labelled after skin puncture, labelling should be performed immediately after blood collection, in front of the patient, while he or she is still sitting in front of the phlebotomist. Failure to follow these procedures increases the risk that the microcontainers will remain unlabelled (20).

The sample should be labelled with a barcode sticker, and the barcode number should be traceable to the following information in the Laboratory Informatics System (LIS):

- patient's first and last names;
- patient's date of birth;
- laboratory identification number;
- patient's health insurance number;
- patient's address and telephone number;
- the name of physician who requested capillary blood sampling;
- requested laboratory tests;
- method of collection (venipuncture or skin puncture);
- time and date of sampling; and



FIGURE 1A. Microcontainer labelled with a barcode.



FIGURE 1B. Capillary labelled with a barcode.



FIGURE 1C. A barcode containing at least two independent identifiers. The patient's first and last names and laboratory identification number must be present on the label.

- Identification of the health professional making the skin puncture.

The more information is printed on the microcontainers, the lower is the risk of incorrect patient identification. Thus, capillary specimens should be identified using at least two independent identifiers. In addition, the patient's first and last names and laboratory identification number must be present on the label (Figure 1c).

The size of the barcode sticker depends on the type of microcollection device and the type of

barcode reader on the analyzer. It is important that identifiers on the barcode sticker be clearly legible.

If a laboratory does not have a LIS, and if labelling samples with a barcode sticker is impossible, the laboratory must establish its own uniform labelling system to ensure traceability to the patient information mentioned above.

Recommendation 8: Positioning the patient

Skin puncture should be performed when the patient is sitting. Chairs should have arm holders to provide support and to prevent falls if the patient loses consciousness. In addition, the patient should not have any foreign objects in the mouth, such as chewing gum or a thermometer, during the skin puncture (3,20).

A pediatric patient should be immobilized with the parent's assistance (Figure 2). A parent should be asked to sit in the phlebotomy chair and to place the child in his or her lap. The parent should then position his or her own legs around the child's in a cross-legged pattern, immobilizing the child's legs. The parent should take his or her own arm opposite to that of the child's arm receiving the skin puncture, extend it across the child's chest and immobilize the child's free arm by tucking it under his or her own. The parent should use the hand on this arm to secure the elbow on the child's arm to receive the puncture, while using his or her other hand to secure the wrist on the child's arm to receive the puncture. The child's hand on the arm to be punctured should remain oriented with the palm facing downward (13).

Recommendation 9: Putting on gloves

Gloves must be worn when performing skin puncture to minimize worker exposure to pathogens. The golden rule is that new gloves should be worn for every patient (3,20,22).

Recommendation 10: Selecting the skin puncture site

Recommended skin puncture sites are the finger for adult patients and older children and the heel for infants and younger children. In young children, whether the finger or heel is pricked de-



FIGURE 2. Recommended procedure for immobilizing a pediatric patient during capillary blood sampling.

A parent should sit in the phlebotomy chair and place the child in his or her lap. For immobilizing the child's legs, parent should put own legs around the child's in a cross-legged pattern. The parent should take own arm opposite to that of the child's arm receiving the skin puncture, extend it across the child's chest and immobilize the child's free arm by tucking it under own. To secure the elbow on the child's arm to receive the puncture, the parent should use the same hand while using other hand to secure the wrist on the child's arm to receive the puncture.

pends on the child's weight and age, because the distance between skin surface and bone varies with age and body weight. Finger pricking is recommended for capillary sampling of children older than 6 months or children heavier than 10 kg, which corresponds to the average body weight of a 12-month-old. For younger children, puncturing the medial or lateral plantar surface of the heel is

recommended. However, this site should not be pricked if the child has already begun to walk. In special situations, such as patients with extensive burns, capillary blood should be sampled from areas of preserved skin, regardless of recommendations. Ultimately, the choice of puncture site depends on the lancet/incision devices available, blade length and incision depth.

10.1. Finger puncture

The following rules apply when capillary blood sampling is performed from a finger:

1. The puncture must be on the palm-up surface of the distal segment (fingertip) of the middle or ring finger (Figure 3a).
2. The puncture must be performed on the side of the fingertip where tissue depth is sufficient to prevent bone injury.
3. The puncture should be made across the fingerprint, not parallel to it (Figure 3b).
4. Under no circumstances should capillary sampling be performed:
 - a) on the smallest finger, because tissue depth is insufficient to prevent bone injury;
 - b) on the thumb or index finger because these are more sensitive than other fingers and may have calluses or scars;
 - c) on swollen or previously punctured sites, because the accumulated tissue fluid will contaminate the blood sample;
 - d) on fingers of the hand where infusion is being performed; or
 - e) on fingers on the side of the body where mastectomy has been performed.

10.2. Heel puncture

Preferred method for blood sampling in term neonates is venipuncture, since heel prick procedure is more painful, less efficient, consumes more time and requires more resampling (28).

When performing heel prick in newborns, pain relief measures should be used, involving a mother whenever is possible. Measures include breastfeeding, skin-to-skin contact, swaddling com-



FIGURE 3. Recommendations for finger pricking. The puncture must be on the palm-up surface of the distal segment (fingertip) of the middle or ring finger (a). The puncture should be made across the fingerprint, not parallel to it (b).

bined with positioning neonates upright. Also, sucrose and non-nutritive sucking can be used to manage pain during the procedure (29-31). Local (EMLA cream) or pre-emptive analgesia (paracetamol) is not recommended as they are ineffective (32,33).

The following rules apply when capillary sampling is performed on an infant's heel:

1. The medial or lateral plantar surface of the heel (Figure 4) is the preferred puncture site for infants up to one year old, including premature newborns. In nearly all infants, the heel bone (calcaneus) is not located below the skin in this area, so the heel bone is protected from injury and related complications.

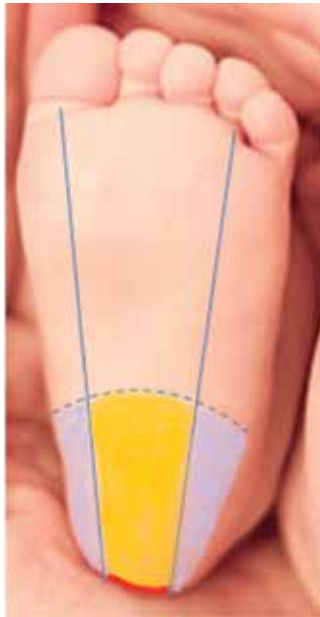


FIGURE 4. Recommendations for heel pricking. The lateral limits of the calcaneus are marked by a line extending posteriorly from a point between the 4th and 5th toes and running parallel to the lateral aspect of the heel, as well as by a line extending posteriorly from the middle of the big toe and running parallel to the medial aspect of the heel. The light blue area indicates the recommended puncture site. The red and yellow areas indicate where puncture must not be performed.

2. Skin puncture on the plantar surface of the heel must be performed at a depth of no more than 2.0 mm to prevent bone injury. This limit is based on the fact that the minimum distance between the skin and perichondrium is 2.4 mm on the plantar surface and 1.2 mm over the posterior surface; the vascular bed of the skin lies 0.35-1.6 mm below the skin surface of the heel (3,13,14,34).
3. Under no circumstances should capillary sampling be performed:
 - a) on the posterior curved part of an infant’s heel (red line, Figure 3) or the central area of the infant’s heel (yellow area, Figure 3) because of greater risk of injury to nerves, tendons or cartilage due to the fact that the distance between the skin and perichondrium is 1.2 mm, only half the value in recommended puncture areas, or

- b) on swollen or previously punctured sites because the accumulated tissue fluid will contaminate the blood sample.

10.3. Earlobe puncture

Earlobe puncture is recommended for blood gas analysis and will be described in Croatian national recommendations for blood gases and acid-base balance. The earlobe is also used occasionally in sports medicine, such as for lactate monitoring, for mass screening and in research studies (13). Relevant recommendations for these specific contexts can be found in the specialized literature.

Recommendation 11.1: Selecting lancet length

The recommended lancet length depends on whether the patient is a child or adult and on the depth of incision (Table 1).

Retractable incision devices are recommended because they minimize risk of patient and health-care worker injury (3). Various retractable incision devices are available commercially, and they are designed to control the blade length and depth of incision.

Healthcare institutions should consider using a retractable incision device with a blade slightly shorter than the recommended incision depth. This is because the pressure applied on the device during puncture results in an incision slightly deeper than the nominal blade length. For exam-

TABLE 1. Recommended incision depth depends on age and recommended puncture site.

	Recommended puncture site	Recommended incision depth up to
Premature neonates (up to 3 kg)	heel	0.85 mm
Infants under 6 months of age	heel	2.0 mm
Child aged 6 months to 8 years	finger	1.5 mm
Child older than 8 years and adults	finger	2.4 mm

ple, if the incision depth should be less than 2.4 mm in the case of older children and adults, the longest blade should be 2.2 mm (13). Regardless of the incision device selected, the incision depths in Table 1 should be respected.

In pediatric and neonatal patients, applying strong pressure to the incision device should be avoided in order to prevent the puncture from being deeper than necessary and thereby damaging bone or nerves. The major blood vessels of the skin are located 0.35-1.6 mm beneath the skin surface (3), and the distance between the skin surface and bone in a 3-kg baby is 3.2 mm on the medial or lateral heel (13). Therefore, punctures that are 2.0-mm deep should penetrate the major skin vasculature without puncturing bone (35). The posterior heel and toe should be avoided as puncture sites because the distance between the skin surface and the bone in each case is only 2.33 or 2.19 mm, respectively, which means greater risk of bone damage (3,13).

Recommendation 11.2: Selecting a microcollection device for capillary blood collection

We recommend plastic microcollection devices for capillary blood specimens. Various microcollection devices are commercially available, and they are designed to control the volume of capillary blood and to contain different additives. Microcontainers with different additives usually bear color-coded caps similar to those on venous sampling tubes. The most appropriate microcollection device depends on the tests requested. The microcontainer or capillary must be filled with the correct volume of capillary blood to ensure the correct final blood-additive ratio.

Recommendation 12: Arterialization of the puncture site

We recommend performing arterialization when the capillary blood sample will be used for blood gas analysis or when the puncture area (hand/finger or heel) is cold or circulation is poor. Arterialization increases the arterial blood flow at the

puncture site and should always be performed when the capillary blood sample will be used to analyze pH and blood gases. The arterialization procedure involves covering the puncture site with a warm, moist towel or other warming device at a temperature of 42 °C or less for 3-5 min prior to puncture. This increases arterial blood flow to the puncture area up to 7-fold (3). Creams containing a hyperemic or vasodilatory agent can be used for arterialization. A warm, well-vascularized puncture area usually provides adequate sample volume without the need to apply pressure to the surrounding tissue.

A survey of medical laboratories in Croatia suggests that 88% of laboratories never apply arterialization before capillary sampling (11).

Recommendation 13: Cleansing the skin puncture site

The skin puncture site must be properly cleansed using sterile cotton or gauze and disinfected with a 70% aqueous solution of isopropanol (3,20). After these steps, the puncture area must be dried to allow the antiseptic to take effect and to prevent discomfort due to residual alcohol.

Povidone iodine should not be used for capillary skin puncture (13) because it can contaminate blood and lead to inflated measurements of potassium, phosphorous or uric acid (36).

Recommendation 14: Performing skin puncture

The retractable incision device is placed upon the cleaned and disinfected skin surface at the puncture site. We recommend that the patient's hand be held firmly to prevent sudden movement. The incision should be made quickly and appropriately according to the manufacturer's instructions.

A pediatric patient should be immobilized with the assistance of the parent or nurse as described in Recommendation 8. The child should be kept warm throughout the procedure, leaving only the extremity of the skin puncture area exposed.

Recommendation 15: Elimination of the first drop of capillary blood sampled

It is crucial to wipe away the first drop of blood with clean gauze, which the healthcare worker should hold in his or her hand during sampling. This applies to all capillary sampling situations, except when the manufacturer of a POCT device specifically requires testing the first drop of blood, as is the case for some self-test glucometers (3). The first drop of blood contains interstitial and intracellular fluid that can contaminate the blood sample.

Recommendation 16: Capillary blood collection

After a site is punctured and wiped, a second drop of blood forms. When the tip of the microcollection device touches the drop, blood flows into the microcollection device by capillary action or the gravity-flow principle, depending on the type of microcollection device (Figure 5a-b). Blood flow can be enhanced by holding the puncture site downwards and applying gentle pressure to the tissue near the puncture site.



FIGURE 5. Recommended steps in capillary blood collection.

After site puncture, wiping and elimination of the first drop, a second drop of blood forms. The healthcare worker touches the tip of the microcollection device to the drop, and blood flows by capillary action when microcollection device is capillaries (or if microcontainer have adapter for capillary sampling) or the gravity-flow principle for microcollection device without adapter.

If blood flow stops during collection, gently tapping the microcontainer on a hard surface can move the blood to the bottom of the tube and restart capillary collection (3). Excessive massaging or squeezing of the puncture site should be avoided in order to prevent hemolysis, contamination of the blood with interstitial and intracellular fluid, and obstruction of blood flow.

16.1: Order of draw in capillary blood collection

When collecting more than one capillary blood samples, special attention must be paid to the order of draw, which differs from the standards for venipuncture.

Multiple capillary blood samples should be collected in the following order (3):

1. samples for blood gas analysis;
2. ethylenediaminetetraacetic acid (EDTA) samples;
3. samples with other additives; and
4. samples without additives (serum).

This order of draw is essential to minimize the effect of platelet clumping.

If more than two capillary blood samples are needed, venipuncture should be requested because it may provide more accurate laboratory results (13).

When blood is collected on filter paper in newborn screening programs, samples should be collected separately and from different puncture sites in order to prevent blood sample quality from being affected by clotting, smearing, contamination, scratching or abrading that can occur during capillary blood spotting (3,37).

Recommendation 17: Disposal of incision device for capillary blood collection

Incision devices must be immediately discarded into a puncture-resistant container with a lid and a prominent biohazard label that satisfies local regulations. We recommend using only safety devices for capillary blood sampling. All disposable equipment used in skin puncture should be disposed of according to the manufacturers' recommendations.

Suspected or confirmed injuries or contamination with patient blood should be handled according to institution policies (3,20).

Recommendation 18: Filling, closure and mixing of microcollection device for capillary blood samples

Capillaries and microcontainers for capillary blood collection should be filled with blood according to the manufacturer's recommendations. Underfilling can cause sample dilution in the case that the additive is a liquid anticoagulant, as well as changes in cellular morphology due to excess anticoagulant. Conversely, overfilling can cause clot formation due to insufficient anticoagulant.

After sample collection, microcollection devices should be capped immediately to prevent exposure to the air, especially if the blood sample will be used for blood gas analysis.

Capped samples should immediately be mixed to prevent clotting. The mixing procedure should follow the recommendations of the microcollection device manufacturer. In the case of blood gas analysis, mixing can be performed as follows: After the capillary has been filled, the capillary end that was submerged in the drop of blood should be closed with the end cap. A metal mixing bar is inserted into the tube, and the other end of the capillary is closed. The sample is mixed by moving the metal bar using a magnet. The magnet should be moved from one end of the capillary to the other five times (38) (Figure 6a). Figure 6b. shows the mixing of microcontainer with adapter for capillary sampling. Number of inversion mixing depend of microcollection device manufacturer. Vigorous shaking should be avoided because it can cause hemolysis (3).

Recommendation 19: Bandaging the skin after capillary sampling

After capillary blood collection and while mixing the tube, the healthcare worker should apply direct pressure to the wound with a clean gauze pad and he or she should slightly elevate the extremity. The person performing the collection, the patient or the accompanying person, should hold

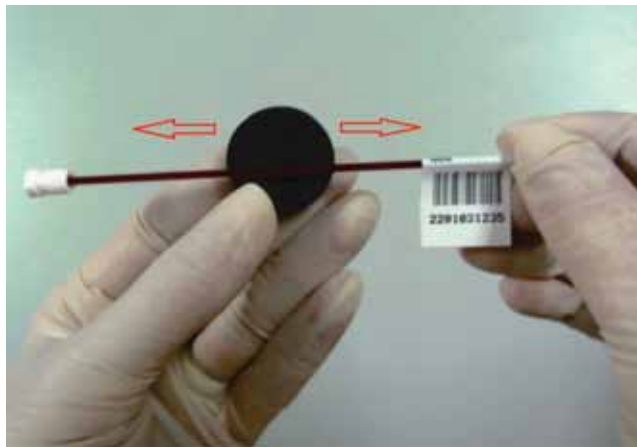


FIGURE 6A. Capillary mixing. After the capillary has been filled, a metal mixing bar is inserted into the tube before ends of the capillary are closed. The sample is mixed by moving the metal bar using a magnet. The magnet should be moved from one end of the capillary to the other five times.

the pad on the puncture site for 30 sec to 1 min. After bleeding has stopped, a bandage can be applied to patients older than 2 years. Adhesive bandages are not recommended for children younger than 2 years because they can irritate the skin (3,20).

Recommendation 20: Glove removal

Before proceeding to the next patient, the health-care worker should dispose of his or her gloves after capillary blood collection and then wash his or

her hands in accordance with local regulations and procedures (22).

Recommendation 21: Recording relevant information during sampling

Any nonconformity that occurs during skin puncture must be recorded according to standard laboratory procedures (20).

For example, excessive crying by babies can alter blood gas tests (39,40), leading to under- or overestimates of pO₂ and of oxygen saturation calculated from pO₂ (3,41), as well as to overestimates of glucose and lactate concentrations (42). Therefore such an event must be recorded on the laboratory test report (3) with a note, such as “Excessive crying during capillary blood sampling. Caution when interpreting pO₂ values.”

Recommendation 22: Dry blood spot sampling

Dried blood spots are widely used in many bioanalyses such as screening for inherited metabolic diseases, diagnosis and treatment of infectious diseases, therapeutic drug monitoring, and pharmacokinetics studies. Spot homogeneity affects accuracy, precision and analyte recovery. This homogeneity depends directly on hematocrit: blood with low hematocrit spreads more rapidly and to a greater extent over the paper surface. Spot homogeneity also depends on the type of spotting paper (35).

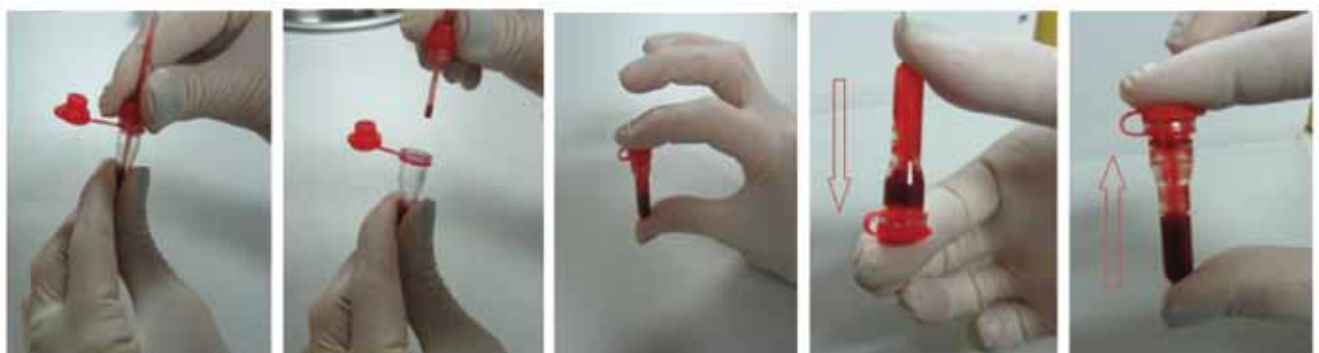


FIGURE 6B. Mixing of microcollection devices with adapter for capillary sampling. After microcontainer has been filled and adapter for capillary blood was removed, microcontainer have to be closed with device cup. Inversion mixing have be preformed acording manufacturer’s instructions.

The following procedure is recommended for collecting dry spot blood samples (43):

1. Clean the sampling site with lukewarm water. Avoid using alcohol-based skin cleansers on babies with immature skin (< 28 weeks), because they can cause burns and blisters (44,45). The sampling site should be completely dry before the sample is collected. The preferred sampling site in full-term and preterm infants is within the external and internal limits of the calcaneus.
2. Wash hands and put on gloves.
3. Use an automated, arch-shaped incision device to make a skin puncture to a depth of 2 mm or less.
4. Fill each circle on the blood spot card by allowing a single blood drop to flow naturally from the front to the back side of the card. Contact between the sampling site and the card must be avoided.
5. Air-dry the blood spot away from direct sunlight or heat.

If necessary, perform a second puncture on the other foot or at a different place on the same foot.

Recommendation 23: Capillary blood sampling for non-medical personnel

The preceding recommendations also apply to capillary blood sampling carried out by non-medical personnel using POCT instruments, which is the case for most diabetic patients who self-monitor blood glucose.

We recommend that non-medical personnel use POCT instruments according to the manufacturer's instructions, especially since the sampling procedure may differ with the device, such as elimination of the first drop (see Recommendation 15).

Recommendation 24: Minimizing the influence of the limitations of capillary blood sampling

Capillary blood sampling is associated with several disadvantages, many of which can lead to greater risk of false test results. A capillary blood sample contains unknown proportions of blood from ven-

ules, arterioles and capillaries (3). Capillary blood samples can also be contaminated to unknown extents by interstitial and intracellular fluid (39). In fact, capillary blood is often sampled into multiple microcollection devices at the same time and from the same puncture site in order to provide sufficient material for several analyses; the risk of contamination with interstitial or intracellular fluid increases as sampling is repeated. Such multiple sampling also increases the risk of hemolysis and clotting (13).

Hemolysis and lipaemia, which can significantly alter blood analysis results, cannot be detected in whole-blood capillary samples because some analyses (e.g. POCT) can consume the entire sample. Hemolysis can occur in such samples due to strong and repetitive squeezing ('milking') of the puncture site, as well as vigorous sample mixing after collection (3). Milking poses particular dangers to assay reliability because it can cause not only hemolysis but also sample dilution with extracellular fluid (15).

Recommendation 24.1: Patients and laboratory tests for which capillary blood sampling is not recommended

Capillary sampling is not recommended for dehydrated patients, patients with poor peripheral circulation or edematous patients (3).

Capillary sampling is not recommended for coagulation analysis or erythrocyte sedimentation rate or for blood cultures (6). In all these cases, venous blood sampling is recommended.

Erythrocyte sedimentation rate and blood cultures require large volumes of blood, making them inappropriate for capillary blood sampling. According to the Croatian Chamber of Medical Biochemists, capillary sampling is not appropriate for determination of erythrocyte sedimentation rate (45).

Concentrations of potassium and calcium in capillary samples differ significantly from values in venous blood samples (46-49). Therefore, when accuracy is critical, the concentrations of these analytes in capillary blood should always be confirmed by venous blood sampling.

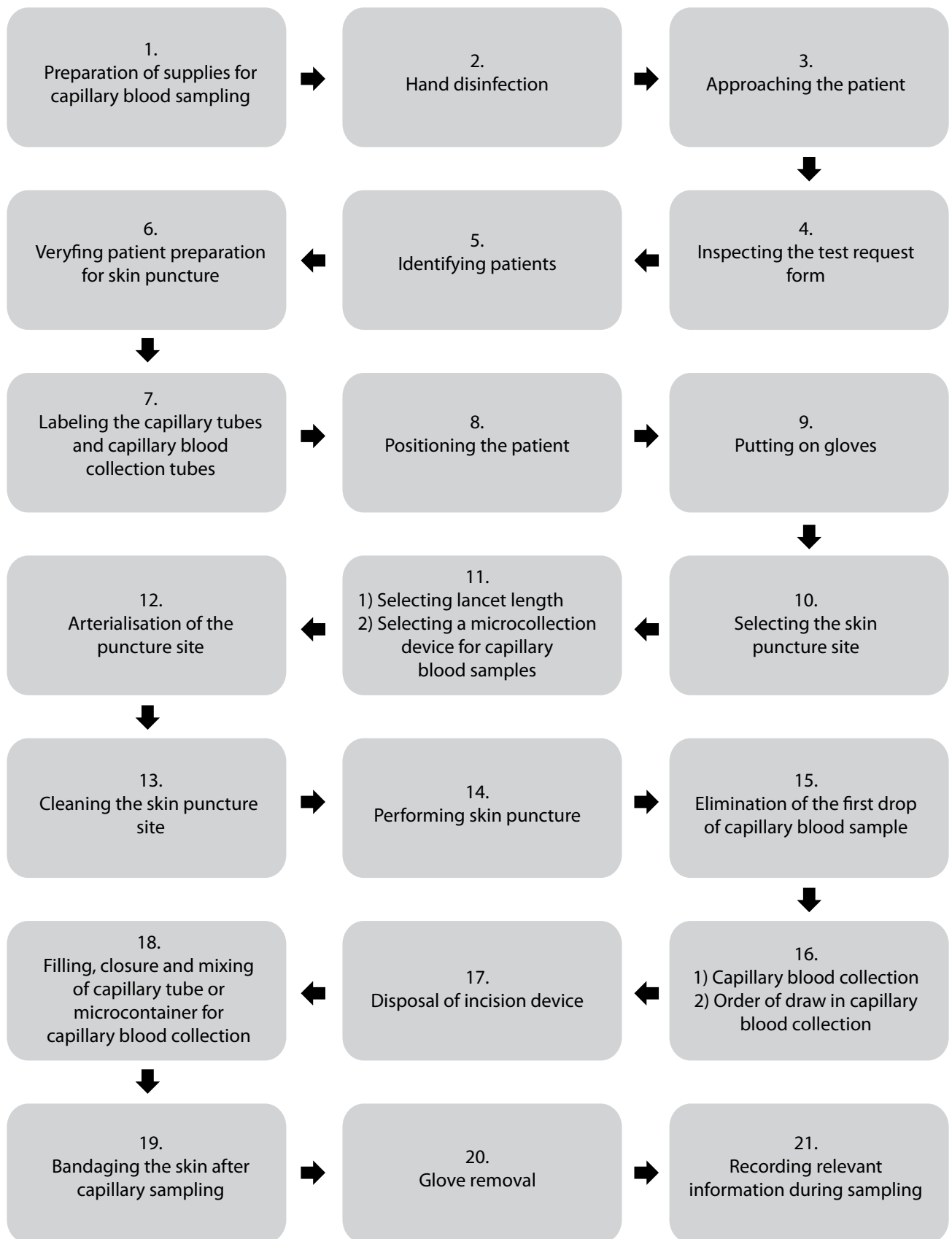


FIGURE 7. Steps in the skin puncture technique.

Recommendation 24.2: Requesting a venous or arterial blood sample instead of a capillary blood sample

Venous blood samples or, if blood gases are requested, arterial blood samples are recommended instead of capillary blood samples when two attempts at capillary sampling fail to give a satisfactory sample, and when more than two microcollection devices for capillary blood are needed for the laboratory tests requested (13).

If necessary, the puncture procedure can be repeated at another site using new equipment (38).

Recommendation 24.3: Rejection of capillary samples with clots in anticoagulant microcollection devices

We recommend rejecting capillary samples with clots in anticoagulant microcollection devices. Healthcare workers should not attempt to remove the clot from the sample. Instead, capillary blood sampling should be repeated.

Microclots in the specimen render it non-homogeneous, affecting the accuracy of analytical results, especially in hematological analysis. Erythrocyte lysis during clot formation can lead to falsely elevated potassium measurements made by blood gas analyzers that can also measure electrolytes. Clots can block the flowpath of the analyzer and give erroneous results or even render the analyzer inoperable.

This highlights the need for thorough mixing of the blood specimen immediately upon collection in order to avoid clot formation. In addition, gentle mixing during collection can help prevent clotting, especially when capillary blood collection is difficult (3,50).

Recommendation 24.4: Differences in analyte concentrations between skin puncture and venipuncture samples

Laboratory test results based on capillary blood samples should be clearly marked as such on the laboratory reports.

Differences between venous and capillary blood analyte concentrations are generally minor,

though clinically important differences have been reported in concentrations of glucose, potassium, total protein, calcium, electrolytes, lactate dehydrogenase and aspartate aminotransferase. Studies suggest that glucose levels are higher in capillary blood samples (46,47,51). Glucose diffuses through the capillaries and is consumed by the cells, so the glucose concentration should be higher in arteries (which feed the capillaries) than in veins (where the capillaries drain). Potassium levels in capillary blood samples can be lower (47), higher (48) or even similar (46) to those in venous blood samples. Levels of total proteins, calcium and electrolytes are lower in capillary blood samples (46-48), while levels of lactate dehydrogenase and aspartate aminotransferase are higher (49).

While CLSI document GP42-A6 (3) reports no significant differences in hematological parameters between capillary and venous blood values, other studies have reported significant differences. Platelet counts are generally lower in capillary blood than in venous blood (52). Capillary values of hemoglobin (Hb), hematocrit (Htc), white blood cells count (WBC), red blood cells count (RBC), mean corpuscular volumen (MCV), mean corpuscular hemoglobin (MCH), are significantly higher than the corresponding venous values; whereas the capillary mean corpuscular hemoglobin concentration (MCHC) value is lower (53). Blood smear is also one of the most frequently performed tests on capillary blood. Native drop or EDTA capillary blood from microcontainer can be used. There is no relevant literature data on the morphological differences between cells from capillary and venous blood sample.

These differences highlight the need to compare analyte concentrations in capillary blood samples with reference values also from capillary blood. However, current practice is to compare capillary blood results against reference values for venous blood. We urge the clinical research community to establish true reference values for analytes determined in capillary blood samples. Until such reference intervals are available, we recommend that all laboratory findings from capillary blood samples be clearly marked as such.

Acknowledgments

The authors are grateful to the Croatian Society of Medical Biochemistry and Laboratory Medicine for providing access to the CLSI guidelines on capillary sampling. The authors also thank Nora Nikolac, PhD and Prof. Ana-Maria Simundic, PhD for critical comments on the manuscript, as well as the reviewers for useful commentaries and sugges-

tions. The authors are grateful to the CLSI for granting permission to use their internationally copyrighted material.

Potential conflict of interest

None declared.

Appendix - expert reviewer comments and author responses.

Reviewer comments	Author responses
REVIEWER 1	
1. Structure of the paper. The manuscript comprises three main chapters: Introduction, Recommendations, and Limitations. In the Recommendation section, the different recommendations are numbered from 1 to 22, then there are three paragraphs (Dry blood spot sampling, Capillary blood sampling for POCT, Recommended capillary blood sampling procedures in specific situations and in the presence of complications) and then a section of Questions and Answers. For an easier reading, I suggest that these three paragraphs should be numbered (from 23 to 25) as they also contain Recommendations; furthermore I suggest including a heading for the Question and answering section.	The section "Questions and Answers" replaced with Recommendations 21-24.
2. Pain control in newborns. In my opinion, the issue deserves more attention in the paper, since the heel pricks is a painful procedure.	New text about pain control in newborns added to Section 10.2.
3. The flow chart indicated as "Steps in the skin puncture technique" is Figure 7.	The flow chart indicated as "Steps in the skin puncture technique" correctly labeled as Figure 7.
4. In the text the word "level" is used to indicate the blood concentration of analytes; the term "concentration" is perhaps more appropriate.	"Level" replaced with "concentration" throughout text.
REVIEWER 2	
5. Both CLSI and WHO documents should be referenced.	CLSI and WHO documents now referenced.
6. Reference (8) is wrong because the paper of Blumenfeld has been referenced as 22. All the involved references must be modified accordingly, including the paper mentioned as ref. 8.	All references checked, corrected when necessary and rearranged, including reference 8.
7. Even though it is self-explanatory (these are the recommendations and no other approach is admitted), it could be useful to stress that no other disinfectant rather than a volatile alcohol must be used to do not affect the capillary blood sampling.	Suggestion accepted and point stressed in Recommendation 13.

Reviewer comments	Author responses
8. Earlobe puncture. This approach is widely adopted in sports medicine to monitor lactate concentrations that may provide a guide to an optimal training intensity. Ideally, lactate concentrations should be measured during a training session and immediately reported to the athlete to ensure that the athlete is working at the desired intensity. In many sports (e.g. cycling, ski, climbing, rowing) earlobe is the only or most accessible site. This is probably outside the scope of the document. Otherwise it could be interesting to mention this specific field of utilization of the earlobe puncture.	Text edited to mention this specific use of earlobe puncture in Recommendation 10.3.
9. Reference (8) on Dried Blood Spots is probably wrong, because I can't find any information on the topic discussed here.	All references checked, corrected when necessary and rearranged, including reference 8.
10. Reference (26) on spuriously haemolysis induced by wipe alcohol is probably wrong here where Dried Blood Spots procedure is described. Please check.	Reference 26 corrected.
11. Ref. 28 and 31 is the same, doubled. Please correct.	All references checked, corrected when necessary and rearranged.
12. The same is for ref. 33 and 37, according to the same web address. Please check.	All references checked, corrected when necessary and rearranged.
13. In the references, when web address is cited often the typo Accessed for Accessed is found. Please correct.	Corrected.
14. In the references, some journal is cited with the full title instead of the official title abbreviation. Please check.	References checked and all journal titles now cited using official abbreviations.
REVIEWER 3	
15. Certain parts of the text are shaded gray. The assumption is that these are the specific recommendations of the working group (WG) that have to be emphasized. Maybe authors should consider having these parts additionally marked as: Recommendation 1, Recommendation 2... Or entitle these for example as a Recommendation for the sample labelling...etc. to clarify to the readers that these are specific recommendations of this Society. Specific comments and suggestions: Text in the grey box. It is a little unclear whether this applies to every referral from or just to specific ones, because it is known that hospital referrals have no information for example about the patient's address but such data is possible to find in an electronic database associated with patients unique hospital number. The text in the grey box. Do the all laboratories have possibility of generating a bar code? Or perhaps do the authors thinking of a unique laboratory number which is associated with appropriate information's on patients? Is it number of insurance really necessary for labelling capillary tubes?	Gray sections removed and reformatted as Recommendations.
16. Furthermore, in the individual sections it should be clearly emphasized what is the recommendation of the authors, or whether the authors for a particular procedure refer themselves to the guidelines that already exist in the literature.	The Working Group's recommendation now clearly emphasized in all Recommendations.
17. My suggestion to the authors is harmonization of names and expressions in the recommendations. For example through the all manuscript authors use different terminology for the personnel involved in phlebotomy or capillary blood drawn: healthcare blood sampling specialist, laboratory technicians, sampling specialist, patient identifiers, healthcare professional, professional, healthcare worker, health worker, workers. To my opinion this should be harmonized.	Terminology harmonized as "health worker" to refer to personnel involved in phlebotomy or capillary blood drawing.

Reviewer comments	Author responses
<p>18. Special attention should be given to the order of presentation of certain facts in the particular chapters which consequently indicate certain recommendation of the Working Group. I suggest to the authors to reorganize the paragraph. It would be better to start at first with the known facts (In Croatia, capillary sampling...) and the recent findings (...A recent survey of clinical labs...) and then make a conclusion on whether is procedure sufficiently standardized, and consequently the need for setting the guidelines.</p>	<p>Text edited according to the reviewer's suggestions, and a new "Recommendations" section added.</p>
<p>19. To my opinion, despite grammatically correct language the quality of the presentation can be improved by using language that is more common in laboratory and in accordance with the writing of the documents (recommendations). Specific comments are listed below. The term "popularity of" should be replaced by growing use or widespread use of...; the term "How the sampling is performed" should be replaced by: The manner in which sampling is performed or the way the sampling is performed; instead with use within. Using the term that you felt the need for something is not common for this type of manuscripts and gives the reader into thinking weather authors at all are standing behind the recommendations. It would be better to use expressions like we want to apply the present recommendations or similar. Instead of accepts I suggest to the authors to state The Working Group for CBS suggests the same procedure that is specified in the Croatian national recommendations... or we support the procedure... Instead of parent use accompanying person or both I suggest instead of constituents to use blood components I suggest to rephrase the sentence into: A survey of medical facilities in Croatia revealed that... I suggest rephrasing the sentence as The above recommendations could be applied also to capillary blood sampling for POCT. I suggest changing expression of estimating coagulation with coagulation testing.</p>	<p>Text edited according to the reviewer's suggestions, and the Working Group's recommendations now clearly indicated in all Recommendations.</p>
<p>20. Specific comment and suggestions: It would be desirable to avoid unnecessary repetition of words in a sentence such as those... sentences could be restructured: With severe burns, obese patients, older patients or anxious patients, patients with a tendency toward thrombosis etc. Only 78% ...well I suggest to omit he word only because 78% is not such a small percent. Pg4Ln36-39 It should be taken into account that paths of the infections are multiple (there are possibilities that staff could also transmit pathogens) and therefore I suggest to change barring sentences in manner: In order to avoid infection standard precautions should be followed. Pg8Ln1-3. Every emergency situation requires immediate sampling, without specific preparation of the patients. In addition to blood gas measurements, it should be taken into consideration that glucose measurement could be required, etc. Pg8Ln31 this is not case only in the pediatric patients, I suppose that this could be related to any patient that is unable to communicate. Pg9Ln5 In the sentence is the word that is missing. (.....the lower is the risk....) Pg9Ln9 I suggest to remove words: pieces of, these are not necessary Pg9Ln27 To my opinion authors should also take into consideration hospital patients that are unable to sitting, as well as adult patients (Pg9Ln 39) as it is stated at Pg10Ln44-49 or if this chapter is related specific to the children provide appropriate title: Positioning on child patient Whether all incision devices are retractable? Pg17Ln29 Order of draw of what?</p>	<p>Text edited according to the reviewer's suggestions.</p>
<p>21. What about non-alcohol disinfectants and it is not clear on how do the lukewarm water should be provided as material at the workplace.</p>	<p>Non-alcohol disinfectant is added as well as lukewarm tap water (for washing hands and for the purposes of arteriolisation proceedings).</p>

Reviewer comments	Author responses
22 Authors stated that capillary tubes should be labelled before skin puncture. Authors stated that capillary tubes should label after the blood collection because label can interfere with collection. Please provide clearer information on this.	Recommendation 7 is restructured. The labelling is performed before or after sampling depends on the policy of the healthcare institution.
23 Pg14Ln10-24 It is not clear whether there is consensus on the recommended puncture depth or not? If there is, I suggest to the authors to give information on logical order recommendation on depth from neonates to adults. In addition, what is recommendation of the Working group?	Table 1 is added for clearly and logical order recommendation on pucture depth.
24 Pg2Ln10-30 It is not completely clear what the authors' intendment to say in this paragraph. Do their recommendations have been issued in accordance with existing guidelines or do the Working group guidelines are taken from the above mentioned guidelines, or do the authors previously studied above-mentioned guidelines in order to set their own guidelines? Nevertheless, given that there is a chapter on Recommendations, it would be desirable to put these information's in section on Recommendations. Pg4Ln46-51 The sentence is not completely clear. I suppose that authors want to tell us that recommendations similar to Croatian guidelines already exist as a part of CLSI and WHO guidelines or the point was different? National guidelines do not form part of the ISO, these could be in accordance with the requirements of the standard ISO 15189. Pg21Ln10-13 The title of the chapter is not completely in concordance to the text provided in the chapter. The authors stated only possible complications due to sampling but there are no recommendations on how to resolve them. The similar text is provided in paragraph at Pg24 entitled Limitations of capillary blood sampling. My suggestion to the authors is to restructure the title of the paragraph at Pg21 and align it with the text in paragraph at Pg24.	The Working Group's recommendations now clearly indicated in all Recommendations.
25 Text in the grey box. It is a little unclear whether this applies to every referral from or just to specific ones, because it is known that hospital referrals have no information for example about the patient's address but such data is possible to find in an electronic database associated with patients unique hospital number. The text in the grey box. Do the all laboratories have possibility of generating a bar code? Or perhaps do the authors thinking of a unique laboratory number which is associated with appropriate information's on patients? Is it number of insurance really necessary for labelling capillary tubes?	Gray sections removed, and the corresponding text marked as Recommendations.
26 Ref 10,13,17,33,37,43 there are unequal space between the words in the references	All references checked and corrected when necessary.
REVIEWER 4	
27 Comment on the text section related to the existing Croatian standards for capillary blood sampling published by the CCMB, page 3, line 2: the existing recommendations available on the web page: http://www.hkmb.hr/povjerenstva/strucna-pitanja.html , are very concise, short and general and do not include all relevant factors that should be taken into consideration for standardized procedures for capillary blood sampling. Further, it is unlikely to expect high compliance of all laboratories with international guidelines published by CLSI and WHO in the absence of national guidelines and recommendations. It is also an important reason for preparing recommendations in the form of the article published in the national journal <i>Biochemia Medica</i> .	This reason, and several others, now highlighted in order to explain why the authors decided to develop these recommendations as a first step to national standardization.
28 Suggestion for the sentence: "The capillary tubes and capillary blood collection tubes should (rather than can) be labelled with labels". But, these labels can be those used for tubes for venous blood sampling or can be appropriate small labels as presented at Figures 1a and 1b.	Text edited according to reviewer's suggestion and presented as Recommendation 7.

Reviewer comments	Author responses
<p>29 The most important comment and suggestion that should be corrected in the present version of the manuscript is related to the text section on page 25, lines 14 to 34: the previous results related to potential differences of hematological parameters between capillary and venous blood samples should be more precise. The authors quoted that several studies showed differences of several hematological analytes while other studies did not. What is the relevant information for the readers in the context of the best evidence literature data? Is there any newer (recent) literature data related to the comparison of platelet count in capillary and venous blood? Instead the term "significantly higher", the exact differences should be listed if the data available from the reference no. 48 or others. It is very important issue related to the capillary blood sampling since the large number of samples for all pediatric population is obtained by capillary sampling. On the other hand, for the same pediatric patient, sample for complete blood count is often obtained by both, venous and capillary blood sampling during hospitalization period. In any case, please, try to give much more precise information related to this important issue on capillary sampling for complete blood count.</p>	<p>More details given about previous studies on differences between venous and capillary analytical results. See also our response to Comment 38.</p>
REVIEWER 5	
<p>30 In Introduction, word "increasingly" is repeated in the same section twice (line two and line five). I would suggest using some of synonyms: more and more, progressively, to an increasing extent, even more.</p>	<p>Text edited according to reviewer's suggestion.</p>
<p>31 In Acknowledgments there are typewrite mistakes. It is not clearly stated if there is one author or more authors. In first sentence it should stand: The authors are grateful (or: The author is grateful); Laboratory should be written with capital letter. In second sentence only one author thanks Nora Nikolac, and then in third sentence there is more than one author.</p>	<p>Text corrected by a native-speaking English editor.</p>
<p>32 Reference 13 cannot be accessed on web page.</p>	<p>All references checked, corrected when necessary and rearranged.</p>
<p>33 Reference 14 cannot be accessed on web page, probably because of typewrite mistake and there is also another typewrite mistake - word Accessed instead of Accessed.</p>	<p>All references checked, corrected when necessary and rearranged.</p>
<p>34 Reference 14: Croatian Chamber of Medical Biochemists. Instructions for capillary blood sampling and capillary tubes. http://www.hkmb.hr/povjerenstva/strucna-pitanja.html_Accessed (accessed) January 15th 2015.) I corrected mistake and open web page but in my opinion it is not precise enough and I was not able to find anything about capillary blood sampling. I had also problems with web pages for references 24 and 25.</p>	<p>Title in the reference corrected and URL confirmed. To obtain information on capillary blood sampling, the user should scroll down on the same page.</p>
REVIEWER 6	
<p>35 Could the authors give some introductory sentence about steps involved in capillary blood sampling before the first paragraph on page 3, line 19?</p>	<p>Text edited according to reviewer's suggestion and presented in new "Recommendations" section.</p>
<p>36 The recommendations are very comprehensive, but can the authors provide a written shorter version, similar to what is shown in Figure 7: Steps in the skin puncture technique? This could be used at every sampling workstation.</p>	<p>Such text added to the Introduction.</p>
REVIEWER 7	
<p>37 Blood smear is also one of the tests mostly performed on capillary blood and I suggest including it to the manuscript (fresh drop of patient's blood or EDTA blood from microteiner is recommended?).</p>	<p>Blood smear is including in Recommendation 24.5.</p>
<p>38 Also, laboratory should not report the results of potassium and calcium from capillary blood except at the insistence of doctors after they meet with possible deviations.</p>	<p>Document focuses on capillary blood sampling, not on post-analytical procedures.</p>

Reviewer comments	Author responses
39 In abstract, one sentence is little clumsy: "How the sampling is performed can influence the test results". For abstract, it would be more appropriate to write: "The way of capillary sampling can influence..." or "The manner in which sampling is performed can influence..."	Abstract edited and rearranged based on reviewer's suggestion.
REVIEWER 8	
40 Expiry dates of all supplies - should they always be checked by the person performing the skin puncture (similar to the H04-A6 document) or may they be checked in other ways?	Text edited according to reviewer's suggestion and source added.
41 Latex allergy check - is this still valid as most countries have abandoned Latex gloves?	This recommendation retained because it forms part of international standards (refs. 3 and 13) and because we are unaware of reliable evidence for or against use of latex gloves.
42 A notation needed that labelled capillary blood collection tube and labelled capillary tube barcodes are congruent?	Rendered unnecessary because of other changes.
43 Figure text to figure 5: Please specify the different steps also in figure legend.	Text added to Figure 5 according to reviewer's comment.
44 Reference list - Please check that abbreviations of publications are correct and punctuation according to <i>Biochemia Medica</i> author instructions.	All references checked and corrected when necessary.
45 The flowchart step figure should be labelled as Figure 7!	The flow chart "Steps in the skin puncture technique" now referred to as Fig 7.
REVIEWER 9	
46 Keywords: I suggest to include the keyword: guideline.	"Recommendations" added to keywords and all text because this document is recommendation for capillary blood sampling, not guideline."
47 Recommendation 1.2: in my opinion the weight of the patient is more important than the age.	Both criteria (age and weight) recommended.
48 A recommend to add a checklist of what to do.	Checklist of capillary blood sampling presented as Figure 7.
REVIEWER 10	
49 Page 4, first paragraph: in the additional material "automatic mixing device" should be added	"Automatic mixing device" added as additional material in Recommendation 1.
50 "Identifying the patient"- shouldn't be the wrist band mentioned?	Text edited according to reviewer's suggestion and presented as Recommendation 5.
51 Page 12, paragraph 3, and point (b): the mistake in the text: "on the palm-up surface of fingertips (!!)" because the distance between the skin surface and bone in the newborns varies from 1.2 to 2.2 mm, so typical lancet depth can easily injure the heel (!!)" bone, and because finger (!!)" puncture in newborns...	Text edited and mistake removed. Table 1 added to simplify data presentation.
52 Arterialization of the puncture site: Many authors support the use of dry heating of the puncture site (not moist towel) as the wet skin make the blood drop forming more difficult.	We recommend cleansing the skin puncture site after arterialization.
53 "Bandaging the skin...": I suppose to use the term of "accompanying person" instead of "parent"	"Accompanying person" used instead of "parent".

Reviewer comments	Author responses
54 Sentence: "If no satisfactory sample can be collected after two attempts, the health care worker should consider venous blood sampling instead" - in many cases the arterial blood is more appropriate sample than venous one for acid-base, etc	Sentence edited according to reviewer's suggestion.
55 Page 26, the second paragraph: the Sentence "Steps in the skin puncture technique" is without any sense here...	Sentence removed.
REVIEWER 11	
56 Page 7 Line 37: In addition, clinical or laboratory staff about to perform...	Sentence rearranged.
57 It is not always suitable to use these fingers because of calluses, scars in certain professions e.g. brick layers, farmers hence may need to indicate that need to go to the side of the finger where skin is thinner/softer or use of the small finger Point 4. a) In my experience the small finger in larger adults is perfectly fine.	We prefer to retain our recommendations because they are consistent with all available literature; the fifth finger is not recommended for puncture because of the short distance between the skin surface and bone.
58 The incision should be made quickly and appropriately according to the manufacturer's instructions.	Sentence edited.
59 Page 24: Line 39: 2. excessive "milking" of the puncture site.	Sentence edited.
60 Page 26 Line 24: The authors are grateful	Text corrected by native English-speaking editor.
REVIEWER 12	
61 I think the paragraph considering who should NOT have performed capillary sampling ought to be placed earlier in the paper?	We prefer not to make statements about who cannot perform capillary blood sampling, since available standards and regulations stipulate only who can perform such sampling.
62 The limitations would be better presented in a Table.	We prefer to present this information within the text.

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