

Tips & Techniques in Preanalytics



SARSTEDT



Prof. Dr. rer. nat. Ralf Lichtinghagen obtained his doctorate after studying chemistry and biology at Ruhr University Bochum in the field of neurobiochemistry. At the beginning of the 1990s he undertook further training at Hannover Medical School ('MHH') to qualify as a Clinical Chemist/European Specialist in Laboratory Medicine (EuSpLM). He acquired his authorization to teach Clinical Chemistry at a university and he currently works as Chief Clinical Chemist in the Central Laboratory of MHH. In addition to his responsibilities in patient care and research, he also gives lectures in Clinical Chemistry / Laboratory Diagnostics in the medicine course of studies.

He is also academic head of the Medical Laboratory Assistants School. Within the national laboratory medicine society ('DGKL') he organises revision courses in the subject of Clinical Chemistry for specialised scientists undergoing further training and for junior doctors. His main areas of research at the MHH's Institute of Clinical Chemistry are Molecular Diagnostics and new biomarkers.

Foreword

The brochure "Tips and Techniques in Preanalytics" is particularly aimed at doctors of medicine, healthcare providers, nurses and medical staff in clinics and doctors' practices.

By working through this brochure the reader should gain a comprehensive impression of the many different aspects of preanalysis.

The sections concerning the collection of analysis materials are specially tailored to the use of Sarstedt systems (S-Monovette®, Microvette®, Minivette®, etc.) and, after specialised familiarisation has taken place, they make it easier to use the described collection techniques properly, particularly for new users.

As a Clinical Chemist I am particularly well aware of the importance of preanalytics within the process as a whole – from the laboratory request and sample collection to the interpreted laboratory findings. After all, preanalytics in particular accounts for a major part of laboratory medicine quality management.

Error-free application of laboratory medicine diagnostics is only possible if the relevant influencing factors and interfering factors are strictly taken into account. This brochure particularly addresses that issue and especially aims to sensitise clinical colleagues to the topic. It is they who, in their capacity as clients requesting medical laboratory diagnostics, make a major contribution towards ensuring that the process as a whole can take place with as little disruption as possible, by performing sample collection properly.

Prof. Dr. Ralf Lichtinghagen

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1 What are preanalytics?



“Preanalytics comprises all the processes that occur before the laboratory analysis.”

1.1 Principles of preanalytics

On average, the preanalytical phase accounts for about 57%¹ of the entire process between the patient and the analysis result. This phase includes the indication, informing and identifying the patient, sample collection with subsequent transport, and storage until centrifugation and sample distribution.

In short, it involves a large number of different steps and areas.

¹ Guder et al.; Proben zwischen Patient und Labor; 2009

The range of possibilities for influencing and changing analytical results during individual steps in this process is correspondingly large.

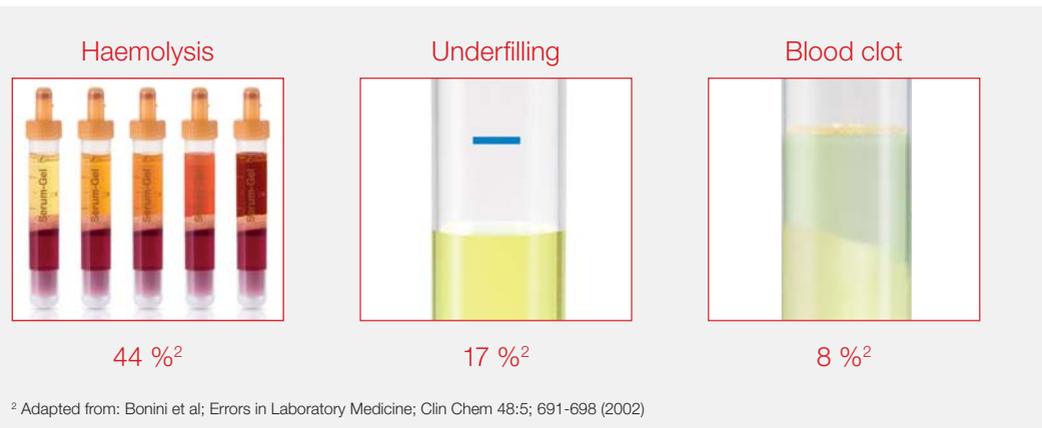
Note: ***About 25% of errors in preanalytics have consequences for the patient!***

It is all the more important for every participant to be informed of the potential influences and sources of error, so that with this awareness they can act appropriately in order to avoid errors. After all, a test result can only be as good as is permitted by the patient sample obtained.

1.2 Common consequences of preanalytical errors

Can values be changed during blood collection?

Common errors

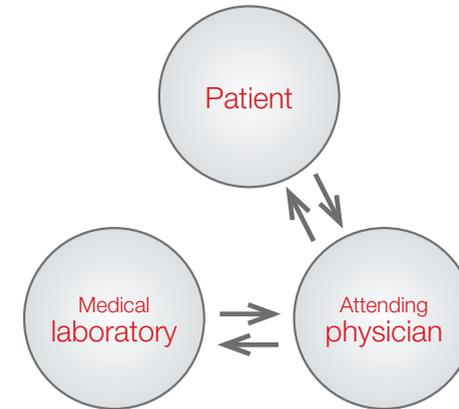


Note: 70-85% of clinical decisions are based on the results of laboratory analyses!³

³ Foubister, Vida. Cap Today Bench press: The Technologist/technician shortfall is putting the squeeze on laboratories nationwide September 2000; Datta, P. Resolving Discordant Samples. Advance for the Administrators of Laboratories; July 2005; p.60.

1.3 Communication as a key to success

Communication between those persons involved in blood collection facilitates work procedures, avoids misunderstandings and prevents preanalytical errors due to missing or incorrect information.



Note: Problems in the area of preanalytics can never be resolved by a single individual but only by close cooperation of the persons involved such as doctors, medical assistants, nursing personnel or the laboratory.

Aim

Standardised conditions for ...

- Preparing for the blood collection
- Blood collection procedure
- Storage/transport to the laboratory

Result

- Safety for patients
- Process cost reduction (working time!)

2 Influencing factors and interfering factors



2.1 Influencing factors

What responsibility does the patient bear?

- Correct details from their medical history
- Medication (list, possibly discontinue)
- Nutrition (particular diet, fasting)
- Correct collection (blood, urine, faeces, etc.)

For correct details concerning medical history, it is important that appropriate questions are asked **before** sample collection.

Taking into account possible influencing factors is important because:

Influencing factors change the concentration of analytes. The effect on the concentration depends on the medical condition and must be considered when evaluating the results.

The list of influencing factors and interfering factors in the following section is not exhaustive. Various examples are presented to illustrate the issues.

“From blood collection and the generation of plausible analysis results to result interpretation, it is absolutely essential to have detailed knowledge of influencing factors and interfering factors, and take them into consideration.”

2.1.1 Non-modifiable influence factors



Population (Race)

Significant differences in blood values can be found in African populations when compared with European populations. In African populations:

- Leucocyte counts are significantly lower
- The vitamin B12 concentration is 1.35 times higher
- The reference ranges for creatinine, CK and alpha amylase are much higher

In Asians the activity of alcohol dehydrogenase is lower than in Europeans. There is also a higher level of lactose intolerance in the Asian population.



Gender

Apart from other gender-specific components (e.g. hormones) muscle mass has an impact on various parameters.

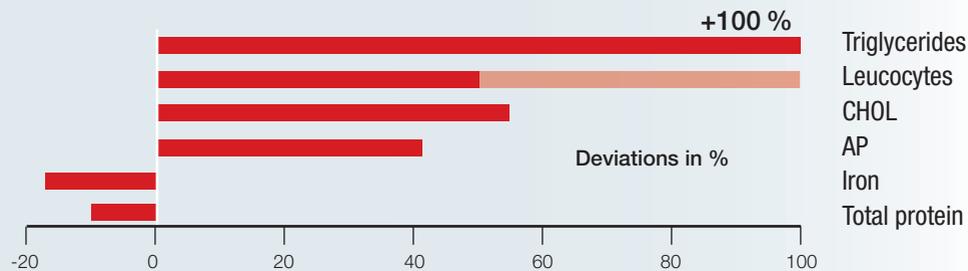
- CK and creatinine are dependent on muscle mass so men are usually found to have much higher levels
- For many parameters it is appropriate to use gender-specific reference ranges



Pregnancy

There is a 5-fold increase in the erythrocyte sedimentation rate during pregnancy.¹

¹ Guder et al.; Proben zwischen Patient und Labor; 2009



⁴ Seelig et al; Präanalytik; 2008

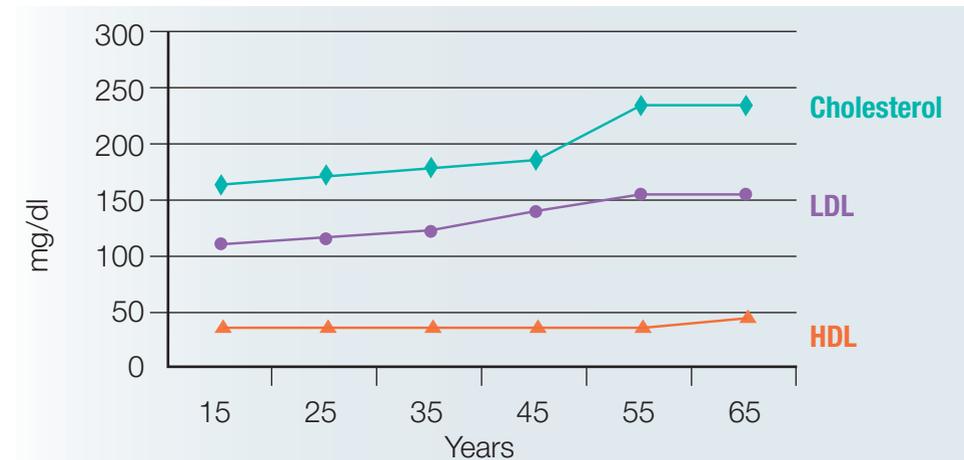


Age

With increasing age there is often an increase in the cholesterol value in both sexes. The activity of alkaline phosphatase in blood plasma is influenced by bone metabolism and is therefore highest in children during the growth phase and after bone fractures.

In infants there are higher bilirubin, haematocrit and HbF levels (for more examples see Section 5 – Blood collection in paediatrics).

That is why age-dependent reference ranges are desirable, but often non-existent, for many parameters.



⁵ Sarstedt; Tips & Techniques in Preanalytics; 2014



Biological rhythm

Vitamin D production (25-OH) fluctuates over the course of a year. In summer, higher UV levels mean that more vitamin D is synthesised than in winter.



Circadian rhythm

Also known as rhythmical daily fluctuation, referring to expected differences in concentration throughout a day for certain clinical chemistry parameters and endocrinological parameters (e.g. renin, cortisol, adrenaline, noradrenaline, VMA and TSH).

With such parameters the time of collection is of fundamental importance. Follow-up measurements should always be collected at the same time of day. As a rule, the time of the collection must be documented and communicated to the laboratory.

Alternatively, 24 hour composite samples (e.g. urine or saliva) can be useful to establish comparable results. Cortisol as a stress indicator is a familiar example. The highest cortisol concentration can be measured in the mornings.



Note:

The circadian rhythm (the biological clock) can be disrupted by travelling to different time zones and/or shift work. If parameters have been affected by daily rhythm, this issue should be included in questions concerning medical history.

⁵ Sarstedt; Tips & Techniques in Preanalytics; 2014

2.1.2 Modifiable influencing factors



Drug use

In the case of regular drug use, e.g. cannabis, heroin or morphines, clinical chemistry parameters can change in the blood. Below are some examples of these changes:

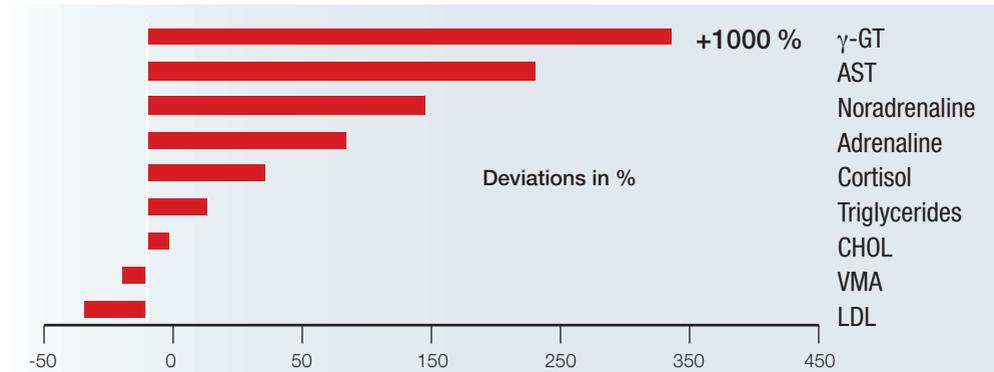
With cannabis use the levels of chloride, urea, insulin, potassium and sodium increase in the blood. In contrast, glucose, uric acid and creatinine levels fall.

The levels of cholesterol, potassium and thyroxine increase during heroin use. During the intake of morphines there is a rise in ALT, amylase, AP, bilirubin, lipase, prolactin and TSH. Insulin and noradrenaline decrease during morphine use.



Substance use: Alcohol

Chronic alcohol abuse causes an increase in liver enzymes, e.g. γ -GT, AST/ALT whilst folic acid and vitamin B6 values decrease.

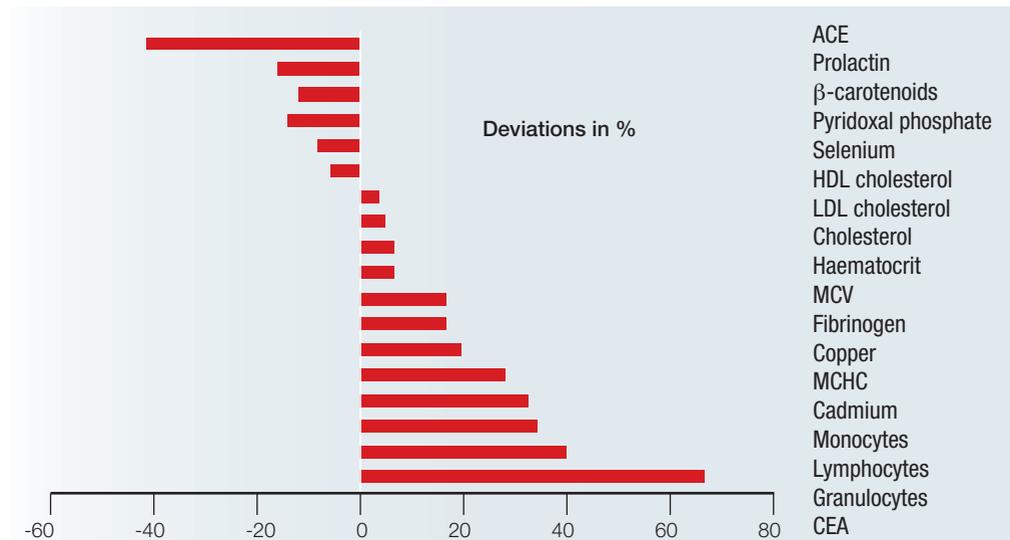


⁴ Seelig et al; Präanalytik; 2008



Substance use: Nicotine

Chronic nicotine use increases the counts of leucocytes, tumour markers such as CEA (highly significant in men) and placental AP (PLAP).



³ Seelig et al; Präanalytik; 2008



Substance use: Caffeine

Even 200 mg caffeine (2 cups robusta coffee or 2-4 cups arabica coffee) increases levels of adrenaline, noradrenaline and cortisol (cortisol +40%).



Medication use

Under the influence of penicillin and ibuprofen, the level of potassium in plasma can increase, whilst under the influence of insulin it decreases. With penicillin use, the thromboplastin time (Quick) increases.

Due to the intake of acetylsalicylic acid (ASA) the levels of AST (GOT), ALT (GPT), creatinine and uric acid rise, depending on the dose.

The medication phenobarbital, which is used to treat epilepsy and for anaesthesia induction, has an enzyme-inducing effect. The activity of AP and γ -GT increases whilst bilirubin concentration in the blood decreases.

Diuretics also have an effect on the electrolyte balance. This is seen, dependent on the substance class, in the levels of potassium, calcium and magnesium, for example.

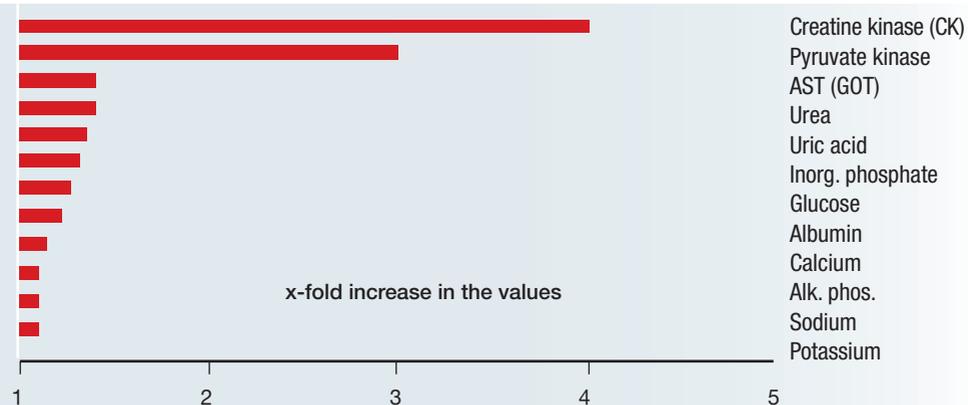
If pantoprazole (proton pump inhibitor) has been administered, the concentration of calcium in the blood may be reduced.

Laxatives can lead to a reduction in potassium.



Physical activity

Physical activity, as compared with the condition at rest, can cause an increase in various clinical chemistry parameters in the blood.



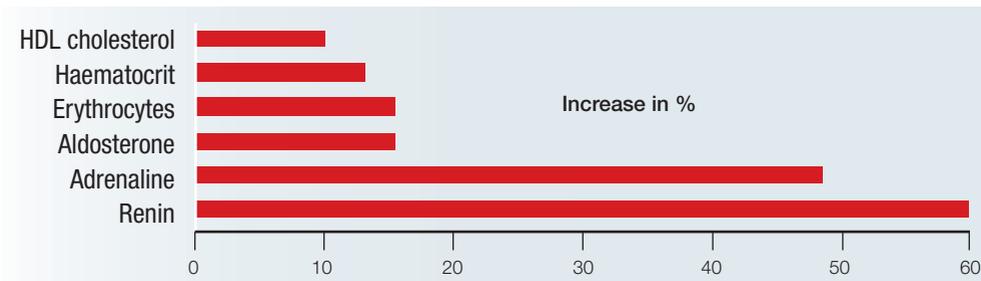
⁵ Sarstedt; Tips & Techniques in Preanalytics; 2014

Physical activity in this case refers to exceptional physical stress. For healthy people, this can be a marathon whereas for bedridden patients just the journey to the clinic can count as exceptional physical stress.



Effect of body position

The distribution of water in the body depends on the position of the body. This leads to parameters such as blood cells, proteins and substances bound to proteins being more concentrated in seated patients than in lying patients.



⁵ Sarstedt; Tips & Techniques in Preanalytics; 2014



Diet-related changes

Changes in analyte concentrations with 4 weeks of fasting or after a standard meal of 800 kcal.

Analyte	Change in %	
	Fasting	Standard meal
Albumin, total protein	- 10	+5
Bilirubin		+ 15
Calcium		+5
γ -glutamyltransferase (γ -GT)	- 50	
Glucose		+ 15
AST (GOT)	+30	+20
ALT (GPT)		+10
Uric acid	+20	+5
Urea	- 20	+5
Potassium		+10
Creatinine	+20	
Phosphorus		+15
Triglycerides	- 40	

⁴ Seelig et al; Präanalytik; 2008

2.2 Interfering factors

Interfering factors can alter test results and cause disruptions, depending on methods used.

By changing the test method it may be possible to eliminate interfering factors.



Image	Description	Possible cause
A	Lipaemia	Disease-related or patient did not fast
B	Jaundice	Syndrome or disease-related
C	Haemolysis	Preanalytical error or disease-related
D	Normal	Good and correct preanalytical conditions

Interference factors are classified as internal (endogenous) or external (exogenous). Examples of interference factors are described below:

Internal interference factors (endogenous)

Cause	Consequence
<ul style="list-style-type: none"> - Gilbert's syndrome - Crigler-Najjar syndrome - Acute hepatitis - Acute liver failure 	<ul style="list-style-type: none"> → Hyperbilirubinemia = jaundice → Possible disruption, e.g. in cholesterol, creatinine, uric acid
<ul style="list-style-type: none"> - Spherocytosis - Immune haemolysis - Haemolytic antibodies - Haemoglobinopathy 	<ul style="list-style-type: none"> → Haemolysis → Significant falsification of a large number of methods of optical measurement → Higher measurements due to the release of erythrocytes (e.g. potassium, LDH, AST)
<ul style="list-style-type: none"> - Hyperlipoproteinemia - Lipid metabolism disorder 	<ul style="list-style-type: none"> → Lipaemia → Patient not fasting at time of blood collection → Significant distortion of a large number of methods of optical measurement False-low levels in electrolyte analyses (sodium, potassium) due to dilution effect
<ul style="list-style-type: none"> - Haematocrit > 65% 	→ Elevation of PTT and aPTT
<ul style="list-style-type: none"> - Haematocrit < 20% 	→ Reduction in PTT and aPTT

⁶ G. Enderl et al; The importance of preanalytics for the coagulation laboratory; Hämostaseologie 2/2010; 30: 63-70

External interference factors (exogenous)

Cause	Consequence
<ul style="list-style-type: none"> - Medication (infusion solution, antibiotics, blood products) - Anticoagulants (contamination due to carryover from preparation) - Contamination (bacteria, fungi, bacterial biofilm from CVC for blood culture) 	→ False measurements (elevation and reduction possible)
<ul style="list-style-type: none"> - Cycling or riding 	→ Can increase the PSA value

3 Venous blood collection



“Venous blood is the most important material tested to answer medical questions. Correct blood collection technique is thus of considerable significance.”

3.1 Patient preparation

Informing the patient

- Informing the patient about the forthcoming procedure helps to alleviate possible anxiety and stress.

Explaining certain regulations

that must be complied with should supplement the patient information, e.g.

- Use of medications
- Adherence to a particular diet
- Sample collection when fasting (except for emergency diagnostics)

Children in particular require careful preparation but the information must be adapted to their ability to understand.

3.2 What is the responsibility of the person collecting the blood?

- Organisation of the blood collection
- Correct documentation (patient identification and time of day)
- Instructing and preparing the patient for the sample collection
- Preparation of the sample (centrifugation if necessary)
- Storage until collection (refrigeration/heating if necessary)

Note: *Communication with the laboratory and, where necessary, with the transport service is essential for the transport and correct storage!*

You can find more information in *Section 10 – Transport & storage.*

3.3 Identification

Patient identification

- Surname
- First name
- Date of birth
- Possibly: admission number, ward, room number

Errors occur not only with common names.

Important: Always ask direct questions.

Never: “You are Mr Miller?”

When asked of patients who are partially/completely deaf or cognitively impaired, such questions may be simply answered with an affirmative nod.

The person seated on the side of the bed may just be a visitor.

If the identity of the patient is not clear, samples should not be collected until there is further clarification.

Identification of the person collecting the blood

It must be possible to determine the identity of the person who collected the sample.

- Place identification on the request form if appropriate

Questions about the type and time of the collection, the patient’s condition and other important details may be of use in case of unclear results.

Identification of the ordering doctor

The identity of the requesting doctor makes it possible to ask questions in the event of

- illegible requests (e.g. referral notes)
- erroneous requests (e.g. prostate phosphatase for a female patient)
- restriction to the most relevant analyses if the volume of the sample material is too small

Identification of the sample

- Never analyse sample containers that are not clearly identified.
- Barcode labels enable reliable identification.
- Identification should always be placed on the primary receptacle.
- Use only waterproof felt-tip pens for glass or plastic containers.
- Additives (anticoagulants, clot activators, gel) are identified by colour coding of the sample container. A lack of international standardisation means that additional identification may be necessary.

Never use the lid, outer packaging or transport container to identify the sample.

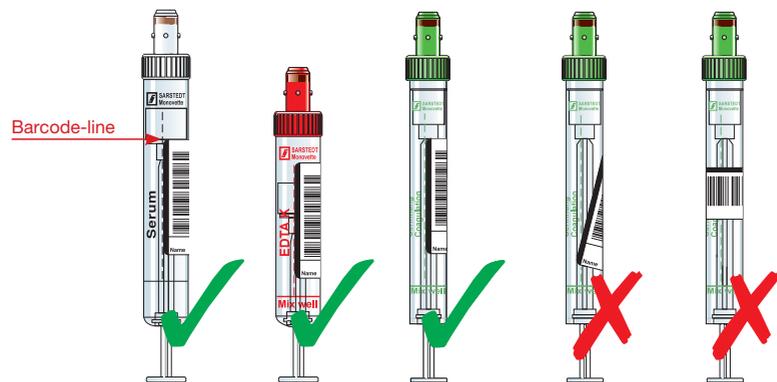


Legal requirements & labelling

- The submitted analysis material and any parts of this material must be able to be clearly assigned to one patient. If this is not possible, the material must not be processed by the medical laboratory.

⁷ RiLiBÄK; § 6.1.7. Part A

Solution: Label sample tube with the barcode immediately before collecting the blood.



- Sample tubes are correctly labelled if:
 - ▶ the contents are still freely visible
 - ▶ it is possible to check the filling volume
 - ▶ the screw cap can be easily removed
 - ▶ the tube and label do not get stuck or stick together in the centrifuge



3.4 Application

Preparation	Application
 Serum	Clinical chemistry, serology, special analyses
 Serum gel	Clinical chemistry, serology (only routine diagnostics)
 Lithium heparin	Plasma collection for clinical chemistry, serology
 EDTA K	Haematology (e.g. Hb, Ht, erythrocytes, leucocytes)
 Citrate 1:10	Coagulation analyses (e.g. Quick, PTT, TT, fibrinogen)
 Citrate 1:5	BSG determination according to Westergren or S-Sedivette®
 Fluoride	Glucose determination (24 h stability) Lactate
 GlucoEXACT	Glucose determination (48 h stability, at RT)

3.5 Order of draw

In the past, the correct order of draw was repeatedly and intensively discussed. The latest findings and studies show that when using a modern blood collection system, carryover of additives is highly unlikely with proper handling of a closed blood collection system. For example, when collecting with the Safety-Needle and the S-Monovette®, no carryover of EDTA is detected.⁸

In case of carryover of EDTA into a serum or heparin tube, potassium may be elevated and calcium lowered, for example.⁹

To ensure the greatest possible safety even for the worst possible conditions during blood collection, we nevertheless recommend adhering to one of the following drawing orders:

⁸ RA Sulaiman, Effect of order of draw samples during phlebotomy on routine biochemistry results; J Clin Pathol. 2011 Nov;64(11):1019-20
⁹ RR Calam et al; Recommended "Order of Draw" for Collecting Blood Specimens into Additive-Containing Tubes; Clin. Chem.; Vol. 28, No. 6, 1982

Recommended order of draw

According to Gurr¹⁰:

- Blood culture
-  Serum/Serum-Gel blood
-  Citrate blood
-  Heparin/Heparin-Gel blood
-  EDTA blood
-  Fluoride/Citrate-fluoride blood

According to CLSI¹¹:

- Blood culture
-  Citrate blood
-  Serum/Serum-Gel blood
-  Heparin/Heparin-Gel blood
-  EDTA blood
-  Fluoride/Citrate-fluoride blood

¹⁰ Gurr et al "Musterstandardarbeitsanweisung Präanalytik" J Lab Med 2011

¹¹ CLSI Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture, Approved Standard, 6th edition GP 41-A6 (former H3-A6), 27 (26) 2007

3.6 Avoiding underfilling

To avoid erroneous measurements or rejection of samples in the laboratory due to underfilling, a precise filling volume is necessary. This should be taken into account for all preparations.

Precise filling of the blood collection system is of particular importance for citrate tubes for coagulation analyses.

Underfilling here results in an excess of citrate in the tube (ratio of blood to preparation). Because citrate binds calcium, more calcium will thus be bound than is expected. This has a direct effect on the analysis results.

If when collecting blood with a Safety-Multify®-Needle, citrate blood is collected first, this leads to underfilling due to the dead volume in the tubing.

Note: *The longer the tubing used, the greater the underfilling*

Dead volume = volume in the tubing:

- 30 cm tubing: > 450 µL
- 20 cm tubing: > 300 µL
- 8 cm tubing: > 120 µL



Therefore, to fill/vent the tubing, a tube (citrate/neutral) is first used and then discarded (empty tube/discard tube). Only then is the actual citrate tube to be used.

4 Carrying out venous blood collection



“The technique for venous blood collection – step by step – for correct procedure in the clinic”

4.1 Standard conditions for blood collection

- No unusual, extreme physical activities in the 3 days prior to blood collection
- No alcohol excess on the day before (abstain from alcohol for 24 hours)
- Fasting between 7 pm and 9 am (i.e. no eating for 12 to 14 hours, drinking water is allowed)
- Rest for at least 10 minutes before the blood collection (sitting or lying)
- Avoid pumping! Opening and closing the fist leads to a considerable increase in the potassium level (up to 2 mmol/L)
- Apply a tourniquet for a maximum of 1 min. (better 30 seconds)
- Puncture vessel, loosen tourniquet, collect blood
- Medications: in consultation with the doctor, take or discontinue

4.2 Obtaining diagnostic samples: 12 steps

1. Disinfect hands! Wear gloves!
2. Apply tourniquet
3. Observe veins and select one
4. Disinfect!
5. Do not touch the puncture site again!
6. Remove the protective sleeve from the Safety-Needle!
7. Face the bevelled edge of the needle upwards!
8. Keep the puncture angle less than 30°!
9. Pull the skin until it is taut, fix the vein!
10. Possibly forewarn the patient!
11. Loosen the tourniquet when the blood starts to flow!
12. Collect sample; note the order of draw!

4.3 Tourniquet application & puncture sites



Disinfect in accordance with a valid hygiene plan



Apply the tourniquet one hand's width above the puncture site

The pulse must be perceptible (tourniquet pressure: 50-100 mmHg)

Maximum constriction time: 1 min.



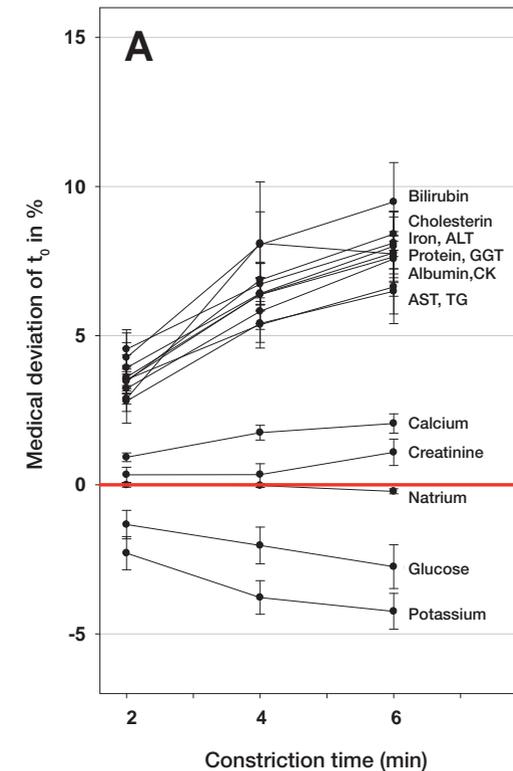
Puncture sites

- 1 Basilic vein
- 2 Median cubital vein (this refers to the non-blue translucent, thick, deep vein that is only visible as a bulge at this point)
- 3 Cephalic vein, runs on the thumb side
- 4 Cephalic vein
- 5 Basilic vein
- 6 Dorsal venous network of hand

Stasis time

Stasis lasting longer than 1 minute can lead to shifts in the concentration of measurements. In the case of high-molecular substances (e.g. total protein) and protein-bound calcium, false-high measurements can occur (generally very relevant for parameters with relatively narrow reference ranges). Potassium levels can drop as constriction time increases.

Comparison – 2 min stasis versus 6 min stasis



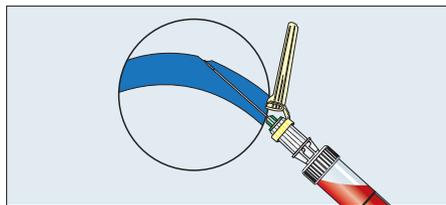
¹² Lichtinghagen et al.: Einfluss der Stauzeit auf normalisierte Laborwerte; J Lab Med 2013; 37(3): 131–137

4.4 Problems before/during blood collection

Very difficult vein conditions

- Look for another puncture site
- Apply a heat pack or warm cloth
- Use Safety-Multify® needle
- Use the aspiration method to collect the blood

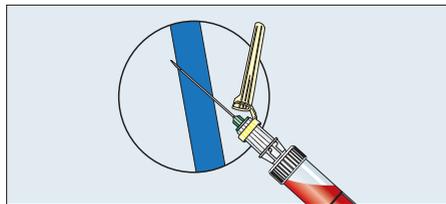
Stopping blood flow during collection



Needle opening is up against the vein wall

Solution:

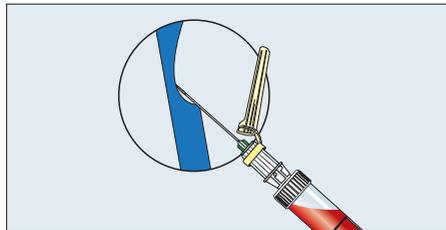
Withdraw the needle slightly until the flow is restored.



Needle has pierced the vein

Solution:

Withdraw the needle slightly until the blood flow is restored.



Vein has collapsed

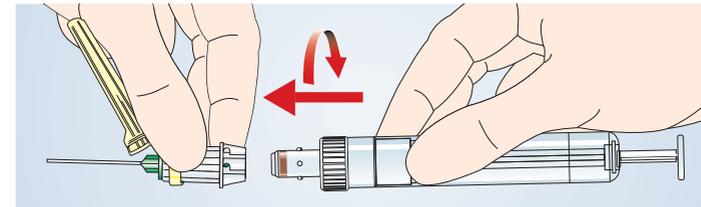
Solution:

Wait until the vein has recovered, then carefully aspirate.

- Pumping the fist leads to an increase in K^+ and Mg^{2+} due to muscle activity
- Extended stasis changes parameters such as K^+ , γ -GT
- Bending the Safety-Needle is not necessary when using the S-Monovette® system because the penetration angle is very flat. Changing the lumen by bending the needle can damage cells (haemolysis).
- Haemolysis can also be caused by using a needle that is too narrow.

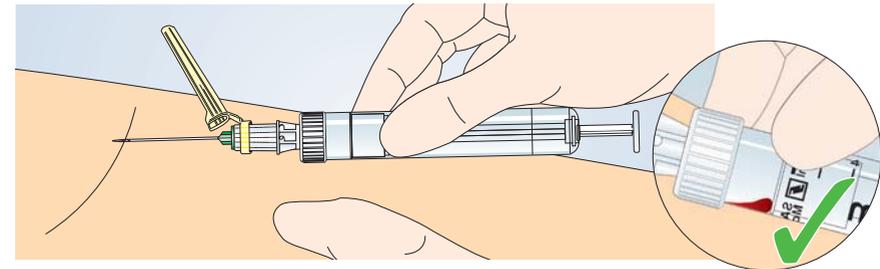
4.5 Aspiration and vacuum technique

4.5.1 S-Monovette® aspiration technique

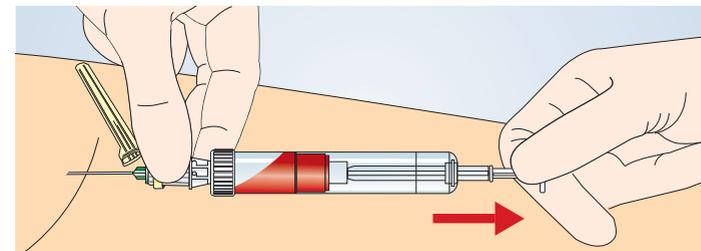


IMPORTANT:

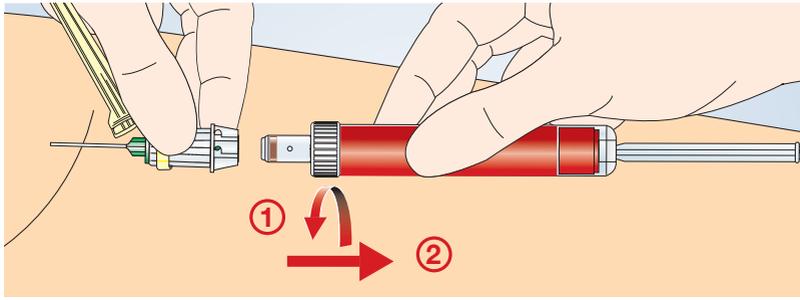
- Just before puncturing the skin, lock the Safety-Needle to the S-Monovette® by twisting it slightly clockwise.



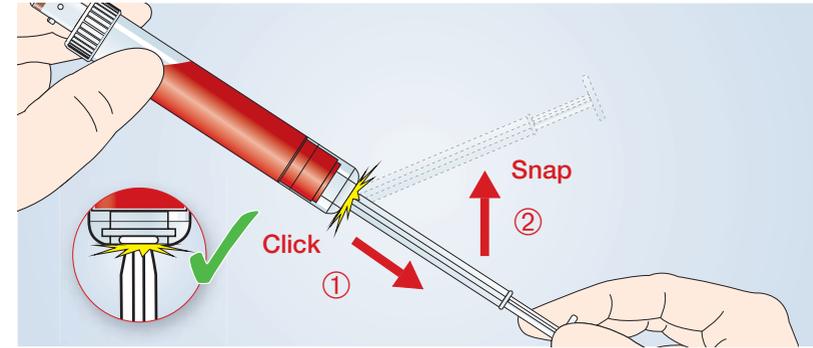
- Use the thumb of the free hand to pull the skin taut. Hold the vein in place. Forewarn the patient and puncture the vein. As soon as the vein is successfully punctured, the first drop of blood enters the S-Monovette®. This lets the user know that the vein has been reached.



- Loosen the tourniquet and slowly withdraw the plunger. Wait until the blood flow stops.

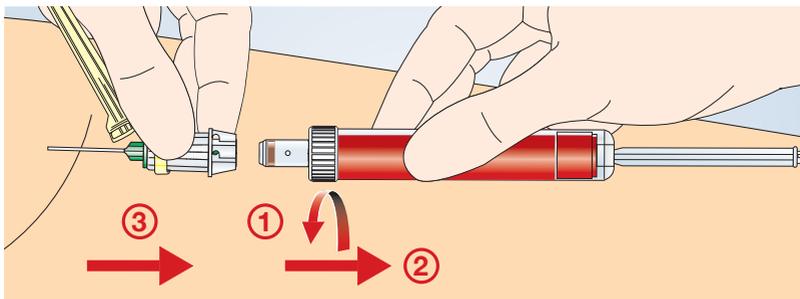


- Change the S-Monovette® for multiple collections. Remove the S-Monovette® from the Safety-Needle by turning it slightly anticlockwise. The needle remains in the vein.



IMPORTANT: For all S-Monovettes, when blood collection is complete, withdraw the plunger into the "snap" position and break off.

After blood collection

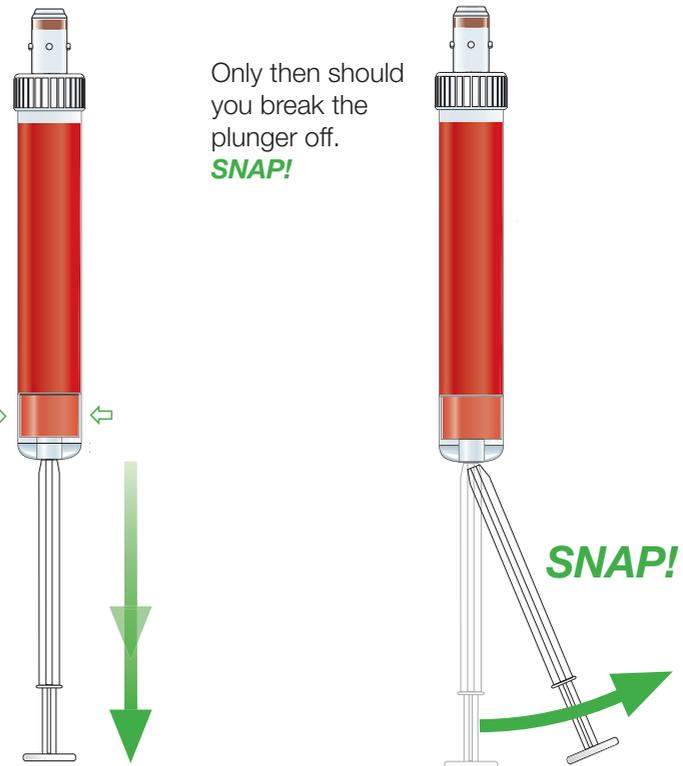


- First remove the S-Monovette® and then withdraw the Safety-Needle from the vein.

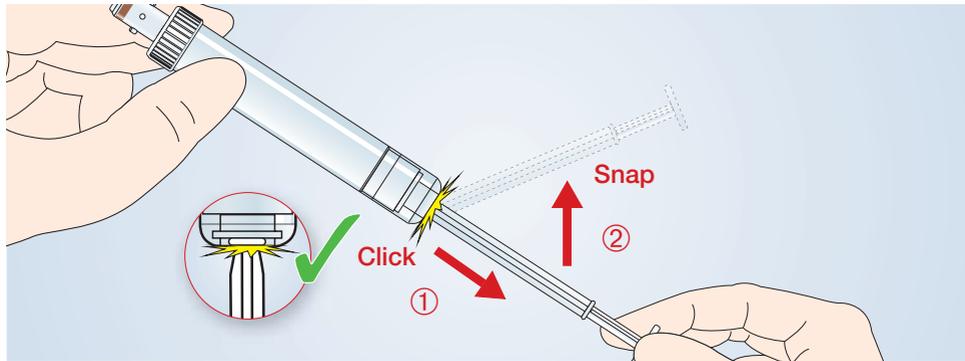
Pull the plunger straight back until the piston locks in with an audible **CLICK**.

Only then should you break the plunger off. **SNAP!**

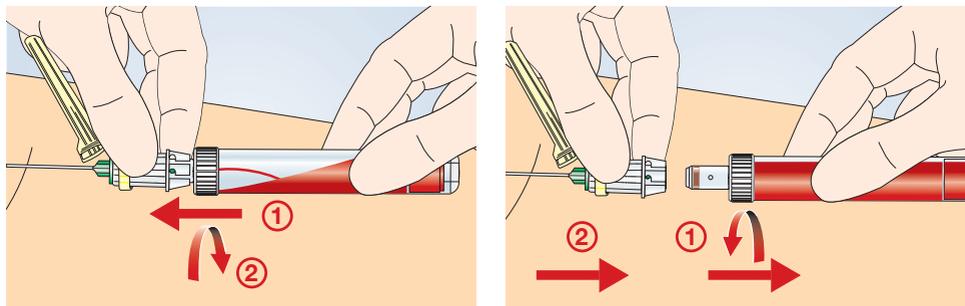
Pull the piston right out to the end



4.5.2 S-Monovette® vacuum technique



- The Safety-Needle must already be in the vein before the collection. As a rule, we recommend filling the first S-Monovette® with the aspiration technique so that the blood collection starts gently. The collection can then continue using the vacuum technique.
- **Immediately** before blood collection, pull the plunger back and lock the piston into the base of the S-Monovette (“click”). Then break off the plunger (“snap”).
- Push the evacuated S-Monovette® onto the Safety-Needle and secure by twisting clockwise.



- Wait until the blood flow stops, remove the S-Monovette® from the Safety-Needle and **then** remove the Safety-Needle from the vein.

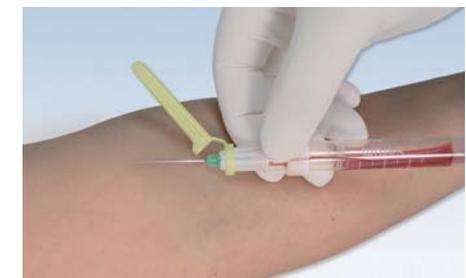
4.5.3 A summary of the 2 collection techniques



Aspiration technique



Vacuum technique



4.6 Blood collection from catheters

Blood collection from catheters should be avoided due to possible distortion of measurements. Haemolysis and contamination from infusions are possible risks. However, if blood collection from a catheter is unavoidable, comply with the following:



- To avoid dilution effects and contamination, at least 15 minutes should elapse between the last infusion and the blood collection. The time depends on the infusion and should comply with internal hospital regulations.⁶
- Recommendations for the time of blood collection after infusions¹

Infusion	Earliest time (hours) for blood collection after ending the infusion ¹
Lipid emulsion	8
Carbohydrate-rich solution	1
Amino acids, protein hydrolysate	1
Electrolytes	1

- If the catheter has been rinsed with solution containing heparin, it should be rinsed with saline before blood collection for coagulation analyses.¹³
- Before the blood collection, at least 5-10 ml of blood should be discarded. To avoid any mix-ups, this tube should be appropriately labelled.¹³

As a rule, a note to the laboratory that the sample was collected from a catheter can simplify the interpretation of any implausible analytical results. For therapeutic drug monitoring (TDM), the risk of a contamination must be noted in particular. See page of traces of medication can lead to erroneously high results.

¹ Guder et al; Proben zwischen Patient und Labor; 2009

⁶ G. Endler et al; The importance of preanalytics for the coagulation laboratory; Hämostaseologie 2/2010

¹³ M. Spannagl et al; Hämostaseologische Globaltests; Hämostaseologi 1/2006

Haemolysis risk factor: Catheter

With blood collection from catheters, the vacuum technique is not recommended due to the high flow velocities of the blood. This is associated with a high risk of haemolysis.¹⁴⁻¹⁷

Using the aspiration technique, **slow, gentle filling**¹⁸ of the S-Monovette® is possible. This greatly reduces the risk of haemolysis.

¹⁴ Margo A et al.; Obtaining blood samples from peripheral intravenous catheters: best practice; AJCC, 9.2009, 18 (5)

¹⁵ Lippi et al.; Prevention of hemolysis in blood samples collected from intravenous catheters; Clin Biochem 46: 561-564, 2013

¹⁶ Heyer et al.; Effectiveness of practices to reduce blood sample hemolysis in EDs: A laboratory medicine best practices systematic review and meta-analysis Clin Biochem 45: 1012-1032, 2012

¹⁷ Grant MS; The Effect of Blood Drawing Techniques and Equipment on the Hemolysis of ED Laboratory Blood Samples; J Emerg Nurs 29:116-121, 2003

¹⁸ Benso S; Can a blood sample for diagnostic exams be drawn from a peripheral venous catheter?; Assist Inferm Ric; 34(2):86-92; Apr-Jun 2015

Multi-Adapter – the direct connection

The S-Monovette® can be directly connected to the catheter with the Multi-Adapter. The use of single-use syringes and the associated risk of haemolysis and cross-contamination can be avoided.



- The Multi-adapter can be connected to the S-Monovette® with Luer connections, e.g. *in vitro* catheter or three-way stopcock.

4.7 Blood collection for blood culture diagnostics

Sepsis is known colloquially as blood poisoning. What is not as well known is that the mortality (lethality) is about 50%¹⁹.

Common symptoms:

- Apathy/weakness
- Fever, chills
- Confusion
- Laboured and rapid breathing
- Rapid pulse, low blood pressure
- Cold hands and feet with poor blood flow (centralisation)

Sepsis is an emergency that requires the earliest possible diagnosis and immediate treatment: international and national treatment guidelines stipulate administration of antibiotics within one hour. Before administering antibiotics, at least 2 blood cultures must be collected.

It is recommended to collect the blood at the start of a fever episode from a peripheral vein.

Blood collection from a venous access (e.g. CVC) is not suitable.

The validity is affected to a high degree by the avoidance of contamination, the transport time, storage conditions and communication of clinical information.²¹

The following information should be communicated to the laboratory²⁰:

- Site of collection
- Date of collection
- Patient identification
- Suspected diagnosis
- Details of the ongoing antibiotic therapy if applicable

¹⁹ Pschyrembel; 2004

²⁰ J. P. Borde et al; Abnahme von Blutkulturen; Dtsch Med Wochenschr; 135:355-358; 2010

²¹ Simon et al; Blutkulturdiagnostik – Standards und aktuelle Entwicklungen; J Lab Med; 36(4):199–207; 2012

4.7.1 Hygiene requirements

False-positive blood cultures result, as a rule, from improper hygiene measures and may be associated with extended hospitalisation, unnecessary antimicrobial therapy, additional diagnostics and considerable extra costs.²¹

Blood collection using blood culture flasks must be done in accordance with the hygiene requirements.

To avoid contamination, the following steps are necessary:

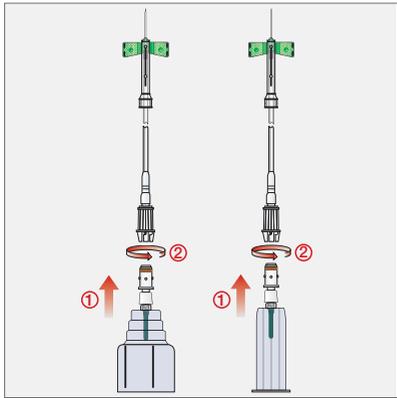
1. Hygienic hand disinfection
2. Wear gloves
3. Disinfection of the puncture site (e.g. with 70% isopropyl alcohol or skin disinfectant)
 - a. Apply the disinfectant and distribute by wiping
 - b. Apply disinfectant again and let it dry for 60 seconds

Important: After the skin disinfection, do not palpate the puncture site again.

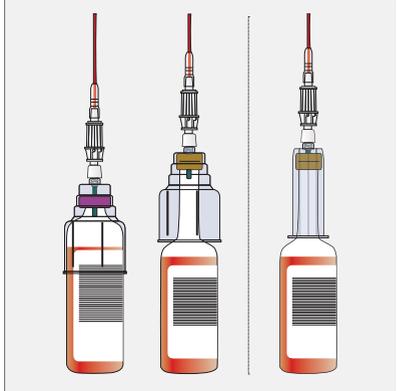
4. Disinfect the blood culture flasks
 - a. Remove the cap
 - b. Disinfect the rubber septum

²¹ Simon et al; Blutkulturdiagnostik – Standards und aktuelle Entwicklungen; J Lab Med; 36(4):199–207; 2012

4.7.2 Handling during blood collection

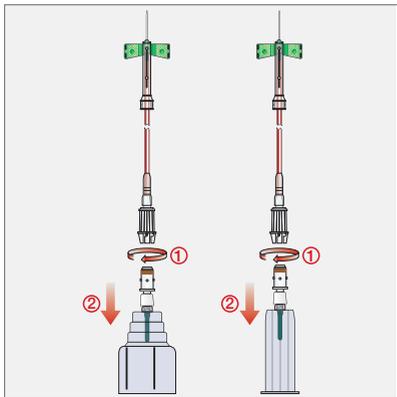


1. Carry out the hygiene steps listed above. Connect the universal blood culture adapter to the guide sleeve of the Safety-Multifly® needle. Puncture the vein and fix the needle in place.

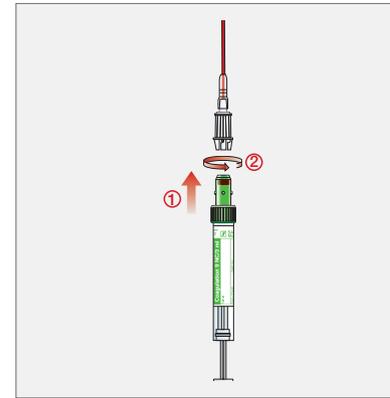


2. Insert the blood culture flask in an upright/vertical position into the holder. The culture medium of the flask must not contact the lid of the blood culture flask. Because of the vacuum in the blood culture flask, it fills automatically.

Note: Note the filling volume.



3. If additional blood collections are required with the S-Monovette®, remove the universal blood culture adapter from the guide sleeve of the Safety-Multifly® needle.



4. You can then carry out the blood collection in the usual manner with the Safety-Multifly® needle.

Important:

- The manufacturer's instructions for handling the blood culture flasks must be followed.
- After the blood collection, the contents must be carefully mixed.
- Do not aerate the flask as this is not necessary.
- The inoculated flask must be sent at room temperature to the laboratory as quickly as possible.

4.7.3 Sample volume and number of flasks

Note:

The blood volume should be checked during the collection using the scale. The vacuum volume of the flask may be larger than the required filling volume.

Marking the filling level on the flask before the collection simplifies checking the blood filling volume during the collection.

The sensitivity of the blood culture diagnostics depends on the number of pairs collected and the sample volume.

There are different recommendations regarding blood volume, number of blood culture pairs and the use of aerobic and anaerobic flasks. Always follow the manufacturer's information for this reason.

5 Blood collection in paediatrics



“Paediatric and neonatal patients have special needs and place high demands on personnel and collection systems”

Paediatrics

Paediatrics is the branch of medicine dealing with children and adolescents. An important focus of paediatrics is neonatology, the treatment of premature infants.

Premature infants are viable from week 23 of pregnancy provided the infants have a birth weight of about 500 grams.

These small patients have special needs and place high demands on personnel and collection systems.

5.1 Medical history²⁴

The details of the paediatric medical history are obtained by asking a third party, usually a parent or legal guardian.

School-aged children should always be asked directly.

The medical history should include the following information

- Details of the current illness
- The complete medical history of the child
- Details of the pregnancy and birth
- The medical history of the families of the parents

Important:

A child may still present in relatively good general condition despite a life-threatening disease. The patient may deteriorate during the recording of the medical history, the clinical examination or even after hospitalisation.

²⁴ Speer et al; Pädiatrie; 2013

5.2 Prerequisites for blood collection

Between 7 months and 3 years of age, resistance from the child may prevent normal blood collection.

To ease the situation, the following tips may help:

- Shorter waiting times
- Bright, warm and child-friendly rooms with toys for all ages
- Small gifts (particularly plasters, bravery awards, etc.)
- Friendly, understanding atmosphere
- If necessary, treat the child on the parent's lap
- Warm hands and equipment
- Consider feelings of embarrassment even in childhood



5.3 Blood collection in paediatrics

The total blood volume of a healthy neonate is about 300 ml. A premature infant of 1,000 g has a total blood volume of about 80 ml. Because of this small volume, it is essential to collect as little blood as possible while still ensuring as much blood as necessary is collected.

In addition, sample collection from premature infants, neonates and infants can be problematical. Choosing the correct collection technique combined with suitable sample tubes eases these difficult conditions as much as is possible.

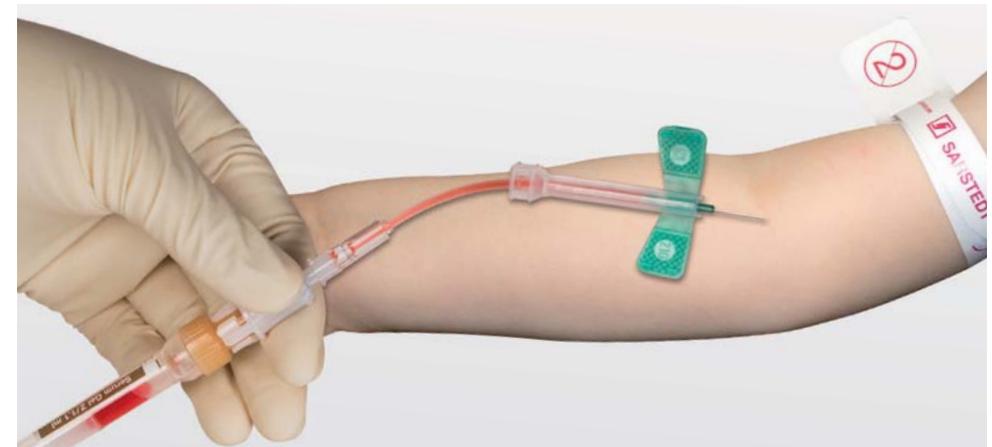
5.3.1 Venous blood collection

For venous blood collection, there is a choice between closed venous blood collection and the drip technique (e.g. from a cephalic vein).

Puncture site	Premature infant	Neonate	Infant	Toddler	School child
Cephalic vein	Only if < 1 week	Recommended	Recommended	-	-
Brachial vein	Perhaps	Perhaps	Perhaps	Recommended	Recommended
Back of hand	Recommended	Recommended	Possible	Recommended	Recommended
Top of foot	Recommended	Recommended	Possible	Perhaps (painful)	-

Closed venous blood collection

Thanks to the option for gentle blood collection using the aspiration technique (see Section 4 – Carrying out venous blood collection), the S-Monovette® combined with the short Safety-Multifly® needle is the optimal solution for difficult vein conditions in paediatrics.



Drip blood collection

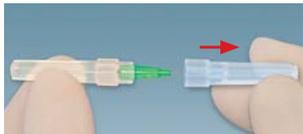
The Micro-Needle combined with the prepared Micro Tubes simplifies blood collection from the cephalic vein.

Difficult handling of broken Luer needles is no longer necessary.

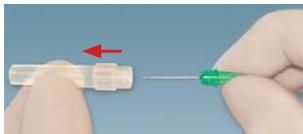
Broken needles are small, cumbersome and may cause haemolysis (formation of burrs in the needle).



Handling the Micro-Needle



1. Remove the protective cap.



2. Remove the Micro-Needle from the protective sheath.



3. Disinfect the puncture site. Puncture the vein and drip the blood into a prepared Micro Tube. If the blood flow stops, the Micro-Needle can be safely rotated by 360° using the handle.



4. Place the Micro-Needle in a suitable disposal box.

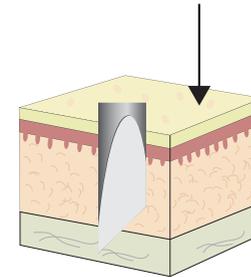
5.3.2 Capillary blood collection

For capillary blood collection, the Neonatal Safety Lancet or the Safety Incision Lancet can be used depending on the patient and the blood volume required.

Comparison of the Safety-Lancet and the Safety Incision Lancet

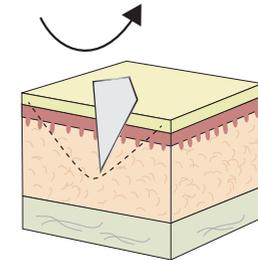
Standard Lancet:

- Vertical firing direction of the blade
- Cylindrical puncture
- Haematoma formation



Incision Lancet:

- Crescent-shaped incision path
- Shallow penetration depth
- Minimises formation of haematoma



Safety-Lancets Mini and Neonatal are suitable for collecting small or medium to high volumes of blood, as required.

	Design	Penetration depth	Needle size	Blood volume
	Neonatal	1.2 mm	Blade 1.5 mm	Medium to high
	Mini	1.6 mm	Needle 28 G	Low

If there is a risk of bone injury, however, Incision Lancets are recommended because these have a shallower penetration depth.

Product range – Safety Incision Lancet

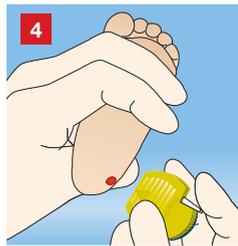
Thanks to the special puncture technique, optimal blood flow with a high blood volume is possible even with a shallow penetration depth. The shallow penetration depth ensures rapid healing and minimises the formation of haematomas.

Design	Application	Penetration depth	Incision length
	Neonates	1.0 mm	2.5 mm
	Premature infants	0.85 mm	1.75 mm

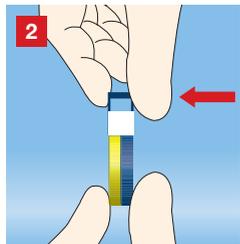
Handling the Safety Incision Lancet



1 Select and disinfect a suitable puncture site.



4 After pressing the firing button, remove the lancet from the heel.



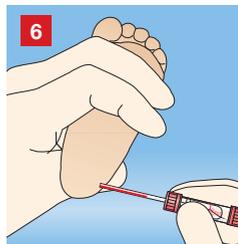
2 Remove the safety mechanism by pressing sideways with the thumb.



5 Dispose of the lancet in a suitable disposal box



3 Lift the foot into a suitable position. Press the blade opening flat against the selected and disinfected puncture site and press the trigger button. The safety incision lancet must always be positioned and triggered parallel to the length of the foot (never across the heel)! The triangle points to the blade exit point.



6 Discard the first drop of blood. Then fill the capillary tube.

Microvette®



Depending on the requirements, Microvette® tubes with a cylindrical or conical inner tube shape and a volume range of 100 to 500 µl are available. There is the option to collect capillary blood using the capillary technique or the collection rim.

The special cap design minimises any aerosol effects when the tube is opened.

Microvette® – Collection methods

To meet the specific requirements of capillary blood collection, there are two collection techniques available:

- 1 Capillary method using the end-to-end capillary
- 2 Gravitational principle using the collection rim

Note: *Letting blood drip into a capillary tube by means of a Luer needle does not constitute capillary blood collection.*

5.4 The difference between capillary blood and venous blood

Taking into account the sample material is important for assessing the analytical results. Between capillary blood and venous blood there are differences in the concentration of various parameters. For example, the serum concentrations of total protein, bilirubin, calcium, sodium and chloride are significantly lower in capillary blood compared to venous blood.²³

Glucose, lactate and CK, however, have higher levels of concentration in capillary blood than in venous blood.

²³ Kupke et al; On the composition of capillary and venous blood serum; Clin Chim Acta. 112(2):177-85; 5 May 1981

5.5 Reference ranges

Depending on the age of the child, concentrations of analytes in different ranges are normal compared to adults. For this reason, it is important to always assess the analytical results relative to the age-appropriate reference/standard ranges⁵⁶.

Some parameters are shown in the following table as examples.

⁵⁶ Kohse et al.; National and international initiatives and approaches for the establishment of reference intervals in pediatric laboratory medicine; J Lab Med 2015; 39(4): 197-212

Analyte	Sample source	SI	Conventional	Remarks
Bilirubin (total)		µmol/L	mg/dL	Indirect bilirubin in neonates may be elevated due to increased breakdown of erythrocytes. Value > 16-18 mg/dl risk of kernicterus. In neonates, direct photometric measurement is possible, direct bilirubin cannot be detected in healthy children.
	Neonate			
	Day 1	< 68	< 4	
	Day 2-3	< 154	< 9	
	Day 3-5	< 239	< 13-14	
	Infant	1.7-14	0.1-0.8	
	Adult	1.7-22	0.1-1.3	
Lactate		mmol/L	mg/dL	Neonates can have higher values on day 1. Elevated in mitochondriopathy, tissue hypoxia, etc.
	Child/Adult	0.5-2.2	4.5-20	
Creatinine	Neonate	µmol/L	mg/dL	Values depend on muscle mass; women have lower values. Creatinine concentration in the serum only increases when the glomerular filtration rate is < 50%.
	Day 1	37-113	0.41-1.24	
	Week 1	14-86	0.15-0.95	
	Week 4	12-48	0.13-0.53	
	Infant	22-55	0.24-0.61	
	Toddler	25-64	0.28-0.70	
	Children	23-106	0.25-1.17	
	Adult	74-110	0.81-1.21	

Analyte	Sample source	SI	Conventional	Remarks
Erythrocytes		Tpt/L (10 ¹² /l)	10 ⁶ /µl	Rapid breakdown after birth. Elevated (polycythaemia) with dehydration and with/after sustained high levels.
	Neonate Week 1	3.9-6.5	3.9-6.5	
	Neonate Week 2	3.6-5.8	3.6-5.8	
	Infant	3.0-5.4	3.0-5.4	
	Toddler Child	4.0-5.4	4.0-5.4	
	Adult (m)	4.5-5.9	4.5-5.9	
	Adult (f)	3.9-5.2	3.9-5.2	
Haematocrit (HCT/Ht)		Fraction l/l	%	Ht elevated with dehydration, lowered with hyperhydration
	Neonate	0.45-0.65	45-65	
	Infant	0.30-0.55	30-55	
	Toddler Child	0.31-0.48	31-48	
	Adult (m)	0.39-0.52	39-52	
	Adult (f)	0.35-0.47	35-47	
Haemoglobin (Hb)		mmol/l	g/dl	
	Neonate Week 1	9.3-13.7	15-22	
	Neonate Week 2	7.8-12.4	12.5-20	
	Infant	5.9-9.9	9.5-16	
	Toddler / Child	6.8-9.9	11-16	
	Adult (m)	8.1-11.2	13-18	
	Adult (f)	7.5-9.3	12-15	

Analyte	Sample source	SI	Conventional	Remarks
Platelets		Gpt/l (10 ⁹ /l)	10 ³ Cells/ μ l	Thrombocytopenia e.g. due to mumps 30 Gpt/L: increased bleeding tendency.
	Neonate	100-250	100-250	
	Toddler	220-500	220-500	
	Children	150-350	150-350	
	Adult	150-400	150-400	
Leucocytes		Gpt/l	Cells/ μ l	Changes in the leucocyte count during the first weeks of life/year. Increases (leucocytosis) are usually caused by elevated numbers of neutrophil granulocytes.
	Neonate Day 1	9-35	9,000-35,000	
	Neonate Weeks 1-4	5-20	5,000-20,000	
	Infant/Toddler/Child	5-18	5,000-18,000	
	Adult (m)	4-10	4,000-10,000	

²⁴ Speer et al; Pädiatrie; 2013

5.6 Haemostasis in paediatrics

Some components of the coagulation system change in childhood and dramatically so, particularly in the first year of life, to adapt to the change in conditions.

Reduced thrombin formation with a simultaneous reduction in thrombin inhibition is a protective mechanism in neonates.

As a rule, neonates have considerably lower values for most coagulation factors than an adult. The reduced liver synthesis rate in the neonate is usually considered responsible but an accelerated turnover is also a possibility, particularly regarding the birth.

Many components reach adult reference values after 1 year of age. Antithrombin is about 10% higher compared to an adult from 1 month of age and into childhood. Values for aPTT are generally longer in childhood than in adults. Factor II and VII remain about 10-20% lower.

Note: *There are a number of special physiological characteristics of children of which the user must be aware so that they can be reliably differentiated from pathological changes.*

Age-related reference values (example reference value)

Age	aPTT [s]*	Age	Antithrombin [%]	D-dimers [μ g/L]
1-3 Months	39 (28-49)	1 day	76 (58-90)	1470 (410-2470)
4-6 Months	36 (31-44)	3 days	74 (60-89)	1340 (580-2740)
7-12 Months	35 (29-42)	1-12 months	109 (72-134)	220 (110-420)
Up to 4 years	33 (28-41)	1-5 years	116 (101-131)	250 (90-530)
5-9 years	34 (28-41)	6-10 years	114 (95-134)	260 (10-560)
10-18 years	34 (29-42)	11-16 years	111 (96-126)	270 (160-390)
Adults	31 (26-36)	Adults	96 (66-124)	180 (50-420)

* measured with Pathromtin SL

²² Barthels et al; Das Gerinnungskompndium; 2012

Due to a physiologically higher haematocrit, the quantity of plasma in neonates is lower.

Haematocrit correction is not necessary here because the age-related reference values were determined under these conditions and a correction therefore does not have to be made.

What is important is that sufficient sample material is collected for the required analyses in light of the low plasma yield.



6 Blood gas



“It is also true for blood gas that the better the preanalytics, the more meaningful the result”

6.1 Type of blood collection

Blood gas collection and blood gas analyses are carried out in many different areas such as emergency admissions, intensive care units, outpatient clinics, operating areas, cardiac catheterisation and pulmonary diagnostic laboratories.

Because the parameters have different concentrations depending on the blood vessel ($p\text{CO}_2$ is higher in venous blood, the concentration of $p\text{O}_2$ and $s\text{O}_2$ is lower in venous blood than in arterial), the puncture site for the sample should be noted and taken into account (e.g. arterial access, CVC, peripheral artery).²⁵ Arterial blood should always be the material of choice.

For children, arterialised capillary blood is often taken from the earlobe or fingertip or for infants from the side of the heel.

For ventilated patients, the setting of the ventilation equipment should also be noted and taken into account.

²⁵ Michael D Davis RRT et al.; AACR Clinical Practice Guideline: Blood Gas Analysis and Hemoximetry: Respiratory Care; 58(10); Oct. 2013

Important: *For calcium measurement using blood gas analysers (ISE method), calcium-titrated heparin (balanced, equilibrated) as in the blood gas capillaries and the Blood Gas Monovette® must be used. Total calcium must therefore not be determined using the Blood Gas Monovette®.*

6.2 Storage

Always aim to measure parameters immediately after the blood collection. If the measurement cannot be done within 15 minutes, the sample should be stored refrigerated (about 4°C).²⁵

²⁵ Michael D Davis RRT et al; AACRC Clinical Practice Guideline: Blood Gas Analysis and Hemoximetry; Respiratory Care; 58(10); Oct. 2013

After storage the samples must be carefully mixed because sedimentation can lead to erroneous measurements of the Hb.

Cellular metabolism may lead to changes in the concentration over extended storage periods.

Lowered	Elevated
pH	pCO ₂
pO ₂	Calcium
Glucose	Lactate

6.3 Troubleshooting

Clotting

Samples with clots cannot be correctly drawn up into the analytical equipment and the results will not be representative.

Solution

- Use liquid-dosed heparin because this mixes more quickly with the sample.²⁶
- Mix the samples carefully and immediately after the samples are collected.
- Use the mixing aid for the blood gas capillaries.

²⁶ Gruber et al; Heparin release is insufficient in syringes with platelets as heparin source; Clinica Chimica Acta, 395: 197, 2008

Air bubbles

To avoid erroneous measurements due to air contamination, air bubbles must be removed immediately after the blood collection (see venting). The longer the samples are stored with air bubbles and the larger the air bubble(s), the greater the change in the values.

Lowered	Elevated
pCO ₂	pH
	pO ₂
	sO ₂



Blood collection from a catheter

Contamination by infusions or flushing solutions are possible risks. Before the blood collection, it must be ensured that an adequate blood volume is discarded.

	Contamination with liquid heparin	Contamination with NaCl solution
Lowered	pO ₂ , Na ⁺ , Cl ⁻	Na ⁺ , Cl ⁻
Elevated	pCO ₂ , K ⁺ , Ca ⁺⁺ , gluc, lactate, tHb	

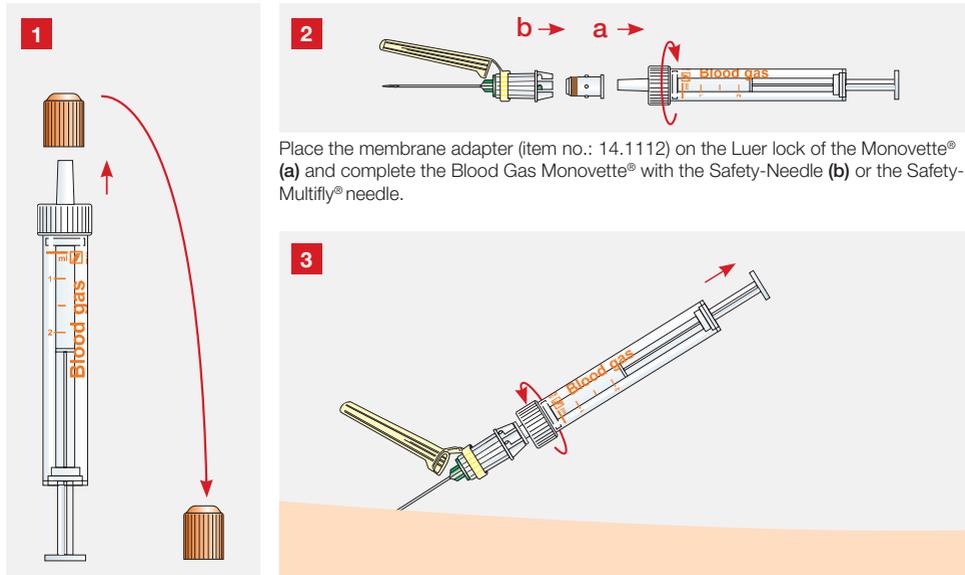
Haemolysis

Haemolytic samples have erroneously high potassium concentrations and erroneously low sodium and calcium concentrations.

Possible causes of haemolysis

- Shearing forces:
 - Sample shaken too hard during mixing or sample transport
- Collection technique:
 - Too much pressure is applied (milking) to the puncture site during collection of arterialised capillary blood
- Temperatures:
 - Extremely high temperatures in summer
 - Extremely low temperatures, e.g. sample frozen or placed directly on ice

6.4 Collection technique – Blood Gas Monovette®

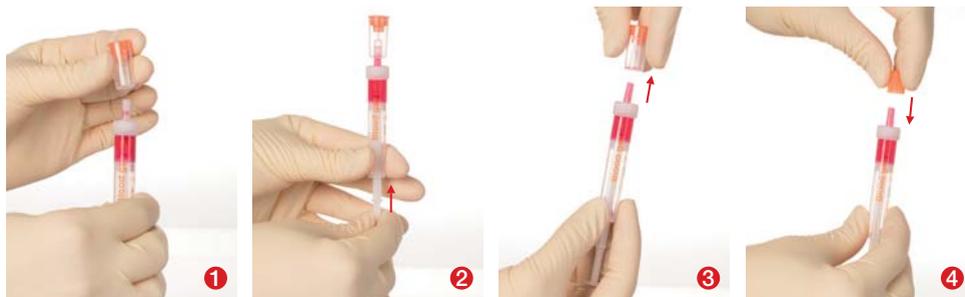


Remove the orange protective cap from the Blood Gas Monovette®.

Remove the blood sample according to the work instructions. When puncturing the artery, a 45° angle is recommended.

Venting the Blood Gas Monovette®

To avoid erroneous measurements due to air contamination, after completing the blood collection the air must be removed from the Blood Gas Monovette® as follows:



Place the ventilator (item no. 14.1148) on the Blood Gas Monovette®.

Carefully push the plunger upwards.

Remove and dispose of the ventilator.

Place the protective cap on for the mixing procedure.

Mixing the Blood Gas Monovette®

In contrast to mixing in the inverted position, which is encouraged by the air bubble in the standard S-Monovettes, the process of mixing the Blood Gas Monovette® is as follows:

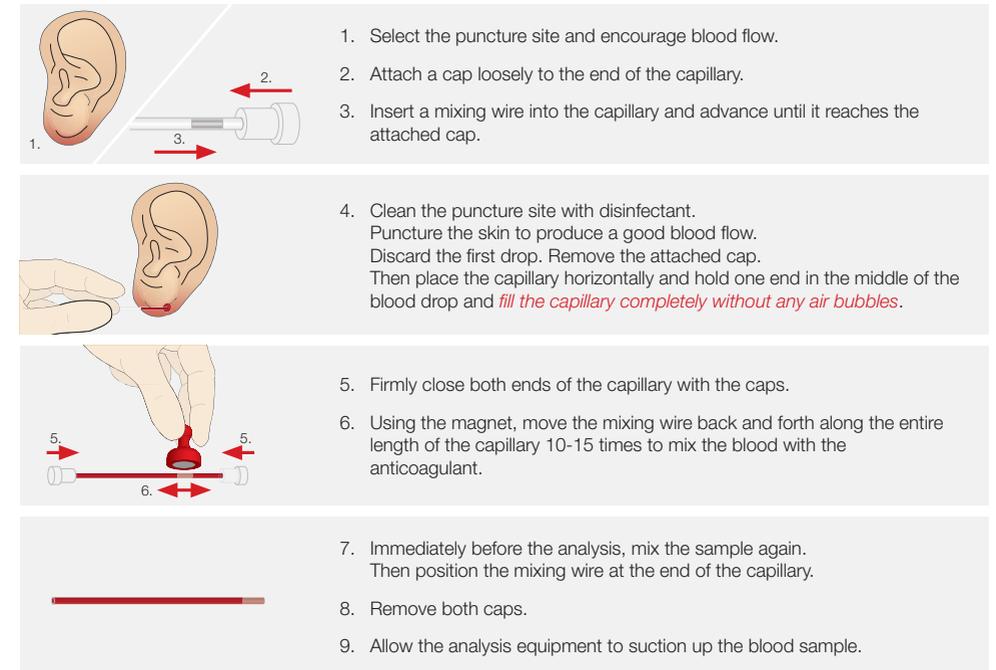


Mix the blood sample immediately after collection by rolling the Blood Gas Monovette® between the palms of the hands. The tubes must be mixed by rolling between the palms of the hands rather than inverting.

Important: *Blood gas analyses should be carried out as soon as possible after blood collection, at the latest 15 minutes after the samples are collected.*

Collection technique – blood gas capillaries

For skin puncture we recommend using the safety lancets, item no. 85.1015 to 85.1019.



7 Safety when collecting blood



“Informing, training and providing safe working equipment are the keys to avoiding needle stick injuries and the associated risk of infection”

Safety – why?

The most important infectious agents that can be transmitted by needle stick injuries (NSI) are hepatitis B virus, hepatitis C virus and HIV.

Using suitable protective measures, these incidents can be almost completely avoided.²⁷

The [EU Directive 2010/32/EU²⁸ Prevention of sharps injuries in the hospital and healthcare sector](#) requires the safest possible working environment for employees in the healthcare sector.

²⁷ The underestimated workplace accident, infection risk due to needle stick injuries; SAFETY FIRST! initiative

²⁸ EU Directive 2010/32/EU of the Council of the European Union from 10 May 2010 Prevention of sharps injuries in the hospital and healthcare sector

Preventive and protective measures

- Introduction of safe working regulations
- Maintain general hygiene
- Vaccinations (against hepatitis B)
- Suitable personal protective equipment
- Wear gloves
- Cover any cuts and grazes with waterproof plasters
- Avoid unnecessary use of sharps
- Provide medical instruments with integrated safety and protective mechanisms
- Forbid the replacement of protective caps on used needles (no re-capping)

Note: *Over half of all needle stick injuries occur during disposal.⁵⁷*

⁵⁷ SAFETY FIRST, Germany - www.nadelstichverletzung.de

7.1 Safety-Needle

The Safety-Needle is ready to use, thus sparing the step of attaching the needle in the needle holder and thus reducing the potential risk of a needle stick injury at the back of the needle.

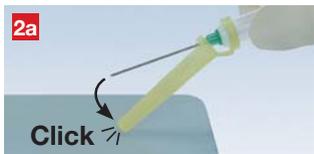


Handling



After blood collection:

Detach the last S-Monovette® from the Safety-Needle and then withdraw the Safety-Needle from the vein.



Hold the Safety-Needle on the adapter, place the needle protector on a stable, flat surface and lock the needle into the needle protector by pressing gently downwards until it makes a noticeable and audible click.



Alternatively, you can also activate the needle protector with your index finger. For reliable function, ensure that this is done at the bottom end of the protector.



After activating the protective mechanism:

Discard the safely locked Safety-Needle in a disposal box.

7.2 Safety-Multifly® needle

The adapter of the Safety-Multifly® needle is already pre-assembled and forms a ready-to-use unit. The single-handed operation of the needle protector of the Safety-Multifly® needle offers maximum protection.

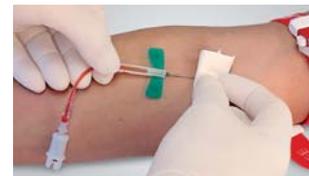


Handling



After blood collection:

Remove the last S-Monovette® from the Safety-Multifly® needle.



Hold the needle protector at its back end with your thumb on top and your forefinger below and withdraw the Safety-Multifly® needle from the vein.



Gently hold the tubing by pressing it slightly into the palm of your hand and push the needle protector over the needle...



...until the needle is noticeably and audibly locked into the protective casing.



After activating the protective mechanism:

Discard the safely locked Safety-Multifly® needle into a disposal box.

Safety activation is **always done** with **one hand only!**

7.3 Multi-Safe disposal boxes

For collecting sharps, waste containers must be provided and used that meet the relevant regulations TRBA 250 (Technical Rules for Biological Materials – German regulations) and ISO 23907.

These regulations define the following features of the containers, for example:

- Shape and appearance
- Container must not rupture when dropped from a particular height in tests
- Container walls must resist penetration up to an applied pressure of 15 N

If the sharps containers are supposed to be disposed of through a medical waste disposal service and are placed on the street, UN certification of the disposal box is mandatory. The certified containers are identified by a multiple digit UN code that is normally located on the top of the lid. Disposal boxes without this identification must be disposed of inside a container with this identification.

Safe disposal

Recommendation:

Only fill the Multi-Safe container to about $\frac{2}{3}$ of its volume.

Do not overfill the Multi-Safe:

Risk of injury!

Note the filling line



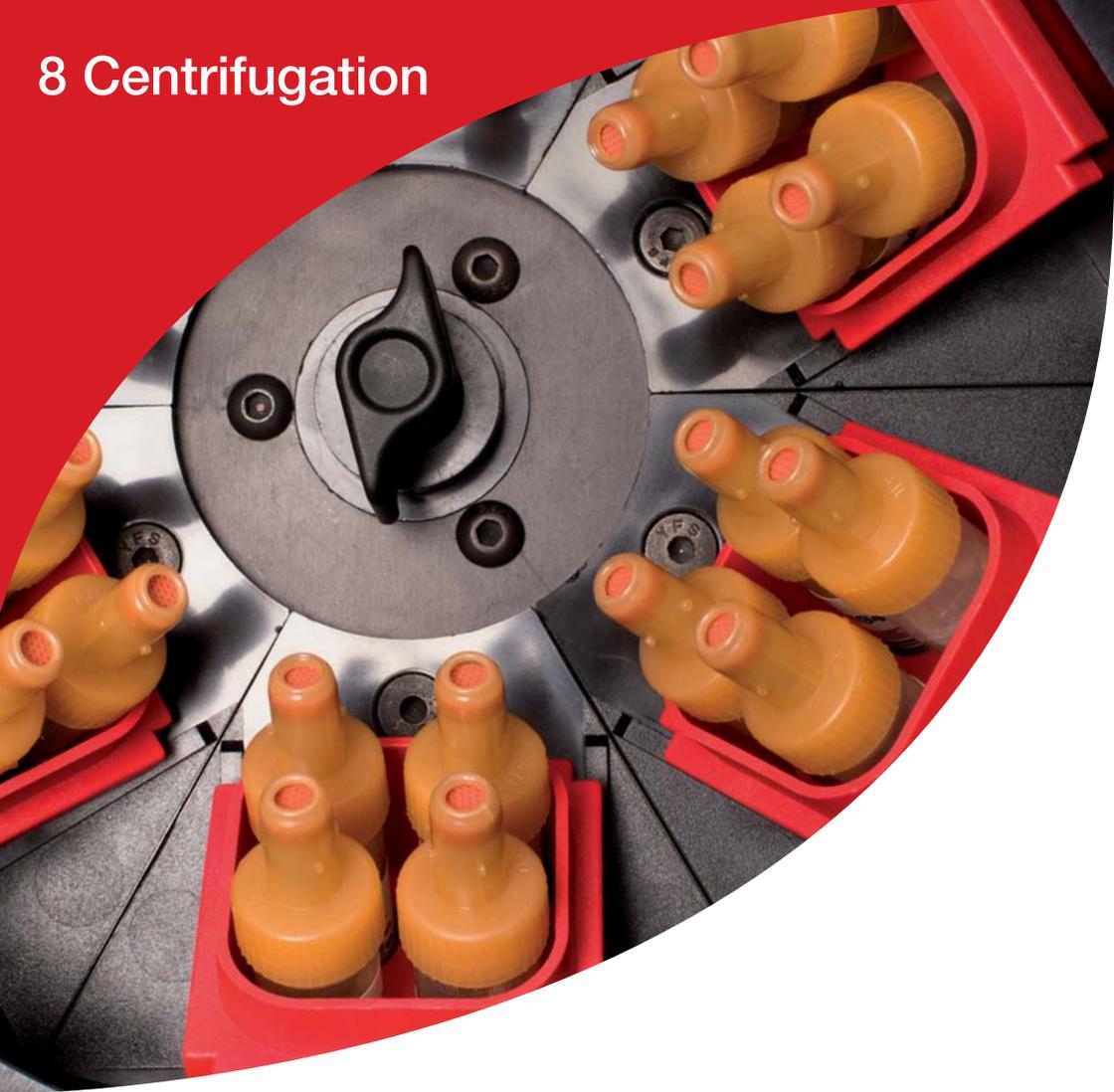
- ▶ As a rule, when disposing of potentially infected medical single-use items, ensure they are disposed of in a **hygienically correct manner**.



Safety instructions

- Only use containers of a size that is suitable for holding the items to be disposed of.
- The lid must be on the container and locked into place before it is filled.
- Connect containers with the recommended adhesive adapter by rotating or fixing by hanging in the wall holder to prevent accidents.
- Do not use the day lid to press down the items to be disposed of.
- Scalpels must be disposed of in the container with particular care. If too much force is used when throwing scalpels in or if other items are placed on top, there is a risk of the angle changing and damaging the container walls or the base of the container.
- Only place items to be disposed of upright in the container.
- Do not press items into the container with force.
- Do not place any liquids in the container.
- Do not place hands or any other objects in the container (risk of injury).
- Do not throw, shake or drop the container.
- Before sealing the container, ensure that no items are projecting through the opening.
- Before disposing of the container, carefully check that the lid is tightly sealed.

8 Centrifugation



8.1 Correct handling for centrifugation

Most laboratory analyses require serum or plasma, the liquid component of the blood. This is obtained by centrifuging the sample. Inside a centrifuge a rotor with tube holders rotates at a speed of several thousand revolutions per minute. This rapid rotation produces a multiple of gravity (g) inside the tube holder. This causes the liquid and solid components of the blood to separate.

What is important is to differentiate between the revolutions per minute and the g force (gravitational force).

The g force is the value that is relevant for a good centrifugation result. For this reason the g force is of particular importance when setting the centrifuge. The g force can be calculated using the radius (cm) and the revolutions per minute (RPM):

$$g = 11.18 \times r \times (\text{min}^{-1}/1,000)^2$$

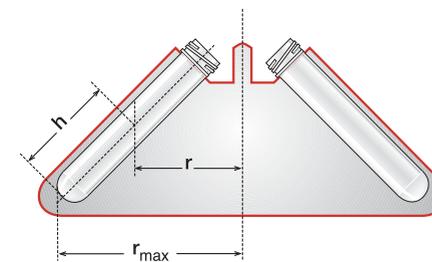
r = radius in cm
 min^{-1} = revolutions per minute

To convert from g force to RPM [min^{-1}] or vice versa, you can use the centrifugation calculator at

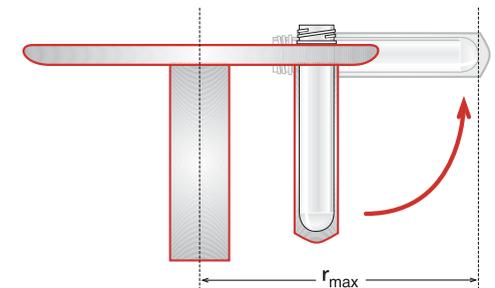
www.sarstedt.com/en/service-consultation/centrifugation-calculator.

The centrifuge radius r can be found in the information provided by the centrifuge manufacturer or it can be determined using the following image:

Fixed-angle rotor



Swinging bucket rotor



“Centrifugation is a physical separation process that is based on the different density characteristics of substances such as blood cells and plasma”

8.2 Difference between fixed-angle and swinging bucket rotors

*For gel-prepared S-Monovettes, we recommend using swinging bucket rotors only. The sample holder is arranged at a fixed oblique angle in a fixed-angle centrifuge. The sample holder in a swinging bucket rotor moves during the centrifugation from a vertical position to a horizontal position. In this way, the force during centrifugation acts evenly from the lid towards the base. The result is a well-shaped, horizontal gel layer.

Fixed-angle rotor



Swinging bucket rotor



8.3 Serum collection



S-Monovette® Serum-Gel with coated beads to accelerate coagulation

After blood collection, the serum samples must coagulate for 15–30 minutes. This means that as the coagulation proceeds, the coagulation factors (e.g. fibrin) are consumed and the blood cells form a blood clot.

The coagulum forms in the shape in which the blood cells are present in the tube. This means that if the S-Monovette® is placed horizontally after the blood collection, the blood cells sediment along the horizontal tube and form a long shape. The resultant shape can be compressed during the centrifugation. After the centrifugation, it springs back in a concertina shape (sausage phenomenon). The serum from such a sample cannot be automatically pipetted. It is therefore important to store serum samples upright after blood collection.



Sample that has clotted upright after centrifugation



Sample that has clotted horizontally after centrifugation

8.4 S-Monovette® centrifugation conditions

	Preparation	Min.	Standard recommendation	Alternative range	Temperature
	S-Monovette® Serum	10	2,000 x g	1,800 - 2,500 x g	18 - 25°C
	S-Monovette® Serum-Gel*	10	2,500 x g	2,200 - 3,000 x g	18 - 25°C
	S-Monovette® Li-Heparin	10	2,000 x g	1,800 - 2,500 x g	18 - 25°C
	S-Monovette® Li-Heparin-Gel* or	10 15	3,000 x g 2,500 x g	2,700 - 3,300 x g 2,300 - 3,000 x g	18 - 25°C
	S-Monovette® EDTA-Gel*	10	2,500 x g	1,800 - 2,500 x g	18 - 25°C
	S-Monovette® Citrate	10	1,800 x g	1,800 - 2,300 x g	18 - 25°C
	S-Monovette® Fluoride/GlucoEXACT	10	2,000 x g	1,800 - 2,500 x g	18 - 25°C

Gel ascent during centrifugation

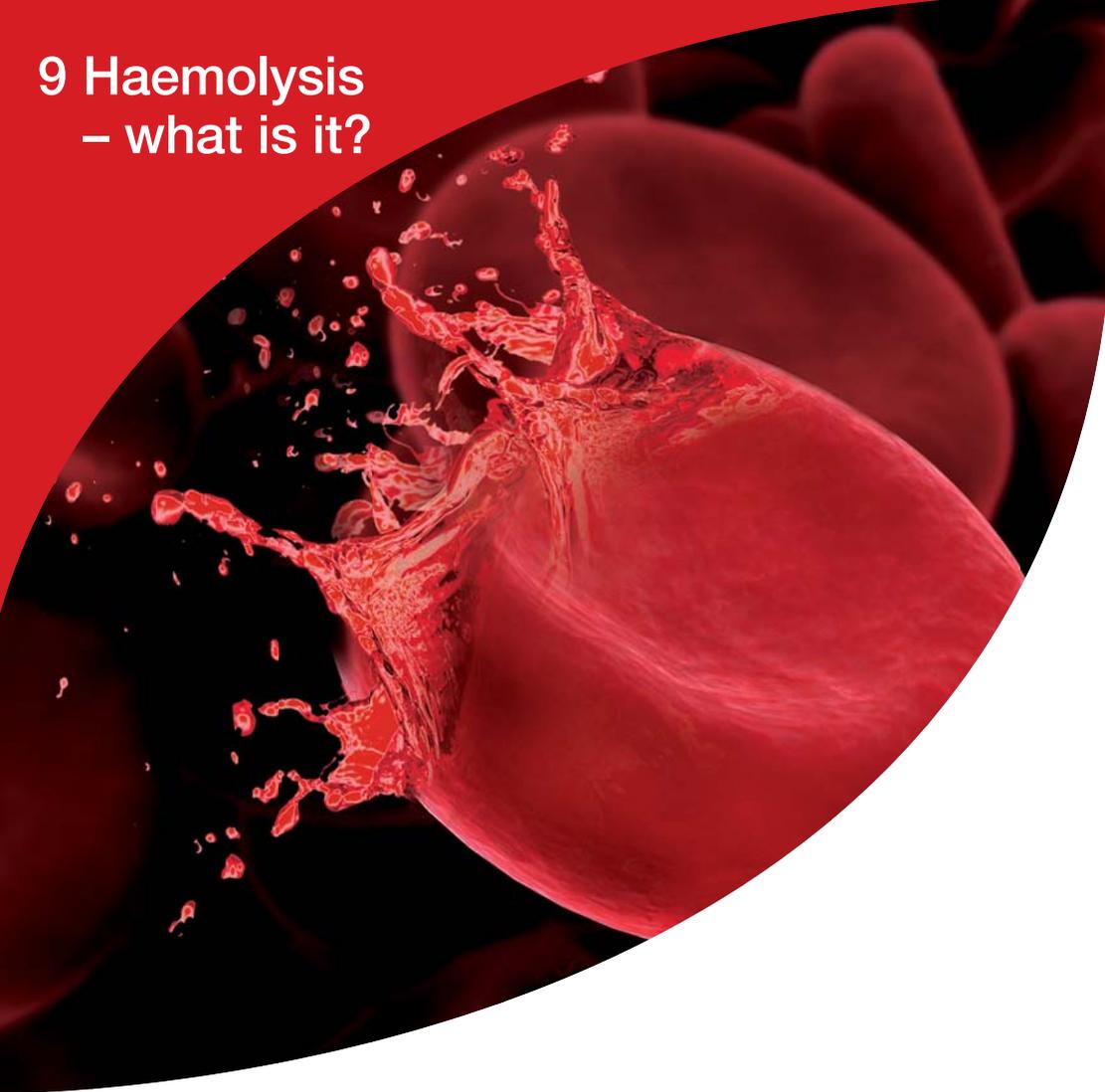


These centrifugation conditions (see p. 72) are recommendations. The values are based on what we consider to be the worst-case conditions, e.g. an older model centrifuge that requires considerably more time to reach the necessary g force than a new high-performance centrifuge. In isolated cases, it may be that the centrifugation conditions deviate from the standard recommendations shown in the table while achieving the same results.

The details of the standard centrifugation conditions are always printed on the label of the inner carton as well.

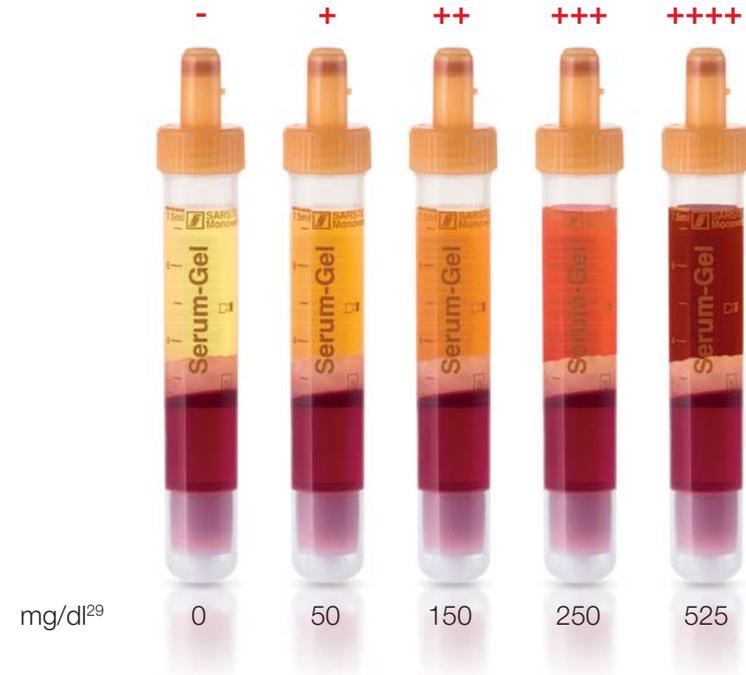
* For gel-prepared S-Monovettes, we recommend using swinging bucket rotors only.

9 Haemolysis – what is it?



Characteristic feature of haemolysis

If more than 0.5% of the erythrocytes are destroyed, the serum/plasma is discoloured.



After centrifugation, this can be seen as a reddish colour of the plasma or serum. The cause is leakage of haemoglobin, which gives the erythrocytes their red colour. Above a concentration of about **20 mg haemoglobin/dl**, haemolysis can be seen in the serum/plasma.

The absence of a red colour does not exclude interference due to haemolysis.

Haemolysis – the destruction of erythrocytes – is classified as *in vivo* haemolysis (pathological) or *in vitro* haemolysis (physiological) based on its cause.

²⁹ CLSI; Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis; Approved Guideline; C56-A; 32(10); 2012

“The destruction of erythrocytes due to damage of the cell membrane leads to leakage of haemoglobin into the plasma/serum. A reddish discolouration of the serum/plasma can be seen.”

9.1 *In vivo* haemolysis

Disease can cause the destruction of erythrocytes **within the body**. This is referred to as *in vivo* haemolysis or haemolytic anaemia.

Such a disease may be inherited or acquired.

Inherited	Acquired
Haemoglobinopathy e.g.: sickle cell anaemia, thalassaemia	Mycoplasma pneumoniae infection Cold agglutinin disease Autoimmune haemolytic anaemia (AIHA) Autoimmune diseases, e.g.: Lupus erythematosus, chronic lymphatic leukaemia (CLL)
Glucose-6-phosphate dehydrogenase deficiency	Infections (e.g.: malaria, babesiosis, Clostridium)
Defects in the erythrocyte membrane (e.g. hereditary spherocytosis or hereditary elliptocytosis)	Mechanical stress in the circulatory system e.g.: Disseminated intravascular coagulation (DIC) Haemolytic uraemic syndrome (HUS) Thrombotic thrombocytopenic purpura (TTP) HELLP syndrome
Pyruvate kinase deficiency = erythrocyte enzymopathy	Burns
	Drugs, toxins
	Blood transfusion from incompatible blood group

³⁰ Lippi et al; In vitro and in vivo hemolysis, an unresolved dispute in laboratory medicine; 2012

9.2 *In vitro* haemolysis

This type of haemolysis develops **outside the body** and is responsible for more than 90% of haemolytic samples. The cause is always due to preanalytics.

Common causes during blood collection

- Prolonged/too tight venous stasis
- Physical shearing forces (needle too thin, bent needle)
- Traumatic venous puncture (poking)
- Blood collection from catheters using the vacuum technique¹⁵
- Intravenous catheter combined with too large vacuum force³¹⁻³⁸
- Infusion solutions (dilution, distortion)

¹⁵ Lippi et al.; Prevention of hemolysis in blood samples collected from intravenous catheters Clin Biochem 46: 561-564, 2013

³¹ Ong, et al. Reducing blood sample hemolysis at a tertiary hospital emergency department. Am J Medicine 2009;122:1054e1-e6.

³² Halm, et al. Obtaining blood samples from peripheral intravenous catheters: best practice? Am J Crit Care 2009;18:474-8.

³³ Wollowitz, et al. Use of butterfly needles to draw blood is independently associated with marked reduction in hemolysis compared to intravenous catheter. Ac Emerg. Med 2013;20:1151-1155.

³⁴ ENA's Translation Into Practice. Reducing Hemolysis of Peripherally Drawn Blood Samples. December, 2012 (Emergency Nursing Association).

³⁵ Heyer et al.; Effectiveness of practices to reduce blood sample hemolysis in EDs: A laboratory medicine best practices systematic review and meta-analysis; Clin Biochem 45: 1012-1032, 2012

³⁶ Grant MS; The Effect of Blood Drawing Techniques and Equipment on the Hemolysis of ED Laboratory Blood Samples; J Emerg Nurs 29:116-121, 2003

³⁷ Straszewski et al J; Use of separate venipunctures for IV access and laboratory studies decreases hemolysis rates; Intern Emerg Med 6(4):357-359, 2011

³⁸ Dugan et al.; Factors Affecting Hemolysis Rates in Blood Samples Drawn from Newly Placed IV Sites in the Emergency Department; J Emerg Nurs 31(4): 338-345, 2005

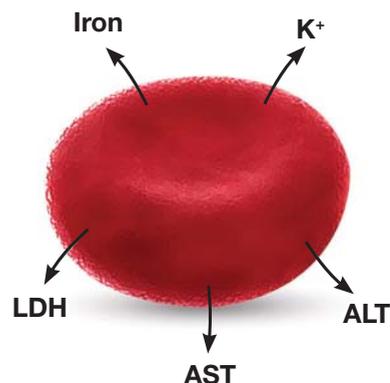
Common causes after blood collection

- Too vigorous mixing/shaking
- Transport influences (too much mechanical stress, e.g. pneumatic tube system)
- Sample too old (the risk of haemolysis increases as the sample ages)
- Sample cooled / heated too much / frozen

9.3 Consequences of haemolysis

Release of cell contents – differences in concentration

Substances that are present in erythrocytes in higher concentration (intracellular concentration) are released into the serum/plasma (extracellular concentration) because the erythrocyte membrane is destroyed during haemolysis. The result is erroneously higher measurements.



Release of cell contents – visual interference

During haemolysis haemoglobin, which gives blood its red colour, is released into the serum/plasma. This can lead to erroneous measurement signals during photometric analyses due to the absorbance of haemoglobin itself.

Erroneous measurement signal = incorrect result

Release of cell contents – method-specific interference

The individual test methods may be affected and interfered with due to the enzymes released from the cells.

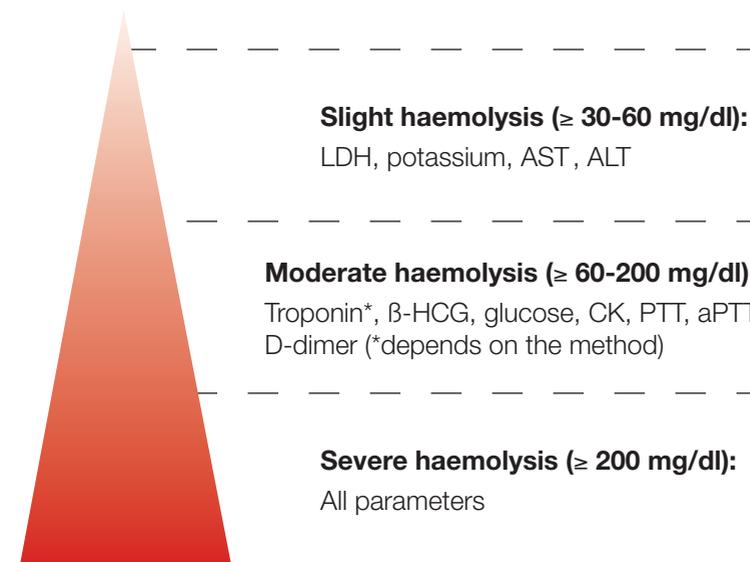
Released cell content	Affects analysis
Free haemoglobin	Bilirubin
Adenylyl kinase	CK, CK-MB
Hydrolase	Coagulation

Release of cell contents – shifts in volume

In cases of extensive or severe haemolysis, there may be an increase in the volume of the liquid fraction in the sample (because there are hardly any or no cells present any more). This leads to a dilution of the serum/plasma.

9.4 Clinical relevance

The following parameters are affected:



³⁹ Lippi et al Hemolyzed specimens: a major challenge for emergency department and clinical laboratories, Crit Rev Clin Lab Sci 48:143–153, 2011

Note: *The analytical results are altered by haemolysis and do not reflect the conditions in the patient. This can lead to an incorrect diagnosis, no diagnosis or unnecessary diagnostics.*

In many cases, repeat blood collection to determine the correct analytical values is necessary.

This causes avoidable patient stress, time wasting and additional costs.^{31,40,41,42}

³¹ Ong, et al. Reducing blood sample hemolysis at a tertiary hospital emergency department. Am J Medicine 2009;122:1054e1–e6.

⁴⁰ Cadamuro et al; The economic burden of hemolysis; CCLM 2015

⁴¹ Jacobs et al; Cost of hemolysis; AnnClinBiochem 2012; 49: 412–413

⁴² P Jacobs et al; Haemolysis Analysis; An Audit of Haemolysed Medical Admission Blood Results; AcuteMed 2010; 9(1): 46-47

10 Storage and transport



“Sample transport and storage must be chosen so that the analytical results are not affected by transport/storage”

10.1 Sample transport

To ensure correct storage, transport conditions and sample shipping, the relevant shipping regulations^{43, 44} and the stability of the individual parameters must be taken into account. This assumes optimal organisation.

Important: *The sender is responsible for shipping the sample and choosing the correct transport system.*

⁴³ P650 IATA/ADR

⁴⁴ TRBA 100

Sample transport compliant with the Packaging Instruction

P650 of the ADR and IATA

Before transporting samples of liquid biological materials in category B in connection with transport boxes and cases, the sender should enquire whether the samples will be shipped via a land, rail or air transport route.

The P650 Packaging Instruction, which is also incorporated in the ADR (European Agreement concerning the International Carriage of Dangerous Goods by Road) and in the IATA (International Air Transport Association), applies specifically for these individual routes. These regulations state that samples must be transported in packaging that consists of 3 components:

- Primary receptacle (leakproof)
- Secondary packaging (leakproof)
- Outer packaging (rigid; with a minimum dimension of 100 × 100 mm; labelled ‘BIOLOGICAL SUBSTANCE, CATEGORY B’ with the UN code ‘UN3373’ printed in a rhombus with a minimum dimension of 50 × 50 mm)

The primary receptacle or the secondary packaging must also be able to withstand an internal pressure of 95 kPa without leakage. There must also be an absorbent material placed between the primary receptacle and the secondary packaging that can absorb the entire contents of the primary receptacle.



In-house transport / TRBA 100

For safe in-house transport of samples of biological working materials and substances, these must be transported in containers that are enclosed, rigid, non-breakable and leakproof and have an external surface that can be disinfected and permanently labelled. These containers must also not be able to be inadvertently opened by external impacts.⁴⁴



⁴⁴ TRBA 100

Transporting “exempt medical samples”

Samples that are not considered infectious substances in category A and B are not subject to the ADR/IATA regulations but must be packaged as follows.

3-component packaging consisting of:

- Primary receptacle (waterproof)
- Secondary packaging (waterproof)
- Outer packaging (minimum dimension 100 × 100 mm with the label “EXEMPT MEDICAL SAMPLE” or “EXEMPT VETERINARY SAMPLE”)

An absorbent material that can absorb the entire contents of the primary receptacle must also be placed between the primary receptacle and the secondary packaging. As a rule, the P650 is the same in both regulations.



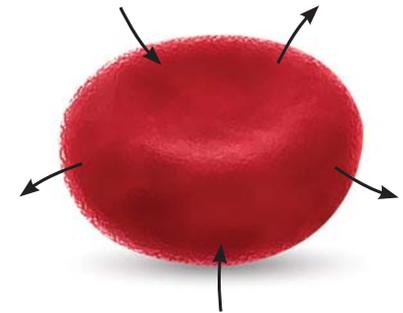
Exception: *Shipping boxes and transport cases that are used to transport samples of biological substances in category B must be tested in accordance with the P650 Packaging Instruction.*

10.2 Influence of temperature, time and cellular metabolism

Concentrations that are measured, change due to the stability of the individual parameters and cellular metabolism. Mechanical or physical stresses placed on the sample materials may also produce changes.

Cellular metabolism

Blood is a living substance. This means that there are metabolic processes, that is, cellular metabolism, occurring in the sample tube after blood collection.



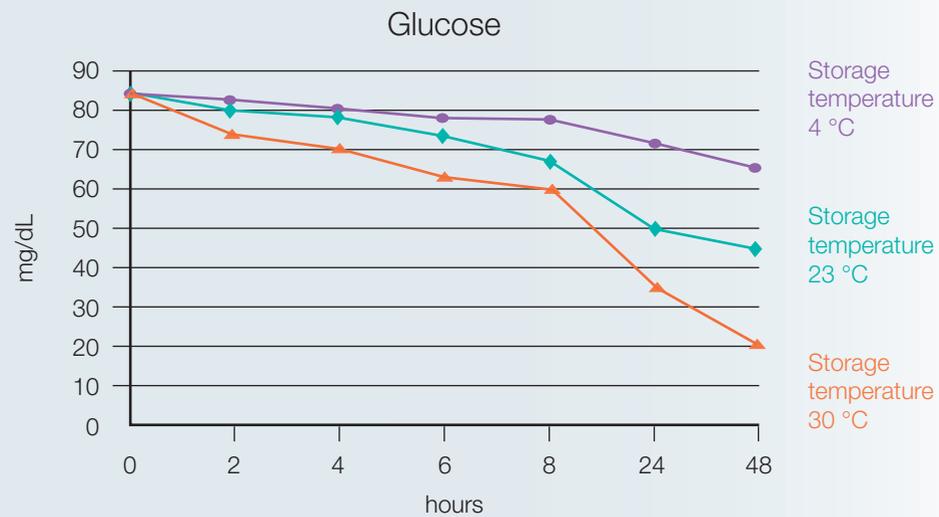
Note: Blood is alive!

Effect of storage on various parameters

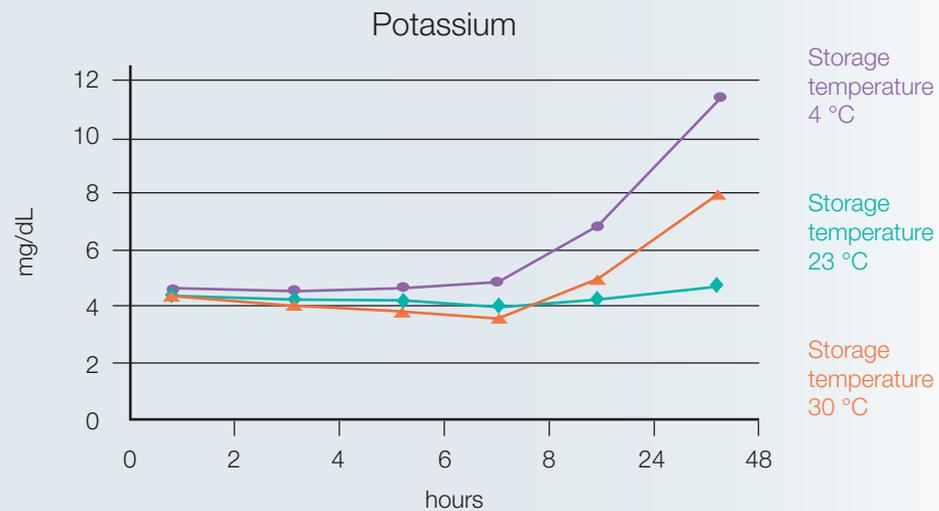
Parameter	Value
Lactate	Increases
Ammonia	Increases
Potassium	Increases
Glucose	Falls
pCO ₂	Falls

Depending on the parameter, the changes in the values may be prevented by special stabilisers in the various preparations or by physical separation (gel, Seraplas® filter, preparing aliquots).

Influence of storage temperature on glucose and potassium



⁵ Sarstedt; Tips & Techniques in Preanalytics; 2014



⁵ Sarstedt; Tips & Techniques in Preanalytics; 2014

Note: *There is no ideal temperature. Correctly collected, fresh samples ensure correct results.*

Sample storage and transport



- Blood samples should be taken to the laboratory for analysis as soon as possible.
- After centrifugation, separating gels or filters prevent diffusion of substances from the erythrocytes into the serum/plasma.

Whole blood without serum/plasma separation using gel or a filter must not be frozen under any circumstances. This would result in complete haemolysis.

Clinical chemistry:

- For long-term storage, the serum should be stored at 2-4°C in closed containers.
- Serum or plasma samples can be stored at -20°C for extended periods.
- Special cool transport containers should be used to protect samples during prolonged transit.
- For some analyses, the sample must be transported promptly (e.g. ammonia within 15 min.).

Coagulation diagnostics:

- For coagulation diagnostics, the sample should be transported at room temperature (18-25°C) as a rule.⁶

Haematology:

- EDTA blood for a blood count can be stored at room temperature (18-25°C) for up to 24 hours.⁴⁵

⁶ G. Ender et al; The importance of preanalytics for the coagulation laboratory; Hämostaseologie 2/2010; 30: 63-70

⁴⁵ N. Tatsumi et al; Specimen Collection, Storage, and Transmission to the Laboratory for Hematological Tests; International Journal of Hematology 75; 261-268; 2002

Checklist for transport

- Seal sample (prevent evaporation)
- Store serum/plasma at 4-8°C
- Store upright
- Store EDTA for blood count at room temperature
- Avoid repeated freezing & thawing
- Protect from exposure to sunlight for light-sensitive parameters (e.g. bilirubin)
- Use special preparation for stabilisation (e.g. S-Monovette® HCY-Z-Gel for homocysteine)



By complying with the following tips, samples can be transported using a pneumatic tube system without significant effects on the values.^{50,51}

- Maximum speed 5 m/s
- “Gentle” curves and shapes
- Brake gently before curves
- Use cushioning lining in pneumatic tube system carriers
- Cushioned horizontal sending and receiving zones
- Send serum samples only after the coagulation is complete

Pneumatic tube transport systems

Pneumatic tube transport systems can considerably shorten the time between blood collection and analytical result.⁴⁶ However, it is not a case of the faster the better. Poor quality or incorrectly set transport systems can lead to haemolysis and activation of coagulation.^{47,48,49}

For monitoring, the LDH values, potassium value, leucocyte count, PTT and D-dimers are compared with and without pneumatic tube transport.



⁴⁶ Koessler et al; The preanalytical influence of two different mechanical transport systems on laboratory analysis; Clin Chem Lab Med; 49(8): 1379-1382; 2011

⁴⁷ Kratz et al; Effects of a pneumatic tube system on routine and novel hematology and coagulation parameters in healthy volunteers; Arch Lab Med; 131: 293-6; 2007

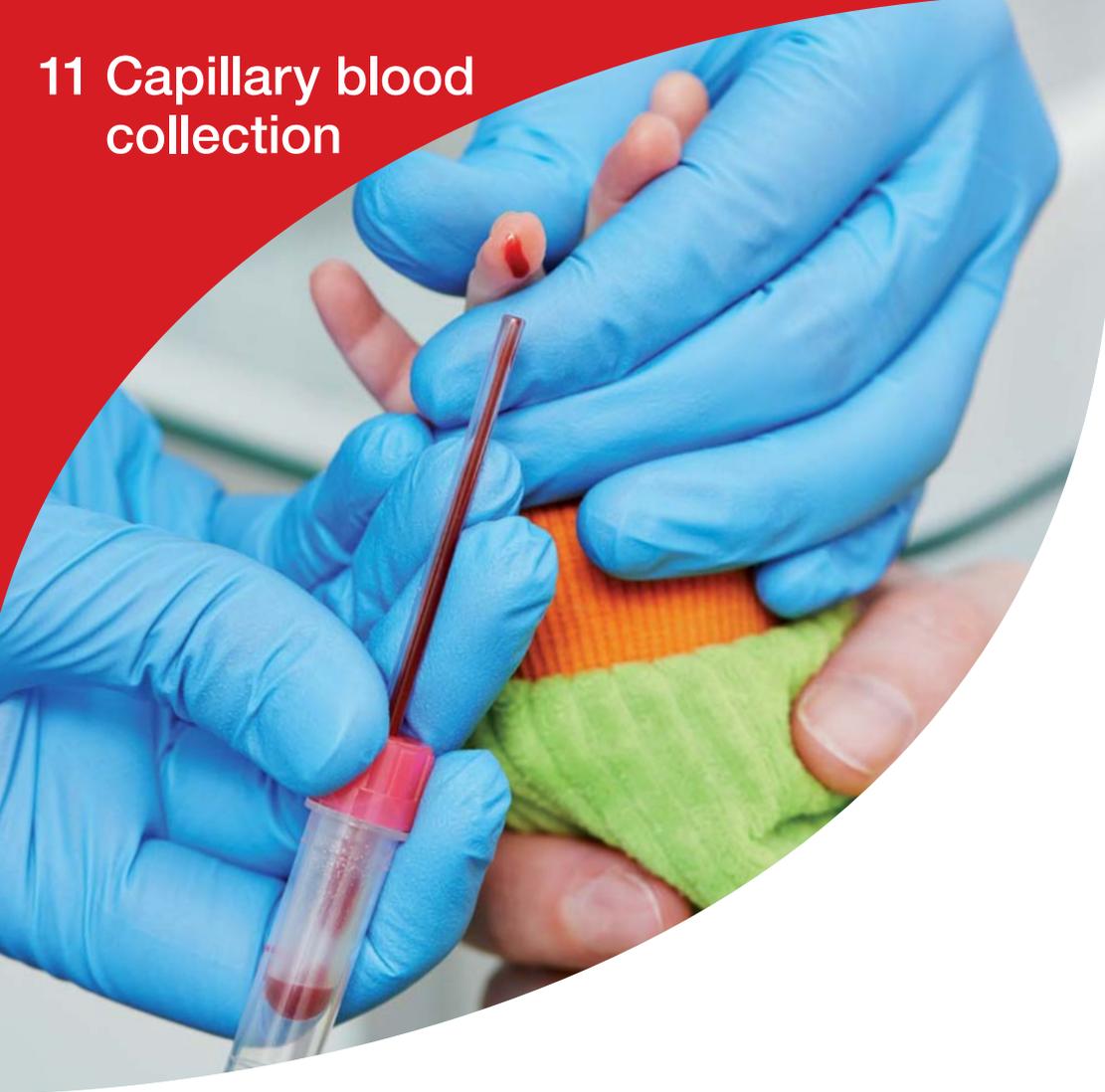
⁴⁸ Sodi et al; Pneumatic tube system induced haemolysis: assessing sample type susceptibility to haemolysis; Ann Clin Biochem; 41:237-40; 2004

⁴⁹ Steige et al; Evaluation of pneumatic-tube system for delivery of blood specimens; Clin Chem; 17:1160-4; 1971

⁵⁰ Koçak et al; The effects of transport by pneumatic tube system on blood cell count, erythrocyte sedimentation and coagulation tests; Biochemia Medica; 23(2):206-10; 2013

⁵¹ Tiwari et al; Speed of sample transportation by a pneumatic tube system can influence the degree of hemolysis; Clin Chem Lab Med; 50(3):471-474; 2012

11 Capillary blood collection



“Particularly in paediatrics and for POCT, obtaining samples from the finger tip, heel or earlobe is of particular importance”

What is capillary blood?

Capillary blood is a mixture of fluids and is comprised of the blood from arterioles, venules and capillaries as well as interstitial and intracellular fluids.

Note: *This mixture of fluids cannot be used for a precise coagulation analysis due to its composition. For this reason, capillary tubes are not provided with a citrate preparation.*

Applications of capillary blood collection

- Paediatrics
- Geriatrics
- In adults for blood gas analyses, glucose and lactate measurements
- Point-of-care testing

Exclusion criteria for capillary blood collection

- Quantities > 1 ml (e.g. blood culture)
- Coagulation analyses
- Inflammations
- Patients in shock

11.1 Carrying out capillary blood collection

- 1 Preparation
 - Materials
 - Patient
 - Puncture site
- 2 Puncture
- 3 Sample collection

Materials required

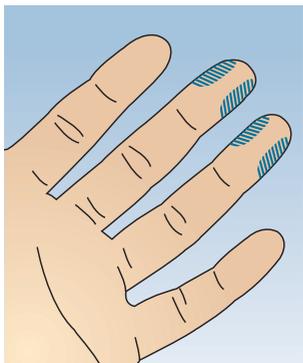
- Gloves
- Swab
- Skin disinfectant
- Semi-automatic single-use lancet (Safety-Lancet)
- Sample tube (BGA capillary tube, Microvettes, bilirubin capillary tubes, etc.)
- Multi-Safe Box for disposal
- Plaster, if required (not advised for small children because they are a choking hazard)

Patient preparation

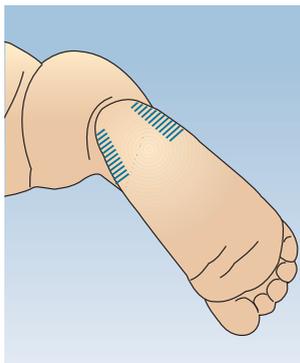
- Identify the patient
- Inform the patient about why the blood is being collected and explain the procedure
- Select the puncture site
- If necessary, encourage blood flow at the puncture site by warming the area

Puncture sites

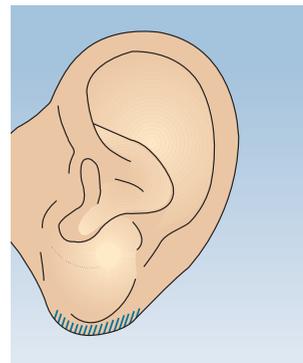
1 Finger tip



2 Heel



3 Earlobe



Advantages of warming the puncture site

- Up to 7x increase in blood flow
- Requirement for capillary blood gas analyses

Encouraging blood flow leads to arterialisation of the capillary blood and thus to acceptable comparability with the analytic values obtained from arterial blood.

Procedure for warming the puncture site

- The foot or hand of the patient is wrapped in a cloth warmed to 39°C to 40°C.
- For the best result, pull a rubber glove over the top.
- Leave for 3-5 min.
- For capillary BGA in adults, the earlobe can be rubbed with a hyperaemia-inducing ointment.

Puncturing and sampling

- Pull on gloves
- Skin disinfection
 - Disinfectant
 - Leave to air dry (until the disinfectant has completely dried)
- Correct hand movement to fix the finger or the foot
- Puncture with a Safety-Lancet

Important information

- Discard the first drop of blood
- Hold the puncture site downwards
- Avoid smearing the blood drop
- Ensure that the sample tube is held in the correct position
- Avoid applying repeated strong pressure (milking)
 - Causes haemolysis and contamination of the samples with tissue fluid

11.1.1 Safety-Lancet and safety incision lancet

The sterile single-use products prevent needle stick injuries because the needle and blade are always safely enclosed within the lancet body before and after use.

The secure trigger button prevents inadvertent, unintentional activation and inactivation of the system.

The Safety-Lancets and the safety incision lancets also comply with the EU Directive 2010/32/EU²⁸, BioStoffV⁵² and TRBA 250⁵³.

²⁸ EU Directive 2010/32/EU of the Council of the European Union from 10 May 2010 Prevention of sharps injuries in the hospital and healthcare sector

⁵² Biological Agents Regulations – BioStoffV; regulations on occupational health and safety at workplaces using biological working materials from 15 July 2013

⁵³ TRBA 250 Biological working materials in the healthcare sector and social welfare organisations; Edition of March 2014 amended on 21.7.2015, GMBI no. 29



Product range – Safety-Lancet

The 5 versions of the Safety-Lancets provide a range of different needle sizes and blades with different penetration depths for sampling from the finger, earlobe and heel.

					
Design	Mini	Normal	Extra	Super	Neonatal
Penetration depth	1.6 mm	1.8 mm	1.8 mm	1.6 mm	1.2 mm
Needle size	28 G	21 G	18 G	Blade 1.5 mm	Blade 1.5 mm
Blood volume	Low	Medium	Medium to high	High	Medium to high

Product range – Safety Incision Lancet

Thanks to the special puncture technique, optimal blood flow with a high blood volume is possible even with a shallow penetration depth. The shallow penetration depth ensures rapid healing and minimises the formation of haematomas.

Design	Application	Penetration depth	Incision length
	Neonates	1.0 mm	2.5 mm
	Premature infants	0.85 mm	1.75 mm

Product range – Safety-Lancet

The handle with its secure, flattened surface enables the lancet to be held in various ways with a defined wing and notch on the serrated lancet body.



1. Twist the protective cap (1/4 turn).



2. Hold the Safety-Lancet against the selected and disinfected puncture site. The small and transparent contact area enables precise puncturing. Press the trigger button.



3. Place the Safety-Lancet in a suitable disposal box.



4. Discard the first blood drop and then collect blood.

11.1.2 Microvette® – order of draw & techniques



Depending on the requirements, Microvette® tubes with a cylindrical or conical inner tube shape and a volume range of 100 to 500 µL are available. There is the option to collect capillary blood using the capillary technique or the collection rim.

The special cap design minimises any aerosol effects when the tube is opened.

Microvette® – order of draw⁵⁴



EDTA



Lithium Heparin /
Lithium Heparin-Gel



Fluoride



Serum /
Serum-Gel

⁵⁴ CLSI Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard – 6th edition GP42-A6 (formerly H04-A6); 28 (25) 2008

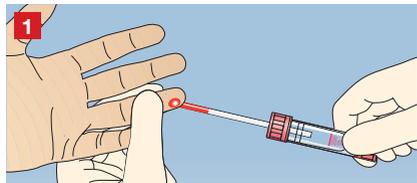
Microvette® – Collection methods

To meet the specific requirements of capillary blood collection, there are two collection techniques available:

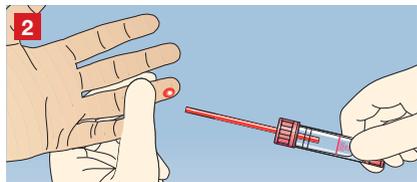
- 1 Capillary method using the end-to-end capillary
- 2 Gravitational principle using the collection rim

Note: *Letting blood drip into a capillary tube by means of a Luer needle does not constitute capillary blood collection.*

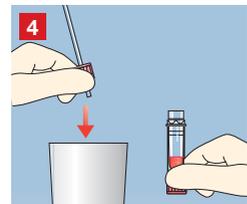
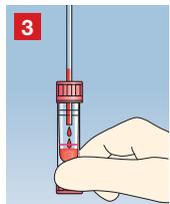
A. Capillary method using the end-to-end capillary



1. Hold the Microvette® in a horizontal or slightly inclined position and collect the blood sample with the end-to-end capillary.

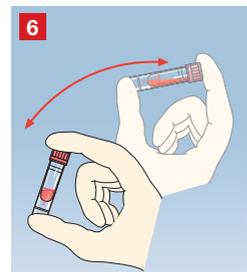
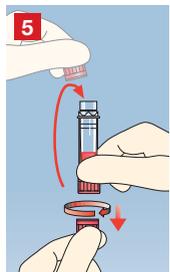


2. Collection is complete when the capillary is entirely filled with blood.



3. Hold the Microvette® upright to allow blood to flow into the collection tube.

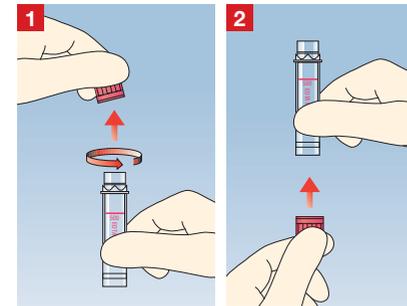
4. Twist to remove the cap including the capillary and discard as a complete unit.



5. Remove the cap from the tube base and close the tube ("click" position).

6. Mix the sample gently but thoroughly.

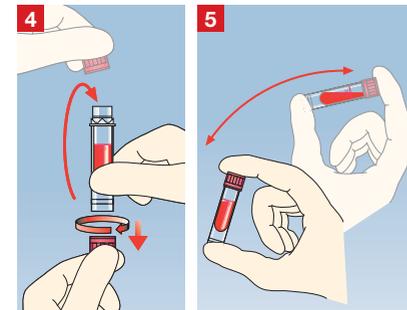
B. Sampling with the collection rim



1. Remove the cap by turning it slightly.
2. Attach the cap to the base of the tube.



3. Collect the blood dripping from the puncture site using the collection rim.



4. Remove the cap from the tube base and close the Microvette® ("click" position).
5. Mix the sample gently but thoroughly.

11.2 Centrifugation conditions for capillary blood collection

Preparation	Min.	Standard recommendation	Min. (alternative)	Alternative range	Temperature
Microvette® Serum Microvette® CB 300 Serum Multivette® Serum	5	10,000 × g	10	2,000-10,000 × g	20°C
Microvette® Serum-Gel* Multivette® Serum-Gel*	5	10,000 × g	10	4,000-10,000 × g	20°C
Microvette® Heparin Microvette® CB 300 Heparin Multivette® Heparin	5	2,000 × g	10	2,000-10,000 × g	20°C
Microvette® Heparin-Gel* Multivette® Heparin-Gel*	5	10,000 × g	10	4,000-10,000 × g	20°C
Microvette® Fluoride Microvette® CB 300 Fluoride Multivette®	5	2,000 × g	10	2,000-10,000 × g	20°C

These centrifugation conditions are recommendations. The values are based on what we consider to be the worst-case conditions, e.g. an older model centrifuge that requires considerably more time to reach the necessary g force than a new high-performance centrifuge. In isolated cases, it may be that the centrifugation conditions deviate from the standard recommendations shown in the table while achieving the same results.

The details of the standard centrifugation conditions are always printed on the label of the inner carton as well.

* For gel-prepared tubes, we recommend using swinging bucket rotors.

11.3 Minivette® POCT

The Minivette® POCT is used for capillary blood collection for immediate bedside diagnostics (also known as POCT).

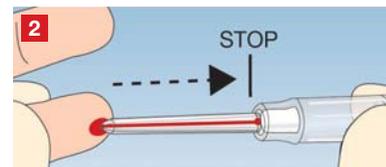
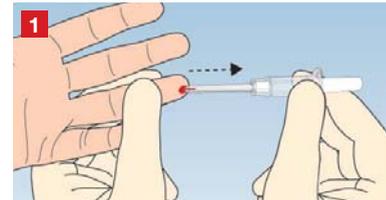
POCT (point-of-care testing) or immediate bedside diagnostics is rapid diagnostics with no preparation of reagents and/or analysis material.

The Minivette® POCT is available in different versions with a range of different volumes and preparations for collecting capillary whole blood, saliva or urine.



Handling the Minivette® POCT

The Minivette® POCT is used for collecting and directly dispensing samples with a small volume. The drip-free handling enables easy sample collection and direct dispensing with no-drip transfer to the test card or sample tubes.



1. The Minivette® POCT is held on the sides below the wings in a horizontal or slightly angled position. When collecting blood drops with the capillary tip, the ventilation hole at the piston end should not be covered. Do not push the piston down - fill the capillary ensuring there are no air bubbles.
2. The blood collection is automatically finished when the capillary is filled with blood to the white blocking filter.
- 3a. Place the capillary tip on the test field and dispense the complete blood sample by gently pressing on the piston.
- 3b. Alternatively, the sample can be dispensed into a micro tube.

12 Urine sample collection



“Hippocrates analysed the odour and colour of urine as long ago as 400 BC and urinalysis still plays a key role in the diagnostic examination”

12.1 Sample collection

Any type of urine sample requires a hygiene procedure that complies with the following rules:

- The patient should have the correct procedure for collecting urine samples explained.
- Before collecting the sample, the patient should thoroughly wash their hands and genital area and then remove any traces of soap.
- To avoid contamination, the sample should be collected from mid-stream urine where possible.
- The urine should be collected in the single-use collection container/bottle⁵⁵ provided.
- The containers must be clean and dry and for bacteriological testing they should also be sterile.
- The containers must be labelled carefully with a waterproof pen to prevent mix-ups.
- Avoid collecting urine during or shortly after menstruation (this results in contamination of the urine with blood).

⁵⁵ CLSI Urinalysis; Approved Guideline – 3rd edition GP16-A3; 29(4) 2009

12.2 Storage and transport

Urine samples should not be exposed to direct sunlight or heat.

The analysis should be carried out within two hours of collection. If this is not possible, the urine should be stored at a temperature of +4°C to +8°C.

Extended storage may cause the following changes, e.g.

- Disintegration of leucocytes and erythrocytes
- Bacterial growth
- Bacterial decomposition of glucose

Before the analysis, samples should be brought to room temperature and thoroughly mixed just before applying to a test strip.

Depending on the parameter, appropriate stabilisers should be used for the storage.

12.3 Types of analyses

Urine can be analysed in a wide range of ways.

Here are some of the most common methods:

Test strips

Depending on the number of test fields, the test strips enable a range of different values to be tested such as specific gravity, haemoglobin, glucose, pH, protein, leucocytes, etc. Information obtained by comparing the colour change of the test field is only an initial indicator and should be specified using additional tests.

What is important is that the test strip is completely and thoroughly wetted and then appropriately dried before reading the result.

The correct incubation times must be complied with and the relevant information about these times can be found in the manufacturer's instructions.



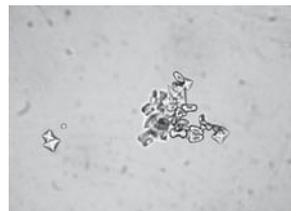
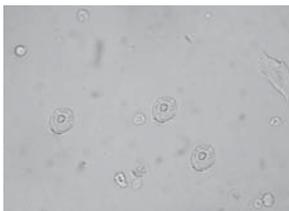
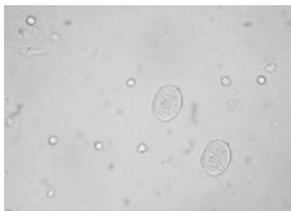
Urine sediment testing

Urine sediment is a preparation of the urine to assess the solid components of the urine using microscopy or flow cytometry. These assessments can provide information about kidney or urinary tract diseases.

To prepare the urine sediment, a defined fraction (e.g. 10 ml) of a urine sample is centrifuged (5 min at 400 × g) and the supernatant is decanted so that about 0.5 ml of urine remains; the sediment is mixed with the residual urine and then analysed microscopically.

The following parameters can be assessed using microscopy, for example:

- Cells such as erythrocytes, leucocytes, epithelial cells, etc.
- Urinary casts such as hyaline tests, granular casts, cellular tests, etc.
- Other elements such as yeast cells, bacteria, urine crystals



Clinical chemistry tests

Clinical chemistry tests provide semi-quantitative and quantitative results for greater specificity for screening tests (e.g. during pregnancy) or when preparing diagnoses for heart, liver or kidney diseases or cancer.

The following parameters can be analysed using clinical chemistry analytics:

Electrolytes, creatinine, albumin, α 2-macroglobulin, α 1-microglobulin, Bence-Jones proteins, glucose, 5-hydroxyindoleacetic acid, immunoglobulins, proteins, catecholamines, porphyrins, vanillylmandelic acid (VMA)

Microbiological tests

With a suspected urinary tract infection after a positive test strip result and abnormal urinary sediment, it is essential to carry out microbial identification (microbial differentiation, bacterial count and subsequent monitoring of the antibiotic therapy).

This provides information about the type and quantity of pathogen (usually bacteria, possibly fungi).

IMPORTANT: *The samples should be collected before starting any antibiotic therapy. For subsequent therapeutic monitoring, provide the laboratory with details of any antibiotic therapy.*



Drug detection

Drug detection is a sensitive examination due to the consequences of a positive test result.

Urine is often used as a sample material because it is easily collected and drugs and their metabolites can be easily detected for long periods after use (compared to blood or saliva). However, urine can also be easily manipulated.

Drug users often try to generate negative results.

This can be caused by excessive drinking, applying third-party urine, addition of acid or mixing in urine-coloured liquids (e.g. apple juice, energy drinks, etc.).

12.4 Types of urine samples

Urine samples are differentiated by the time and type of collection.

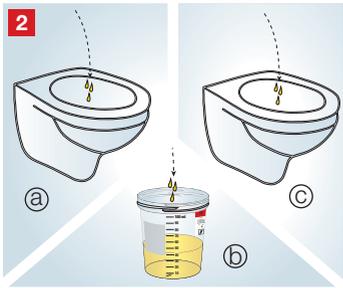
Mid-stream urine

Collecting a urine sample using mid-stream urine is recommended in principle to obtain the purest possible sample

Correct sample collection:



1. Correct cleaning and drying of the hands and external genital area.



2. Release the first urine in the toilet (a) and then collect the mid-stream urine with the urine container (b). The remaining urine is also disposed of in the toilet (c). Avoid contamination.



3. Securely close the container with the lid.

Note:

- Particularly important for microbial tests
- Requirement: Cooperative patient

Mid-stream urine collection is divided into:

First morning urine

The components in the first urine passed in the morning are more concentrated.

- **Application:**
Suitable for bacterial tests, test strips, sediment, clinical chemistry tests, protein diagnostics.
- **Advantage:**
Due to the long retention time in the bladder, the morning urine is ideally suited to nitrite and protein measurement.

Second morning urine

The second morning urine can possibly provide the mean values for individual parameters and can be used in isolated cases as a substitute for 24-h urine collection.

- **Application:**
Test strips, glucose, protein
- **Drawback:**
Not suitable for the nitrite test

Spontaneous urine

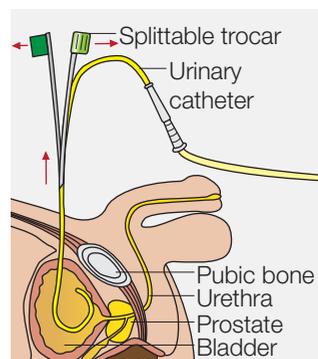
The urine can be collected at any time. Spontaneous collection is useful in cases of suspected urinary tract infection or intoxication.

- **Application:**
Is completely adequate for many chemical and microscopic parameters
- **Advantage:**
Easily collected
- **Drawback:**
Dilution errors – for correct assessment always take the specific gravity (density) into account

Suprapubic aspiration of urine

The bladder is punctured suprapubically while ensuring strict aseptic technique. The invasive nature of this type of urine collection means that although this method has the smallest risk of contaminating samples, it is rarely used.

However, in paediatrics the advantages of this method can outweigh the drawbacks of classic collection (particularly for bacterial tests).



Catheter urine

For sample collection from catheters, single-use catheters and indwelling urinary catheters are differentiated.

Intermittent catheter urine

Collecting urine using intermittent catheterisation is very rarely carried out because it is painful for the patient and the risk of infection is high.

Indwelling catheter urine

For patients with an indwelling urinary catheter, this type of urine sample collection is the easiest and most hygienic method. The urine should be collected from a special adapter on the inlet tube and not from the collection bag, however.

Note: For diagnostic purposes, urine should not be collected from the urine bag.



24 Hour urine collection

The entire urine produced is collected over a period of 24 hours. Collection over this period compensates for any variations in concentration of parameters during the day.

Typical applications for 24 hour urine collection include measuring catecholamines or creatinine clearance. When measuring catecholamines and other unstable parameters, adding a stabiliser (e.g. 20% HCl) to the urine is necessary.

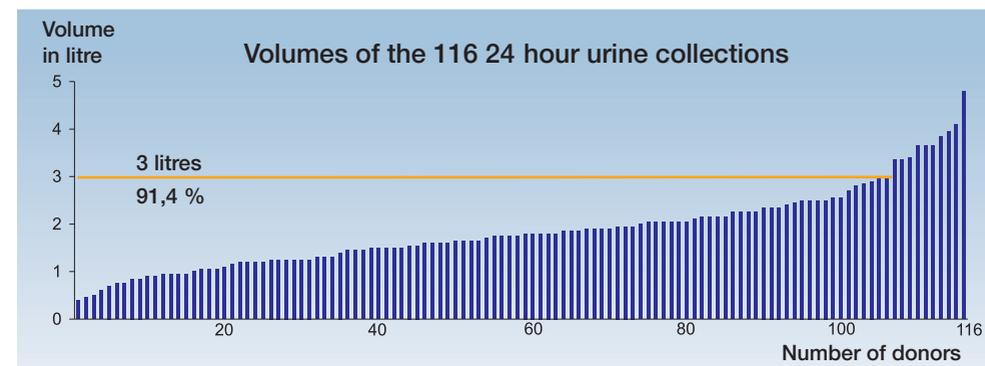
There are ready-to-use products such as the UriSet 24 available for this purpose.



Collected urine volume

Because the patient is usually responsible for collecting the urine, it is essential to provide the patient with clear instructions for correct handling.

The volume of the bottle is of particular importance here. Studies have shown that collection bottles with a volume of 2,000 ml were only adequate for 60% of all volunteers.



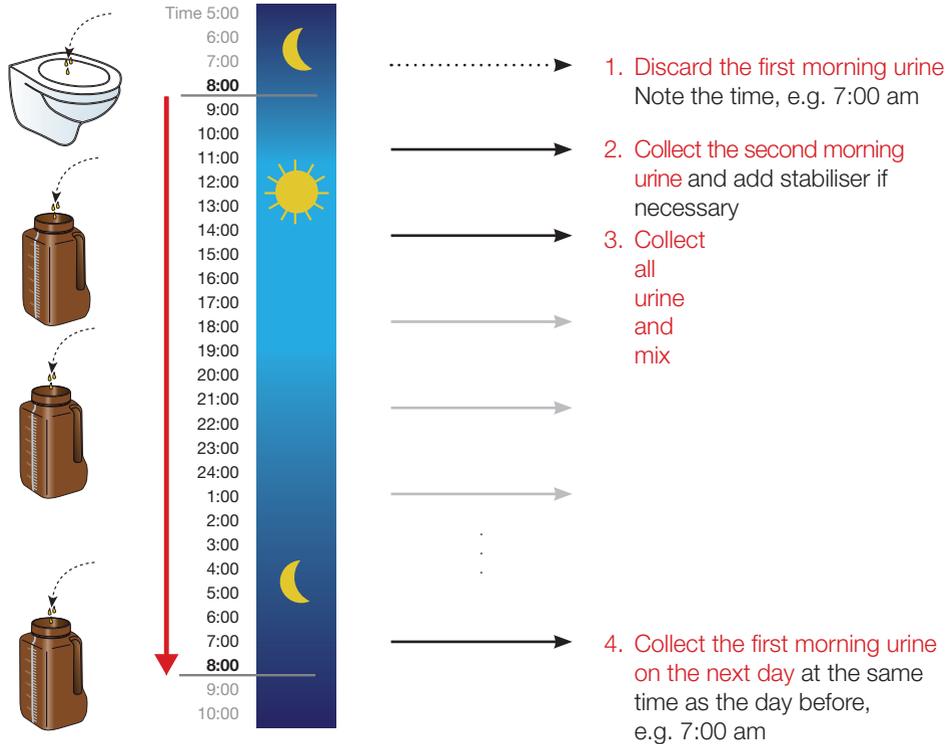
That is, in these cases a second bottle must be used and a tube must be filled from each bottle. The particular quantity of urine in the collection bottle must then be noted on both tubes. The urine from the two tubes is then mixed in the laboratory in the correct ratio.

To circumvent this potentially error-prone process, a collection bottle with a volume of 3,000 ml should be used.

12.5 Handling urine sample collection systems

Collection procedure for 24 hour urine

START



END
(24 hours)

IMPORTANT: During the collection period, about 1.5-2 litres should be drunk throughout the day. Thoroughly wash hands and the genital area before each collection step and rinse away any traces of soap.

Urine-Monovette®

The Urine-Monovette® is suitable for sample collection, transport, as a container for immersing the test strip and for centrifugation.



Insert the suction tip into the cup and draw up the Urine-Monovette® piston to the base line.

Hold the Urine-Monovette® with the suction tip upwards and withdraw the plunger in downward direction until the suction tip is empty.

Withdraw the tip, break off the plunger, attach the cap.

Urine-Monovette® with boric acid



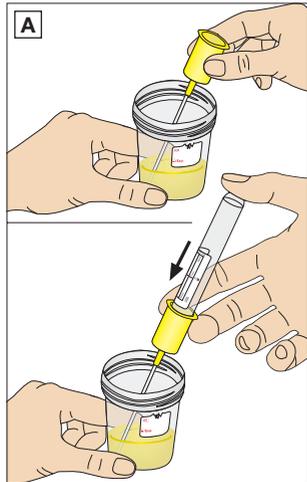
In a filling volume of 10 ml, the boric acid concentration is 1.5%. Microorganisms are stabilised for up to 48 hours when stored at room temperature.

Important:

- Adhere to the stated volume
- Thoroughly mix after urine aspiration
- Not suitable for clinical chemistry tests, strip tests, etc.

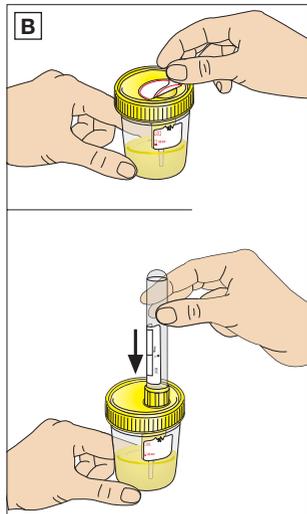
V-Monovette® Urine

By using a closed system, hygiene and comfort is significantly improved both for the patient and for the user.



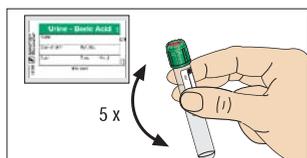
A: Immerse the transfer device into the urine sample.

Insert the V-Monovette® into the transfer device and press firmly until the needle penetrates the cap.

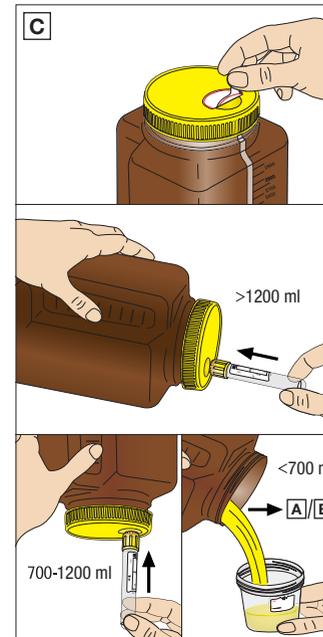


B: Remove the safety label from the lid using the flap. Please do not touch the collection area in the lid. Risk of injury!

First insert the V-Monovette® Urine with the cap in the collection area and press firmly. The tube fills with urine by itself. Only remove the tube when the flow stops.



Mix the V-Monovette® Urine with preparation, e.g. boric acid.



C: Grasp the safety label on the flap and remove from the lid of the collection bottle. Please do not touch the collection area in the lid. Risk of injury!

The collection bottle is placed with the recessed grip facing upwards on a flat surface. Insert the tube into the collection area and press firmly.

For small collection volumes between 700 and 1200 ml, the V-Monovette® Urine can also be filled upside down. For collection volumes < 700 ml, the collection bottle must be opened. The collected urine is then transferred to a container.

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15 Imprint

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© 2015 · SARSTEDT AG & Co.
 Postfach 12 20 · D-51582 Nümbrecht
 Telefon +49 22 93 305 0 · Telefax +49 22 93 305-3450
 info@sarstedt.com · www.sarstedt.com

If you have any questions,
we'll be happy to help!

A thin red circular line is drawn around the contact information text.

SARSTEDT Ltd.
68 Boston Road
Leicester LE4 1AW
Tel.: 0116 235 9023
Fax: 0116 236 6099
info@sarstedt.co.uk
www.sarstedt.com