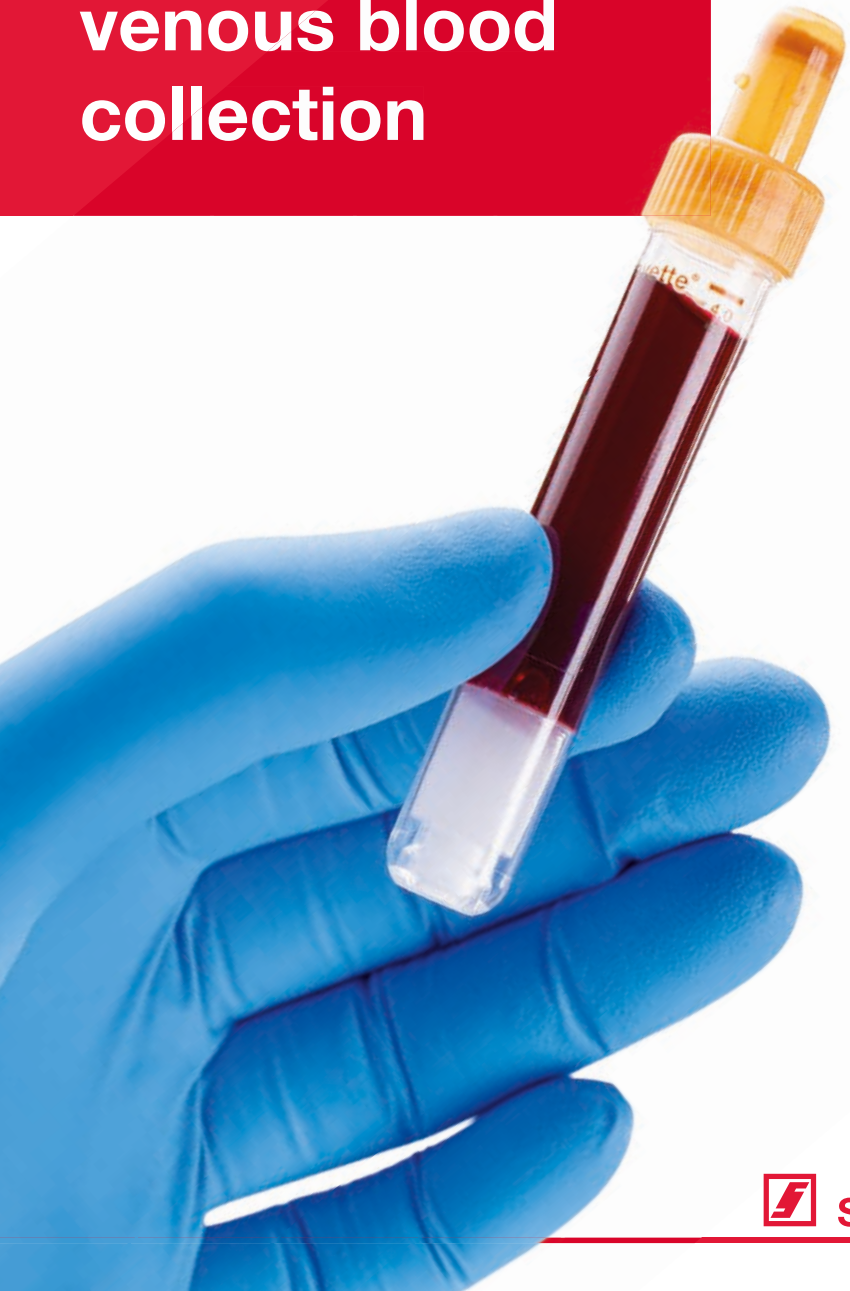
Basics of venous blood collection

Pre Analytical Workflow



SARSTEDT

The “Fundamentals of venous blood collection” is intended for doctors, laboratory physicians, healthcare professionals, phlebotomists, laboratory personnel and medical assistants in hospitals and medical practices.

The reason for this guide is that “According to reliable data, preanalytical errors still account for nearly 60%–70% of all problems occurring in laboratory diagnostics, most of them attributable to mishandling procedures during collection, handling, preparing or storing the specimens. Although most of these would be ‘intercepted’ before inappropriate reactions are taken, in nearly one fifth of the cases they can produce inappropriate investigations and unjustifiable increase in costs, while generating inappropriate clinical decisions and causing some unfortunate circumstances.”*

Awareness should be heightened concerning the large number of influencing factors in preanalytics, with new focus placed on the points of contact with venous blood collection.

Collection of venous blood with the SARSTEDT S-Monovette® blood collection system is explained and, especially for new users after they have been instructed, should facilitate the correct collection of venous blood, in particular using the aspiration technique.

The significance of preanalytics is essential from the perspective of laboratory medicine – starting from the laboratory order, to specimen collection up to the interpreted laboratory result, since it has a critical impact on preservation of sample integrity.

* Lippi et al. Preanalytical quality improvement: from dream to reality CCLM 2011 49(7):1113-26. DOI: 10.1515/CCLM.2011.600

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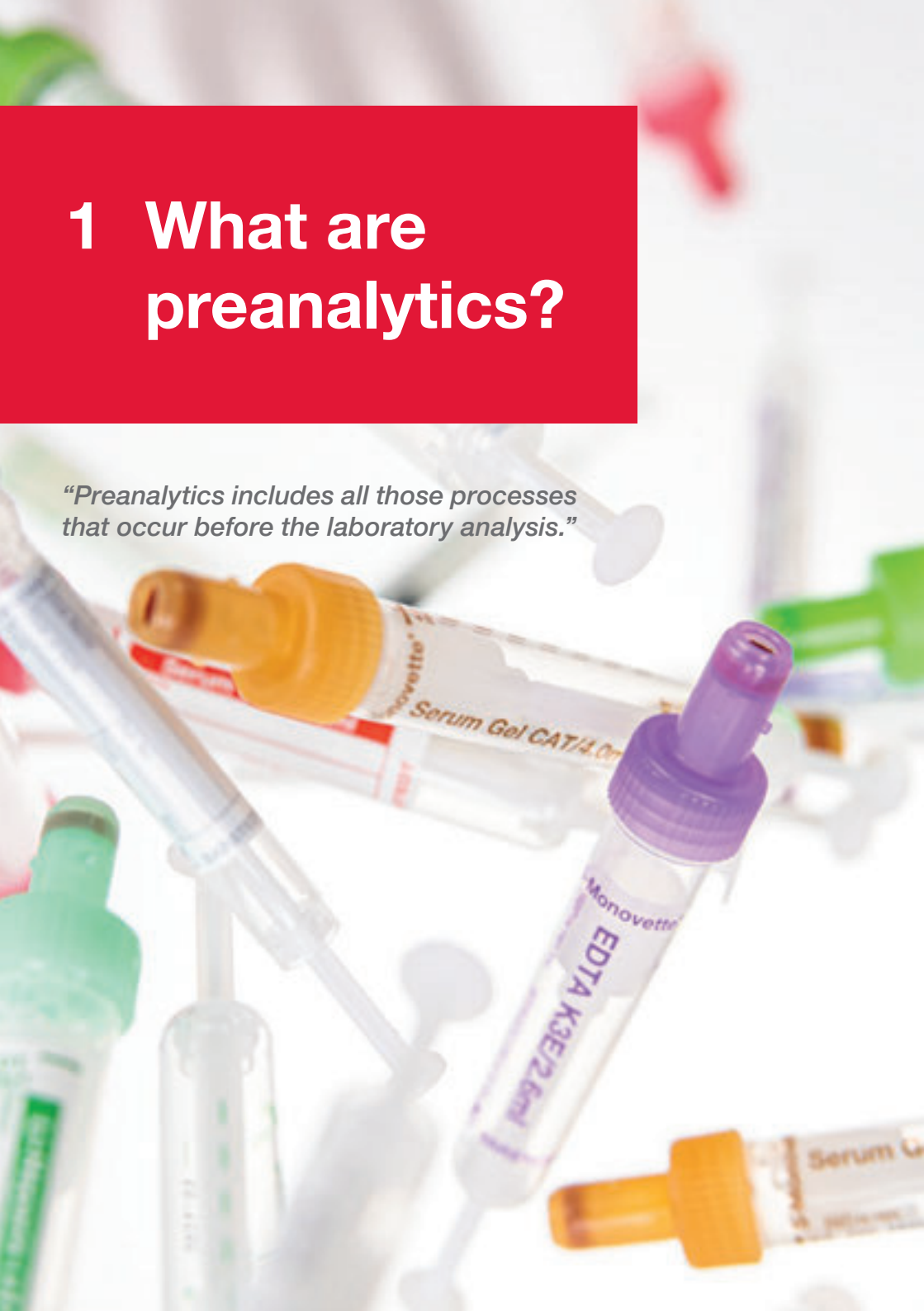
1 What are preanalytics?

“Preanalytics includes all those 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 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

Haemolysis

44 %[2]

Underfilling

17 %[2]

Blood clot

8 %[2]

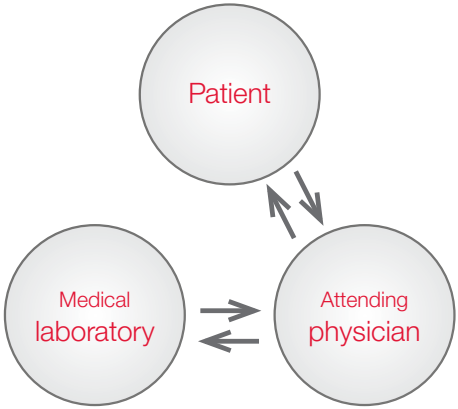
[2] Bonini et al.; Errors in Laboratory Medicine; Clin Chem 2002; 48(5): 691-98

Note: 70–85% of clinical decisions are based on the results of laboratory analys≤es![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; 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 alone but only by close cooperation between the people involved such as doctors, medical assistants and nursing personnel or the laboratory.***

Aim

Standardised conditions for …

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

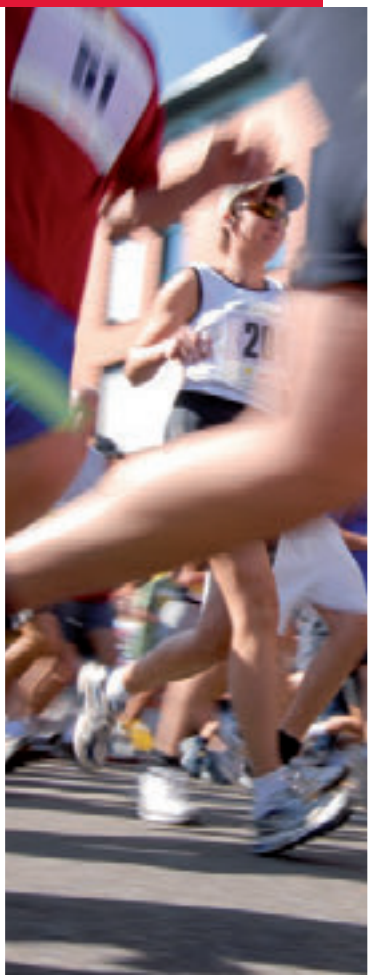
Result

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



2 Influencing factors & interference factors

“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 to take them into consideration.”


2.1 Influencing factors

What responsibility does the patient bear?

- Correct details from the medical history
- Specify medication (e.g. Marcumar, contraceptive – the pill, dietary supplements)
- Diet (e.g. vegan, vegetarian, on a 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 does not depend on the medical condition and must be considered when evaluating the results.

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

2.1.1 Non-modifiable influencing factors

Population

Significant differences in blood values can be found in the African population when compared with the European population.

– 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 an increase in lactose intolerance in the Asia population.

Gender

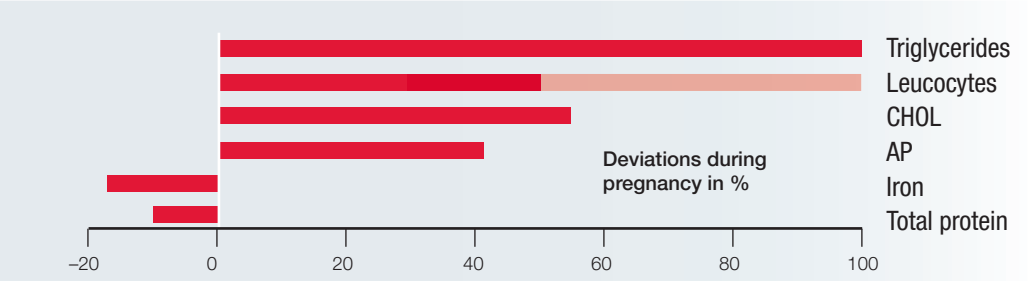
Apart from other gender-specific components (e.g. hormones), muscle mass has an impact on various parameters.

– 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 blood sedimentation rate over the course of a pregnancy.[1]

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



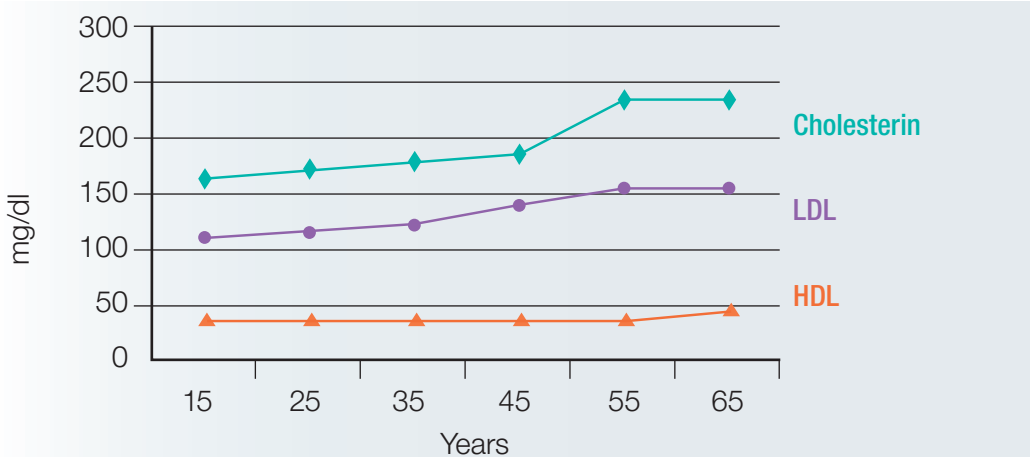
[4] Seelig et al.; Präanalytik; 2008

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] Guder, Narayanan; Pre-Examination Procedures in Laboratory Diagnostics: Preanalytical Aspects and their Impact on the Quality of Medical Laboratory Results 2015 DOI:10.1515/9783110334043

Biological rhythm

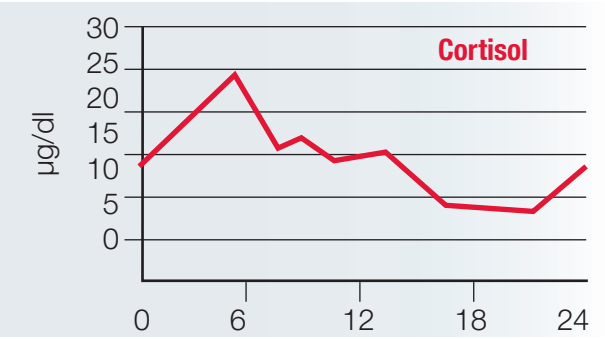
Vitamin D production (25-OH) fluctuates over the course of the year. In summer, higher UV levels mean that more vitamin D is synthesised than in winter.

Circadian rhythm

Also known as rhythmical daily fluctuation, referring to expected differences in concentration within 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. 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 familiar example. The highest cortisol concentration can be measured in the mornings.



Note:

The circadian rhythm (the biological clock) can be shifted by travel 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] Guder, Narayanan; Pre-Examination Procedures in Laboratory Diagnostics: Preanalytical Aspects and their Impact on the Quality of Medical Laboratory Results 2015 DOI:10.1515/9783110334043

2.1.2 Modifiable influencing factors

Drug use

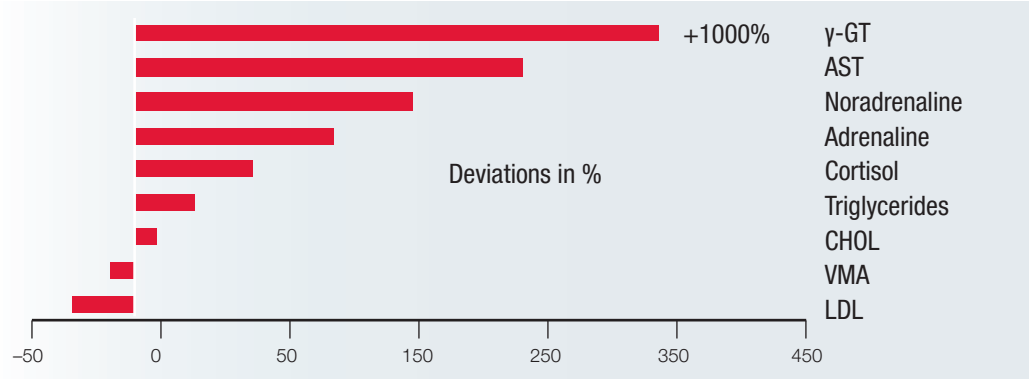
In the case of regular drug use, e.g. cannabis, heroin or morphines, these change clinical chemistry parameters in the blood as follows:

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

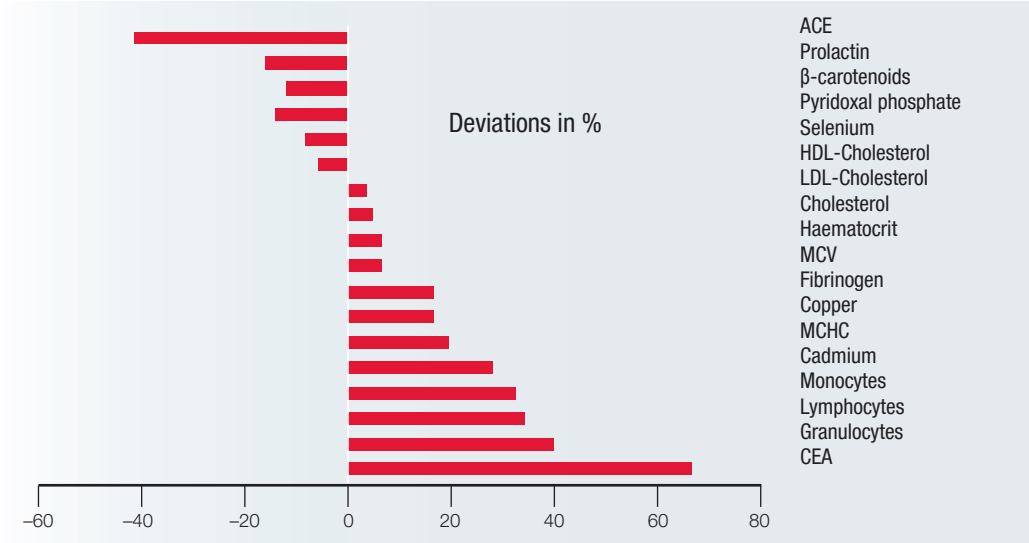
Chronic alcohol abuse causes an increase in the activity of liver enzymes, e.g. γ-GT, AST/ALT, whilst folic acid and vitamin B6 values decrease.



[4] Guder, Narayanan; Pre-Examination Procedures in Laboratory Diagnostics: Preanalytical Aspects and their Impact on the Quality of Medical Laboratory Results 2015 DOI:10.1515/9783110334043, chapter 3.3.3

Substance use: Nicotine

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



[4] Guder, Narayanan; Pre-Examination Procedures in Laboratory Diagnostics: Preanalytical Aspects and their Impact on the Quality of Medical Laboratory Results 2015 DOI:10.1515/9783110334043, chapter 3.3.3

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-inhibiting effect. The activity of AP and y-GT increases, whilst bilirubin concentration in the blood decreases.

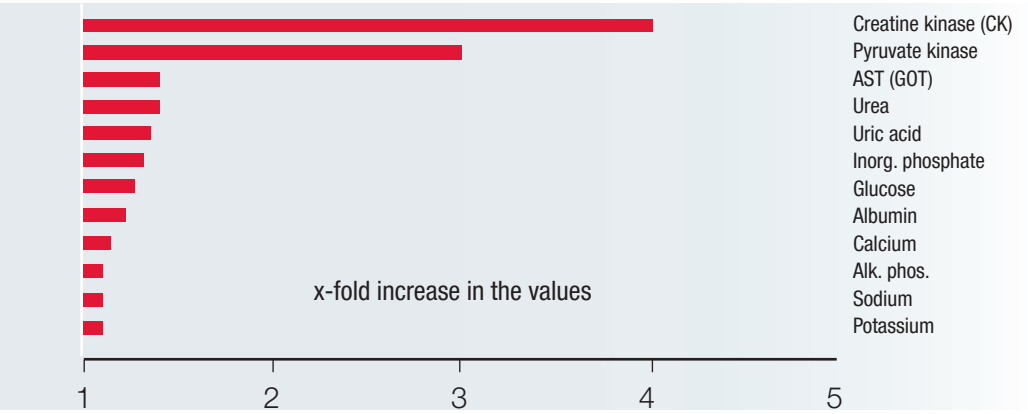
Diuretics also have an effect on the electrolyte balance. This is seen, dependent on the substance class, in the levels of potassium, calcium and magnesium, for example.

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.

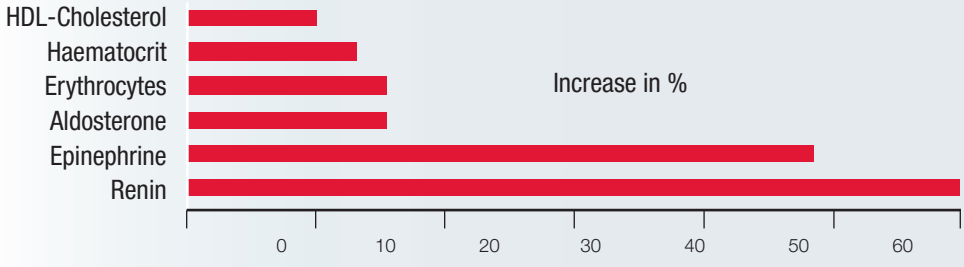


[5] Guder, Narayanan; Pre-Examination Procedures in Laboratory Diagnostics: Preanalytical Aspects and their Impact on the Quality of Medical Laboratory Results 2015 DOI:10.1515/9783110334043

Physical activity in this case refers to exceptional physical stress. For healthy people this could be e.g. a marathon, whereas for bedridden patients just the journey to the practice 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] Guder, Narayanan; Pre-Examination Procedures in Laboratory Diagnostics: Preanalytical Aspects and their Impact on the Quality of Medical Laboratory Results 2015 DOI:10.1515/9783110334043

Diet-related changes

Changes in analyte concentrations with 4-week 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-glutamyl transferase (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 Interference 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.

A B C D

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
– Gilbert's syndrome – Crigler-Najjar syndrome – Acute hepatitis – Acute liver failure	→ Hyperbilirubinaemia = jaundice → Possible disruption, e.g. in cholesterol, creatinine, uric acid
– Spherocytosis – Immune haemolysis – Haemolytic antibodies – Haemoglobinopathy	→ 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)
– Hyperlipoproteinaemia – Lipid metabolism disorder	→ 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
– Haematocrit > 65%	→ Elevation of PTT and aPTT6
– Haematocrit < 20%	→ Reduction in PTT and aPTT

[6] Endler et al.; The importance of preanalytics for the coagulation laboratory; Hämostaseologie 2010; 30(2): 63-70

External interference factors (exogenous)

Cause	Consequence
– Medication (infusion solution, antibiotics, blood products) – Anticoagulants (contamination due to carryover of preparation) – Contamination (bacteria, fungi, bacterial biofilm from CVC for blood culture)	→ Incorrect measurements (elevation and reduction possible)
– 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 and why it is needed helps to alleviate possible anxiety and stress.

An explanation of certain regulations that must be complied with should be added to 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 level of comprehension.

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 and storage*.

3.3 Identification

Patient identification

- Surname
- First name
- Date of birth
- Perhaps: Admission number, ward, room number

Errors occur not only with common names.

Important: Always ask direct questions.

Never: "You are Mr Miller, aren't you?"

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

The person sitting at the specified bed may just be a visitor.

If the identity of the patient is not clear, no samples should be collected or samples should only be collected with reservation.

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, any problems during collection, the patient's condition and other important details may be of use in the case of unclear results.

Identification of the requesting 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.

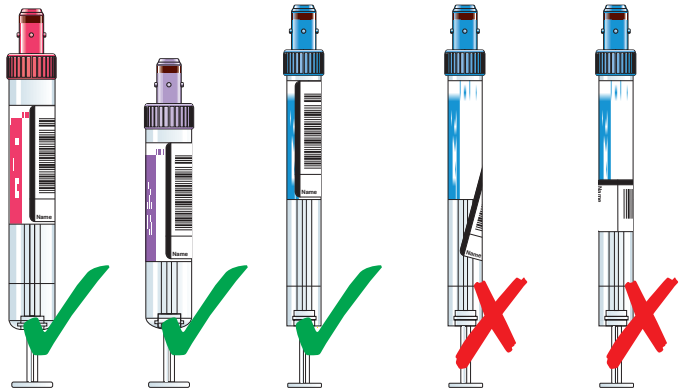
Never use the lid, outer packaging or transport container to identify the sample.

Legal requirements and 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 A5

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

Description	Based on BS 4851 (EU code)	ISO 6710:2017	Application
S-Monovette® serum			Clinical chemistry, serology, special analyses
S-Monovette® serum gel			Clinical chemistry, serology (only routine diagnostics)
S-Monovette® citrate (1:10)			Coagulation analyses (e.g. Quick, PTT, TT, fibrinogen)
S-Sedivette® BSR (1:5)			BSR determination according to Westergren or S-Sedivette®
S-Monovette® lithium heparin			Plasma collection for clinical chemistry, serology
S-Monovette® lithium heparin gel			Plasma collection for clinical chemistry, serology
S-Monovette® EDTA KE			Haematology (e.g. Hb, Ht, erythrocytes, leukocytes)
S-Monovette® glucose FE/FH (Fluoride/EDTA)			Glucose determination and enzymatic lactate
S-Monovette® GlucoEXACT (fluoride/citrate)			Glucose determination (48 h stability, at RT)
S-Monovette® metal analysis			Metal analysis

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] Sulaiman, Effect of order of draw samples during phlebotomy on routine biochemistry results; J Clin Pathol. 2011; 64(11): 1019-20
[9] Calam et al.; Recommended "Order of Draw" for Collecting Blood Specimens into Additive-Containing Tubes; Clin. Chem.; 1982; 28(6): 1399

Recommended order of draw

According to Gurr[10]:

Based on BS 4851 (EU Code)	ISO 6710:2017	
		Blood culture
		Serum/serum-gel blood
		Citrate blood
		Heparin/heparin-gel blood
		EDTA blood
		Fluoride/citrate-fluoride blood

According to CLSI[11]:

Based on BS 4851 (EU Code)	ISO 6710:2017	
		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 2007, 6th edition GP 41-A6 (former H3-A6), 27 (26)

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.

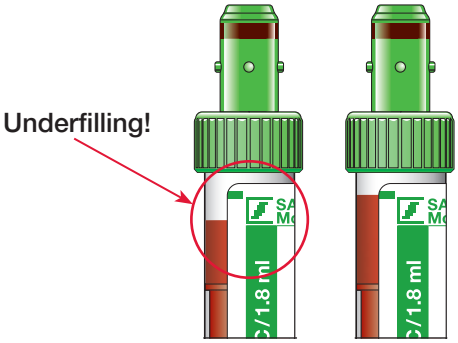
Underfilling here results in an excess of citrate in the tube (ratio of blood to preparation). Because citrate binds calcium, more calcium will thus be bound than is expected. This has a direct effect on the analysis results.

If, when collecting blood with a Safety-Multifly®-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***

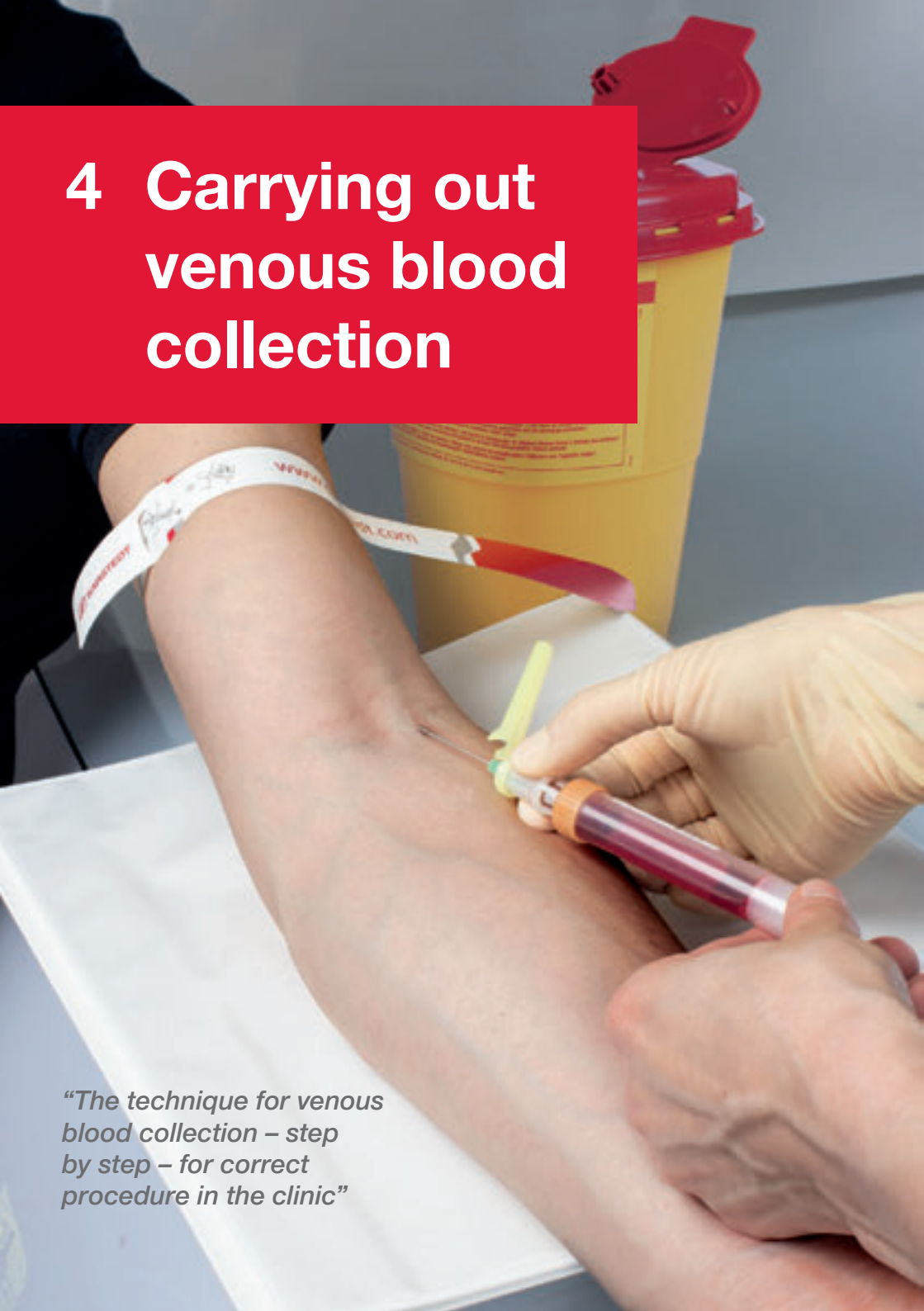
Dead volume = volume in the tubing:

30 cm tubing:	approx. 450 µl
20 cm tubing:	approx. 300 µl
8 cm tubing:	approx. 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”

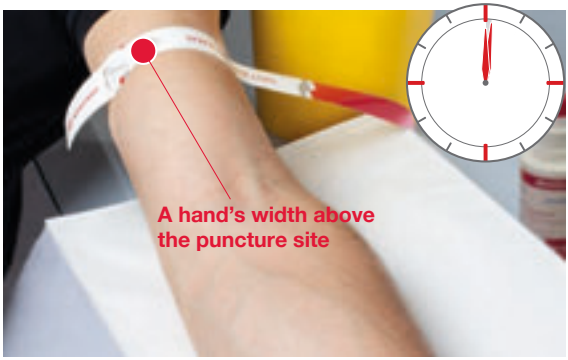
4.1 Standard conditions for blood collection

- No unusual, extreme physical activities in the 3 days prior to blood collection
- No alcohol excess on the day before (abstention 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 rise in the level of potassium (up to 2 mmol/l) in serum/plasma
- Apply a tourniquet for a maximum of 1 minute (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!

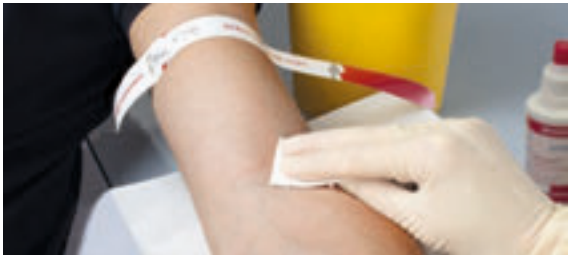
4.3 Tourniquet application and puncture sites



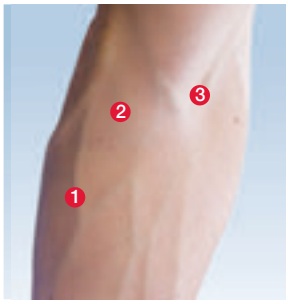
Apply the tourniquet one hand's width above the puncture site

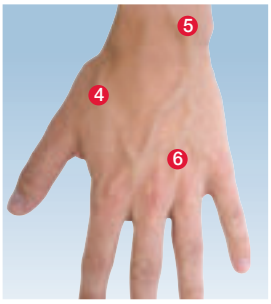
The pulse must be perceptible (tourniquet pressure: 50–100 mmHg)

Maximum constriction time: 1 minute.



Disinfect in accordance with a valid hygiene plan





Puncture sites

❶ Vena basilica

❷ Vena mediana cubiti (this refers to the not blue translucent, thick, deep vein that is only visible as a bulge at this point)

❸ Vena cephalica, runs on the thumb side

❹ Vena cephalica

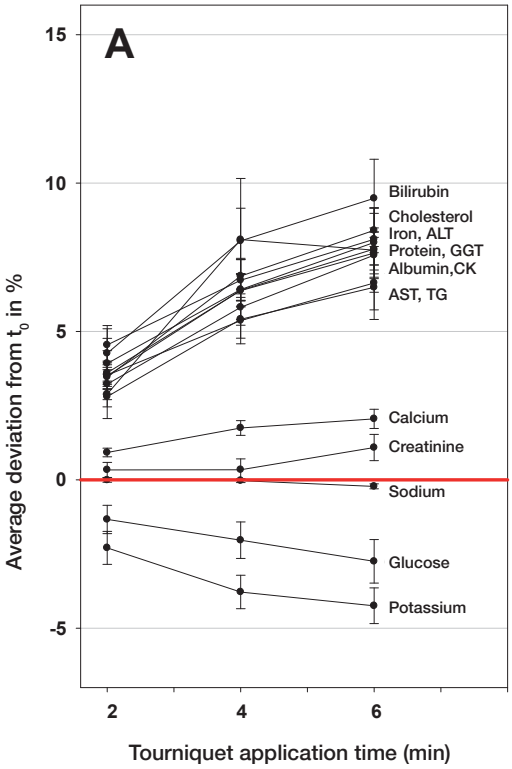
❺ Vena basilica

❻ Rete venosum dorsale manus

Tourniquet application time

Applying a tourniquet for 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 minute tourniquet application versus 6 minute tourniquet application



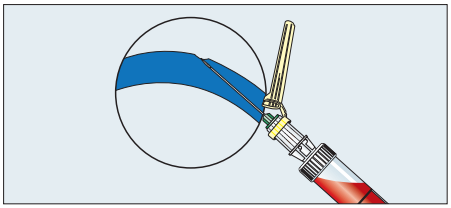
[12] Lichtinghagen et al.: Einfluss der Stauzeit auf normalisierte Laborwerte; J Lab Med 2013; 37(3): 131-37

4.4 Problems before/during blood collection

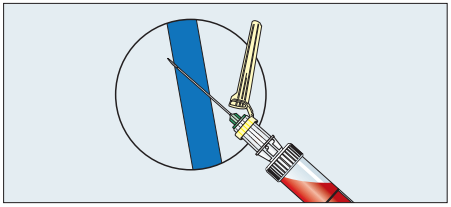
Difficult vein conditions

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

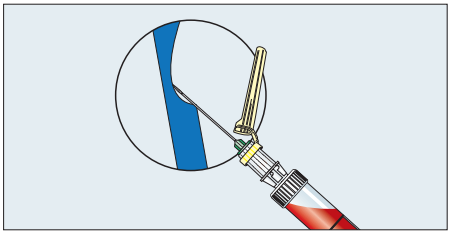
The blood flow stops during the collection



Needle opening is on 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 flow is restored.

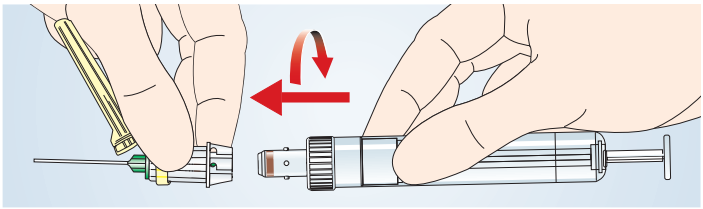


Vein has collapsed
Solution:
Wait until the vein has recovered, then carefully aspirate.

- Pumping the fist leads to an increase in K^+ and Mg^{2+} due to muscle activity
- Extended tourniquet application 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 as a standard. Changing the lumen by bending the needle can damage cells (haemolysis).
- Haemolysis can also be caused by using a needle that is too narrow.

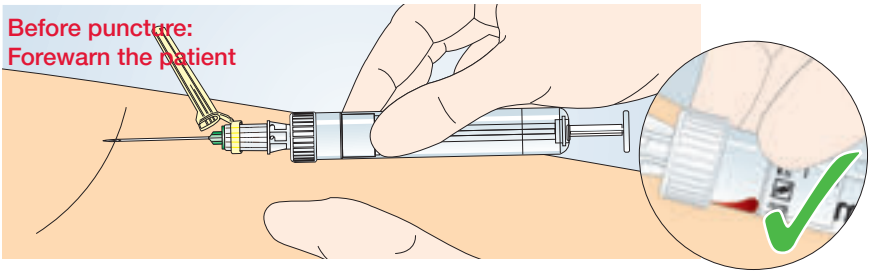
4.5 Aspiration and vacuum technique

4.5.1 S-Monovette® aspiration technique

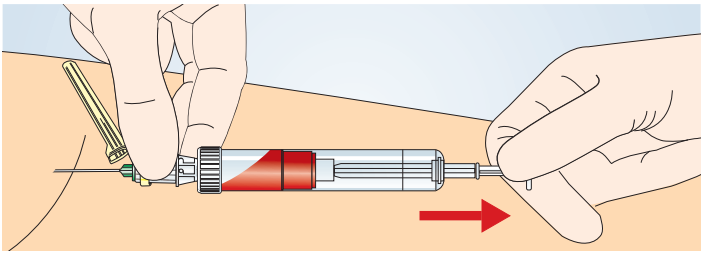


IMPORTANT:

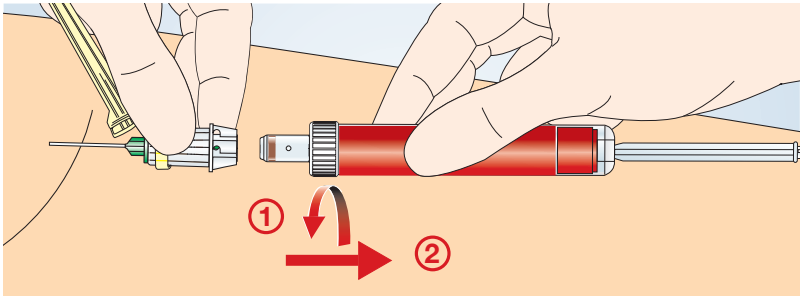
- Just before puncturing the skin, lock the Safety-Needle to the S-Monovette® by twisting it clockwise slightly.



- 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.

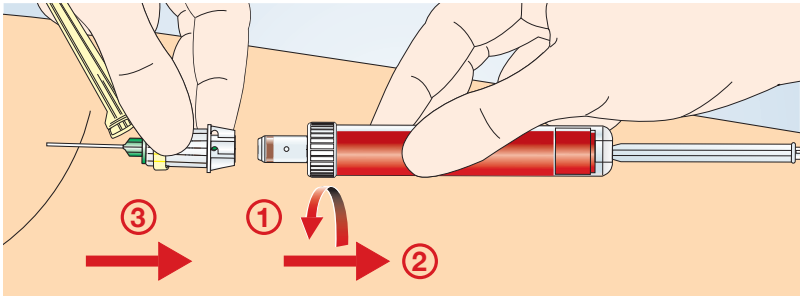


- Loosen the tourniquet and slowly withdraw the plunger. Wait until the blood flow stops.

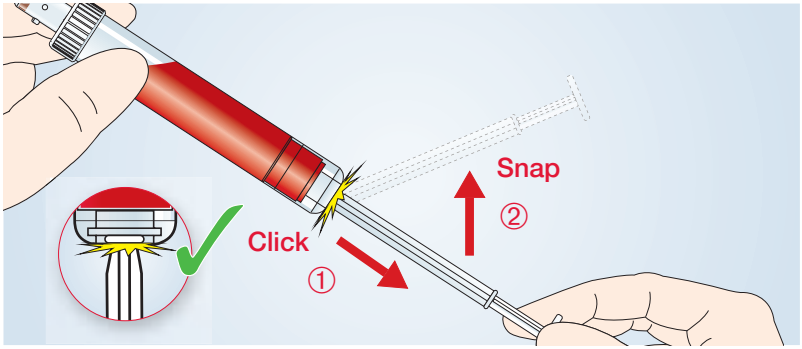


- Change the S-Monovette® for multiple collections. Remove the S-Monovette® from the Safety-Needle by turning it slightly anticlockwise.
  The Safety-Needle remains in the vein.

After blood collection



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

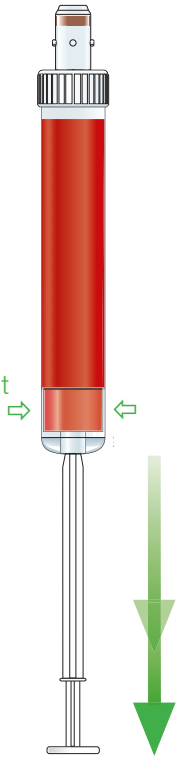


IMPORTANT:

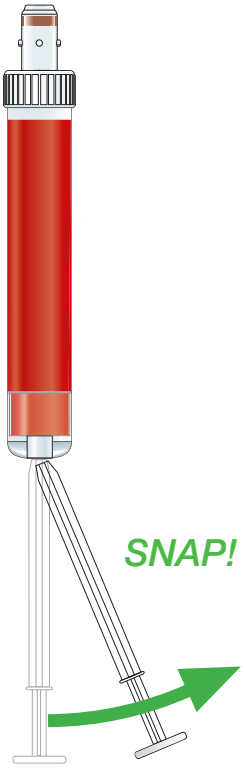
For all S-Monovettes, when blood collection is complete, withdraw the plunger into the 'snap' position and break off.

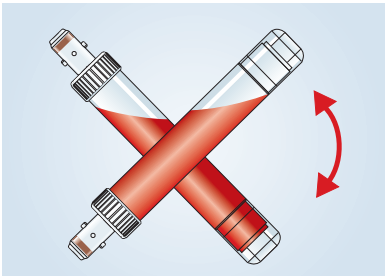
Pull the plunger straight back until the piston locks in with an audible ***CLICK***.

Pull the piston right out to the end



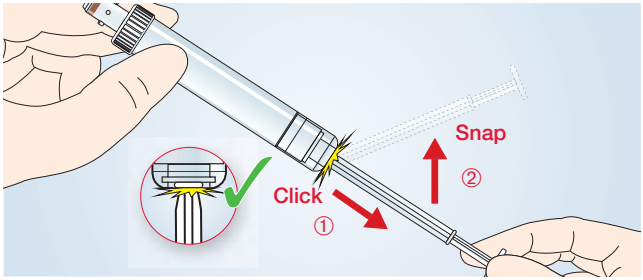
Only then should you break the plunger off. ***SNAP!***



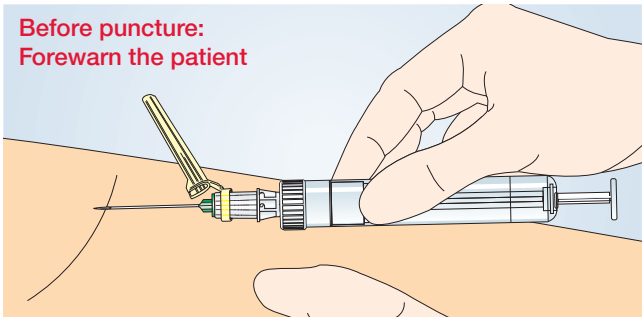


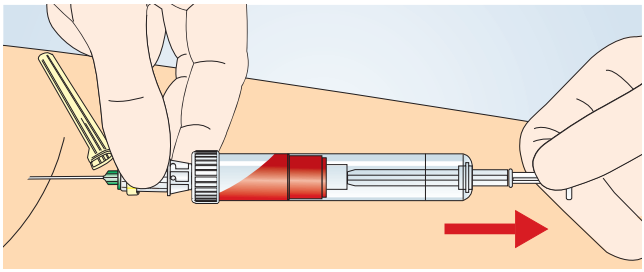
- After ending the **entire** blood collection, thoroughly invert all S-Monovettes.

4.5.2 S-Monovette® vacuum technique

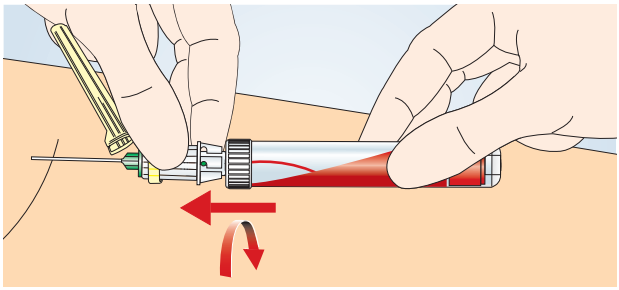


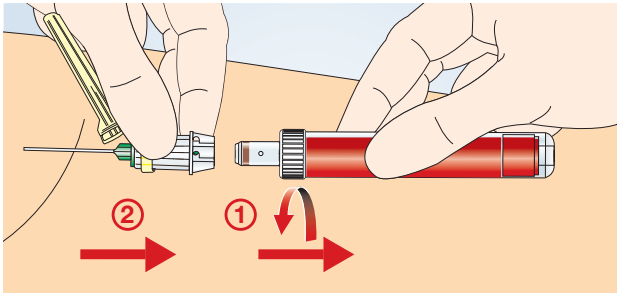
- Prepare S-Monovettes – produce a fresh vacuum
  In order to do this, pull the plunger back and lock the piston into the base of the S-Monovette (‘click’). Then break off the plunger (‘snap’).
- As a rule, we recommend filling the first S-Monovette® with the aspiration technique so that the blood collection starts gently.



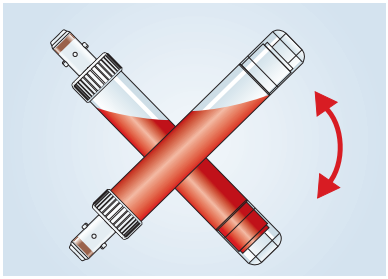


- Now the S-Monovette® can be removed in the vacuum technique.
  When doing so, push the available S-Monovette® onto the Safety-Needle and secure by twisting clockwise.



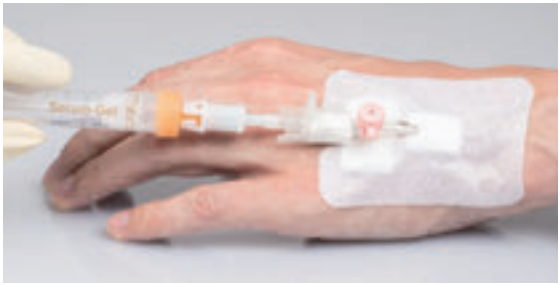


- Wait until the blood flow stops, remove the S-Monovette® from the Safety-Needle and then remove the Safety-Needle from the vein.
- After ending the **entire** blood collection, thoroughly invert all S-Monovettes.



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 or 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 infusions[1]

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. Seepage of traces of medication can lead to erroneously high results.

[1] Guder et al.; Proben zwischen Patient und Labor; 2009
[6] Endler et al.; The importance of preanalytics for the coagulation laboratory; Hämostaseologie 2010; 30(2): 63-70
[13] Spannagl et al.; Hämostaseologische Globaltests; Hämostaseologi 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 et al.; Obtaining blood samples from peripheral intravenous catheters: best practice; AJCC, 2009; 18(5)
[15] Lippi et al.; Prevention of hemolysis in blood samples collected from intravenous catheters; Clin Biochem 2013; 46(7-8): 561-4
[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 2012; 45(13-14): 1012-32
[17] Grant; The Effect of Blood Drawing Techniques and Equipment on the Hemolysis of ED Laboratory Blood Samples; J Emerg Nurs 2003; 29(2):116-21
[18] Benso; Can a blood sample for diagnostic exams be drawn from a peripheral venous catheter?; Assist Inferm Ric; 2015; 34(2): 86-92

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.

- Multi Adapter – To connect 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 (fatality rate) 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 laboratory[20]:

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

[19] Pschyrembel 2004
[20] Borde et al.; Abnahme von Blutkulturen; Dtsch Med Wochenschr; 2010; 135: 355-58
[21] Simon et al.; Blutkulturdiagnostik – Standards und aktuelle Entwicklungen; J Lab Med; 2012; 36(4):199–207

4.7.1 Hygiene requirements

False-positive blood cultures resulting from improper hygiene measures 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:

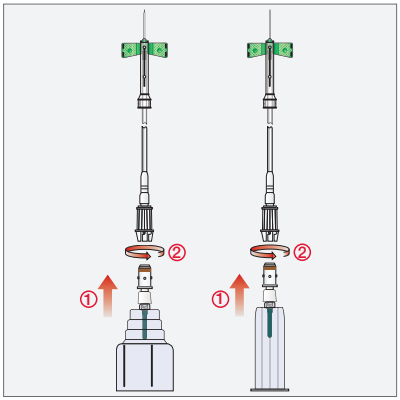
1. Hygienic hand disinfection
2. Wear gloves
3. Disinfection of the puncture site (e.g. with 70% isopropyl alcohol or skin disinfectant)
   a. Apply disinfectant
   b. Apply disinfectant again and let it dry

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

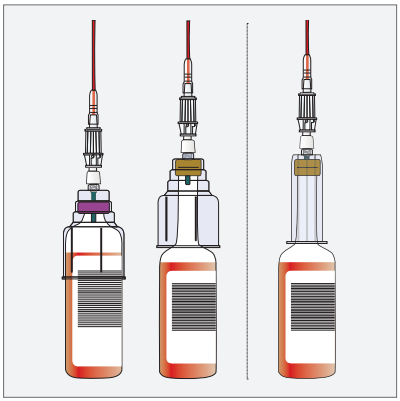
4. Disinfect the blood culture flasks
   a. Remove the cap
   b. Disinfect the rubber septum

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

4.7.2 Handling during blood collection



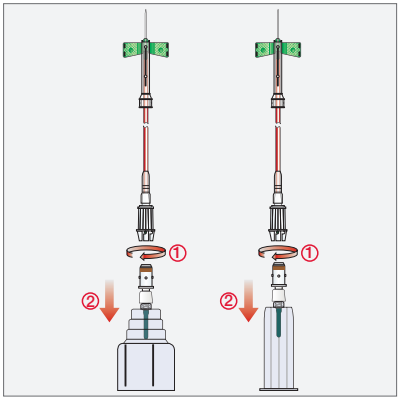
1. Carry out the hygiene steps listed above. Connect the blood culture adapter to the guide sleeve of the Safety-Multifly®-Needle. Puncture the vein and fix the needle in place.



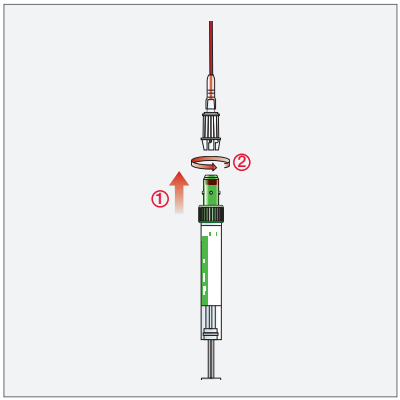
2. Insert the blood culture flask in an upright/ vertical position into the holder. The culture medium of the flask must not come into contact with the lid of the blood culture flask.

   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 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 & 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.

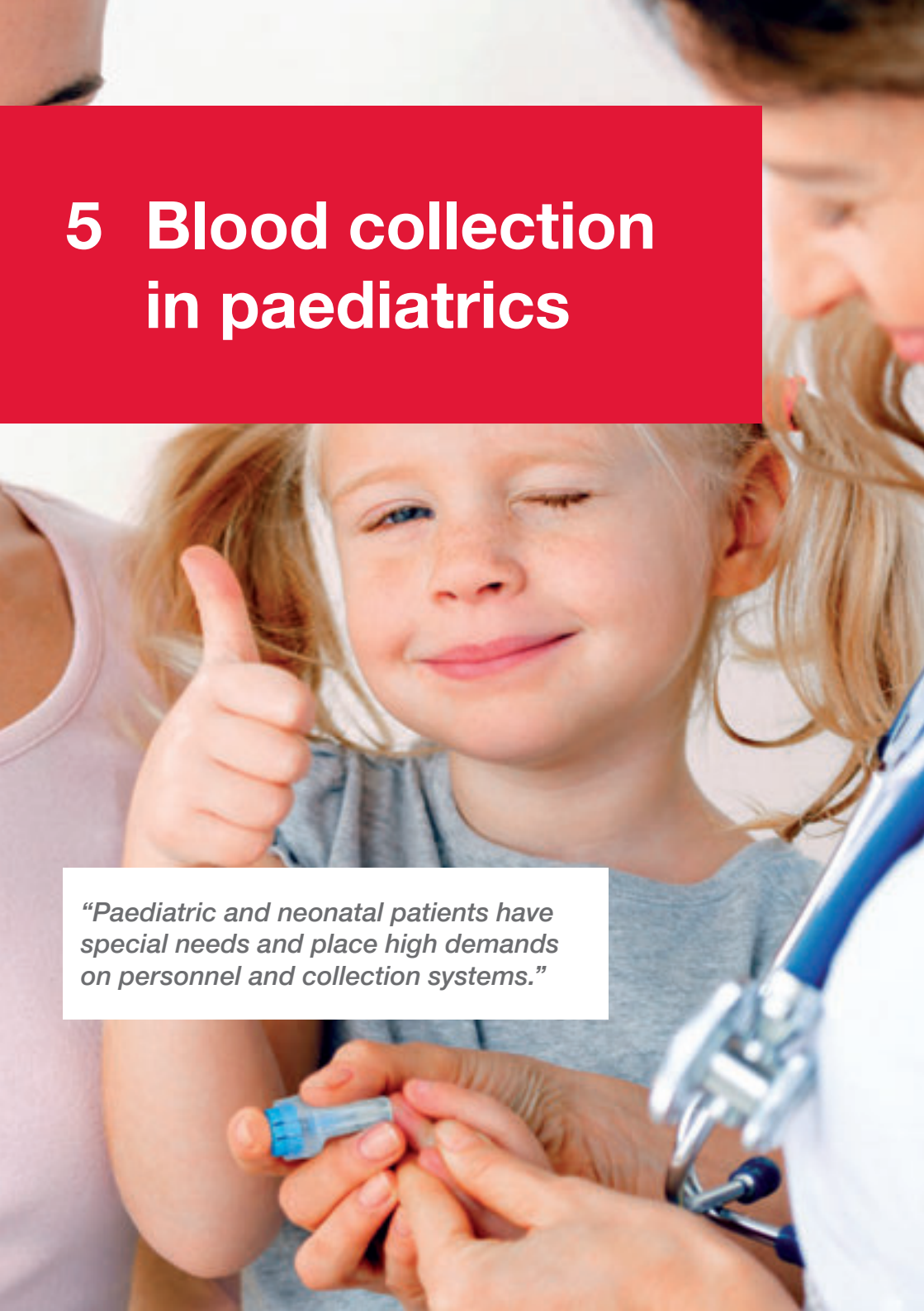
Marking the filling level on the flask before the collection simplifies checking the blood filling volume during the collection.

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.

The viability of premature infants begins in the 23rd week of pregnancy, when neonates 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 history[22]

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's condition may deteriorate during the recording of the medical history, the clinical examination or even after hospitalisation.

[22] 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:

- No long 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 babies, neonates and infants can be problematic. Choosing the correct collection technique combined with suitable sample tubes eases these difficult conditions as much as is possible.

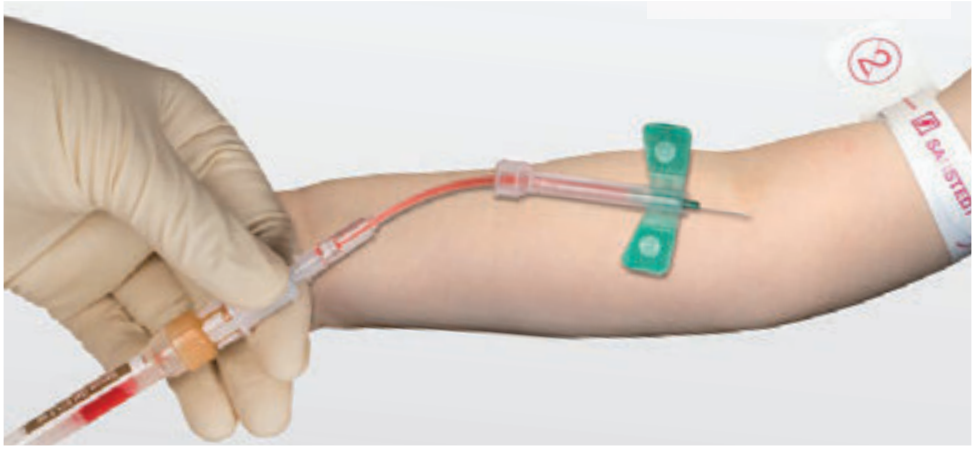
5.3.1 Venous blood collection

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	Possible	Possible	Possible	Recommended	Recommended
Back of hand	Recommended	Recommended	Possible	Recommended	Recommended
Top of foot	Recommended	Recommended	Possible	Possible (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 paediatric 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 sample 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 sample 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.

5.4 The difference between capillary blood and venous blood

Taking into account the sample material is important for assessing the analytical results. There are differences between capillary blood and venous blood in terms of the concentration of various parameters. For example, the serum concentrations of total protein, bilirubin, calcium, sodium and chloride are significantly lower in capillary blood than in venous blood.[23]

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

5.5 Reference ranges

Depending on the age of the child, concentrations of analytes are normal in different ranges compared to adults. For this reason, it is important to always assess the analytical results relative to the age-appropriate reference/standard ranges[24].

Some parameters are shown in the following table as examples.

[24] 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.
	Neonates			
	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 may have higher values on day 1. Elevated in mitochondriopathy, tissue hypoxia, etc.
	Child/ Adult	0.5–2.2	4.5–20	

Analyte	Sample source	SI	Conventional	Remarks
Creatinine	Neonates	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 < 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	
Erythrocytes		Tpt/L (10^{12}/l)	10^6/µl	Rapid breakdown after birth. Elevated (polycythaemia) with dehydration and with/after sustained high levels.
	Neonates week 1	3.9–6.5	3.9–6.5	
	Neonates 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)		Fraction l/l	%	Hct elevated with dehydration, lowered with hyperhydration
	Neonates	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	

Analyte	Sample source	SI	Conventional	Remarks
Haemoglobin (Hb)		mmol/l	g/dl	
	Neonates week 1	9.3–13.7	15–22	
	Neonates 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	
Platelets		Gpt/l(10^9/l)	10^3 Cells/µl	Thrombocytopenia e.g. due to measles 30 Gpt/L: increased bleeding tendency.
	Neonates	100–250	100–250	
	Toddler	220–500	220–500	
	Children	150–350	150–350	
	Adult	150–400	150–400	
Leucocytes		Gpt/l	Cells/µl	Changes in the leucocyte count during the first weeks of life/year. Increases (leucocytosis) are usually caused by elevated numbers of neutrophil granulocytes.
	Neonates day 1	9–35	9,000–35,000	
	Neonates week 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	

[22] 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 metabolism is also a possibility, particularly during 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 [µg/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
[25] Barthels et al.; Das Gerinnungskompendium; 2012

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

Haematocrit correction is not necessary here because the age-appropriate 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 Safety around 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 significant infectious agents that can be transmitted by needle stick injuries (NSI) are hepatitis B virus, hepatitis C virus and HIV.

However, by using suitable protective measures these incidents can be almost completely avoided.[26]

The EU Directive 2010/32/EU[27] Prevention of sharps injuries in the hospital and healthcare sector requires the safest possible working environment for employees in the healthcare sector.

[26] The underestimated workplace accident, infection risk due to needle stick injuries; SAFETY FIRST! initiative
[27] EU Directive 2010/32/EU of the Council of the European Union from 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)

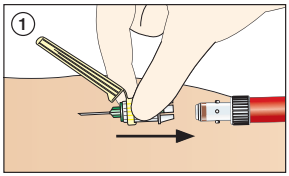
Note: ***Over half of all needle stick injuries occur during disposal.***[28]

[28] SAFETY FIRST, Germany – www.nadelstichverletzung.de

6.1 Safety-Needle

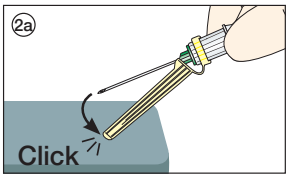
The Safety-Needle is **always ready for use** as the holder (adapter) is already integrated. This reduces the potential risk of a needle stick injury at the back of the needle.

Handling

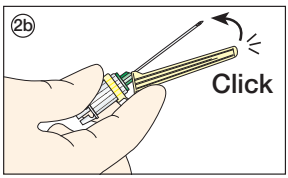


After blood collection:

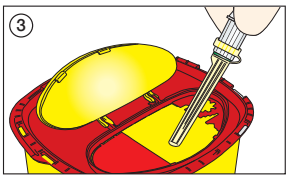
Detach the last S-Monovette® from the Safety-Needle and then withdraw the Safety-Needle from the vein.



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.



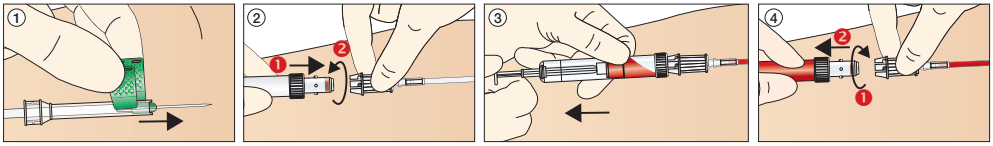
After activating the protective mechanism:

Discard the safely locked Safety-Needle in a sharps container.

6.2 Safety-Multifly®-Needle

The Safety-Multifly®-Needle with integrated holder (adapter) is **ready for use**. Due to the single-handed operation of the needle protection of the Safety-Multifly®-Needle, maximum protection is guaranteed.

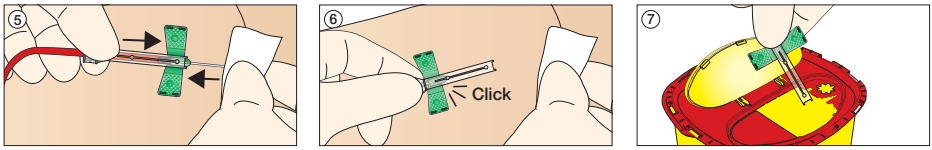
6.2.1 Handling during blood collection



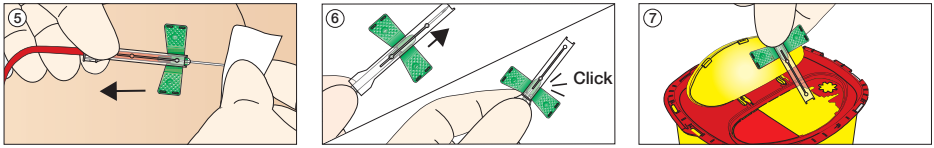
Activation of the needle protection...

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

1)...in the vein:
Activate the needle protection in parallel to removing the Safety-Multifly®-Needle from the vein.



2)...outside the vein:
Pull the Safety-Multifly®-Needle out of the vein and activate the needle protection.



6.2.2 Use of short-term infusion

The Safety-Multifly®-Needle without integrated holder (adapter) can be used directly for the short-term infusion as well as for the connection to Luer adapters.

6.3 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, 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

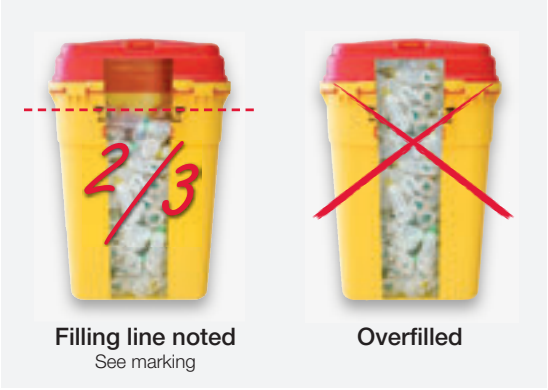
Recommendation:

Only fill the Multi-Safe container to about 2/3 of its volume.

Do not overfill the Multi-Safe:

Risk of injury!

Note the filling line



Filling line noted
See marking

Overfilled

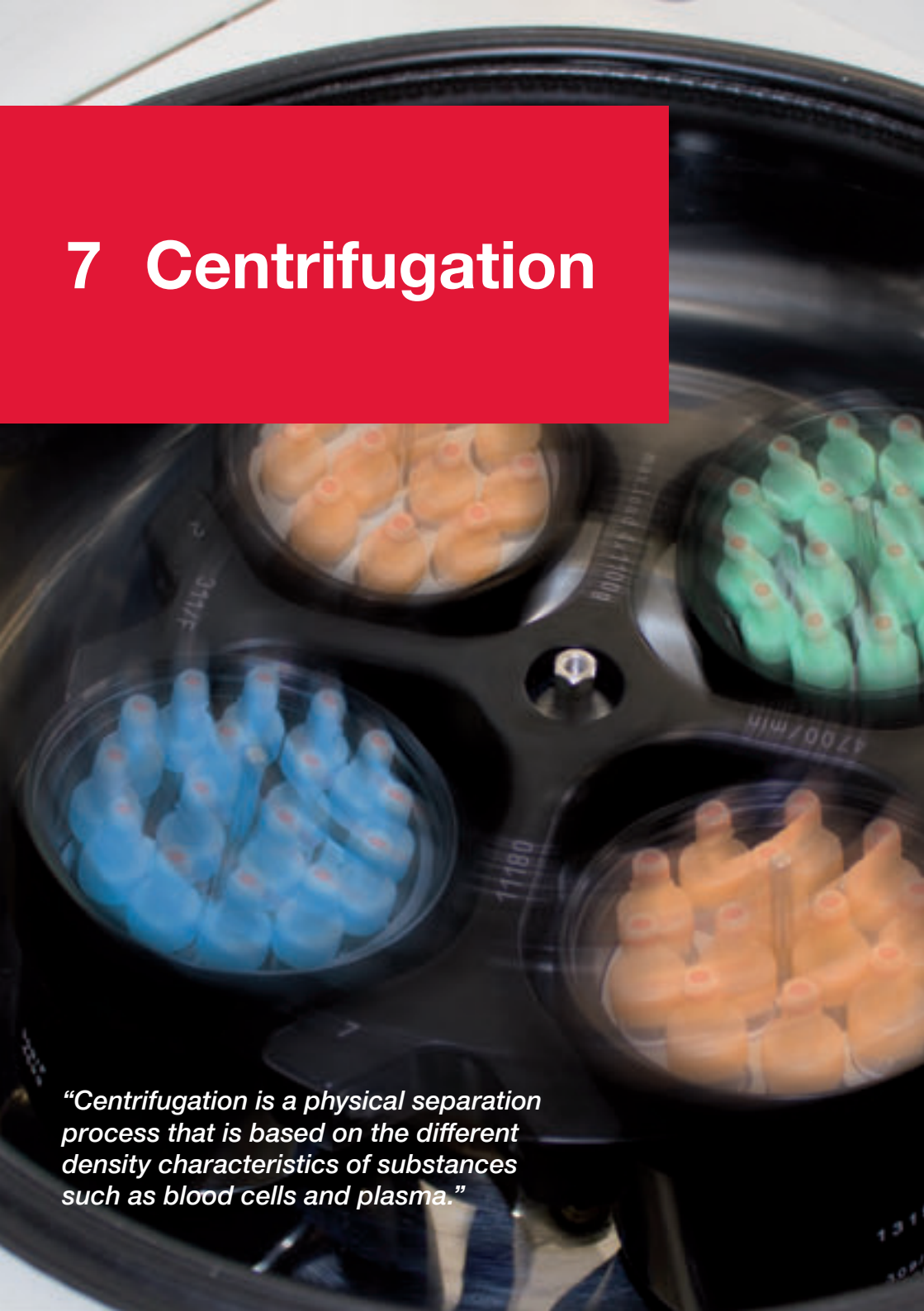
➤ As a rule, when disposing of potentially infected medical single-use items, ensure they are ***disposed of in a hygienically correct*** manner.



Safety instructions

- Only use containers of a size that are 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 on 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.
- Do not press items into the container with force.
- Do not place any liquids in the container.
- Do not put your 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.

7 Centrifugation



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

7.1 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 gravitational acceleration (g) inside the tube. 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):

$$g = 11.18 \times r \times \left(\frac{n}{1{,}000}\right)^2$$

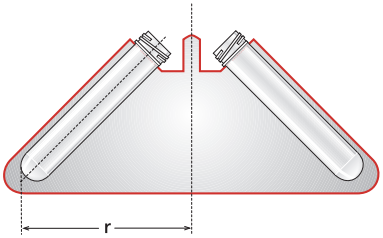
r = radius in cm

n = RPM (min^{-1})

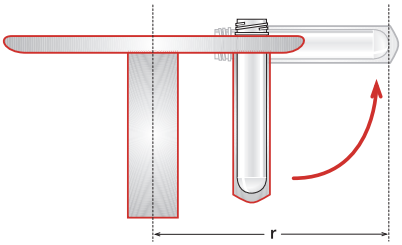
To convert from g force to RPM [min^{-1}] or vice versa,
you can use the centrifugation calculator at
www.sarstedt.com/en/service-consultation/centrifugation-calculator.

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



7.2 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

7.3 Serum collection

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

After blood collection, the serum samples must coagulate for 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, however, 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

7.4 S-Monovette® centrifugation conditions

The centrifugation process is a key element of the pre-analytical phase. The simultaneous centrifugation of different S-Monovettes is a prerequisite in routine laboratories for meeting the requirements of rapid patient care.
Our optimised centrifugation ranges for the S-Monovettes give you the opportunity to select the ideal centrifugation condition for you.

The optimum sample quality

In order to guarantee reliable sample quality within these centrifugation ranges, we carry out extensive and carefully validated tests. To assess the sample quality, meaningful criteria such as the integrity of the gel layer, the haemolysis, the cell counts (generally thrombocytes) and the stability of three cell-sensitive parameters (phosphate, glucose, LDH) are selected. For the S-Monovette® Citrate, a platelet count of < 10,000/µl (PPP) is a criterion in accordance with DIN 58905-1:2015-12.

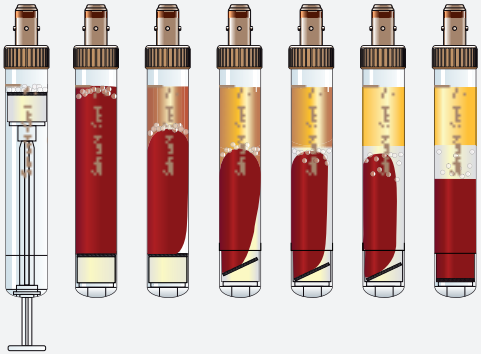
Minimum centrifugation time

Based on BS 4851 (EU code)	ISO 6710:2017	S-Monovette®	Relative centrifugal acceleration (g)				
			2000 × g	2500 × g	3000 x g*	3500 x g*	4000 x g*
		**Serum**	10 min	10 min	6 min	4 min	4 min
		**Serum gel**	15 min	10 min	4 min	4 min	4 min
		**Li Heparin**	10 min	10 min	7 min	7 min	7 min
		**Lithium heparin gel**	15 min	15 min	10 min	7 min	7 min
		**Lithium Heparin Gel**+	8 min	7 min	5 min	4 min	4 min
		**EDTA**	n. v.	n. v.	7 min	6 min	5 min
		**EDTA Gel**	15 min	10 min	10 min	7 min	7 min
		**Citrate**	9 min	8 min	7 min	6 min	5 min
		**Fluoride**	9 min	8 min	7 min	6 min	5 min
		**GlucoEXACT**	9 min	8 min	7 min	6 min	5 min
		**Citrate PBM 1.8 ml** Centrifuge radius > 17 cm	9 min	8 min	7 min	6 min	5 min
		**Citrate PBM 1.8 ml** Centrifuge radius > 9 to ≤ 17 cm	n.v.	n.v.	10 min	n.v.	n.v.

n.v. = not validated
Centrifugation at 20ºC
* Applies for all S-Monovettes with the exception of 8 mm diameter (S-Monovettes paediatrics)

7.5 Gel ascent during centrifugation

Gel ascent for the S-Monovette® Serum Gel



With the S-Monovette® Serum Gel, the coagulation process is already complete prior to centrifugation. Thus, the gel can ascend rapidly, unhindered and uniformly compact between the blood clot and the vessel wall. Subsequently, the serum and blood clot are separated from one another.

Gel ascent for the S-Monovette® Lithium Heparin Gel

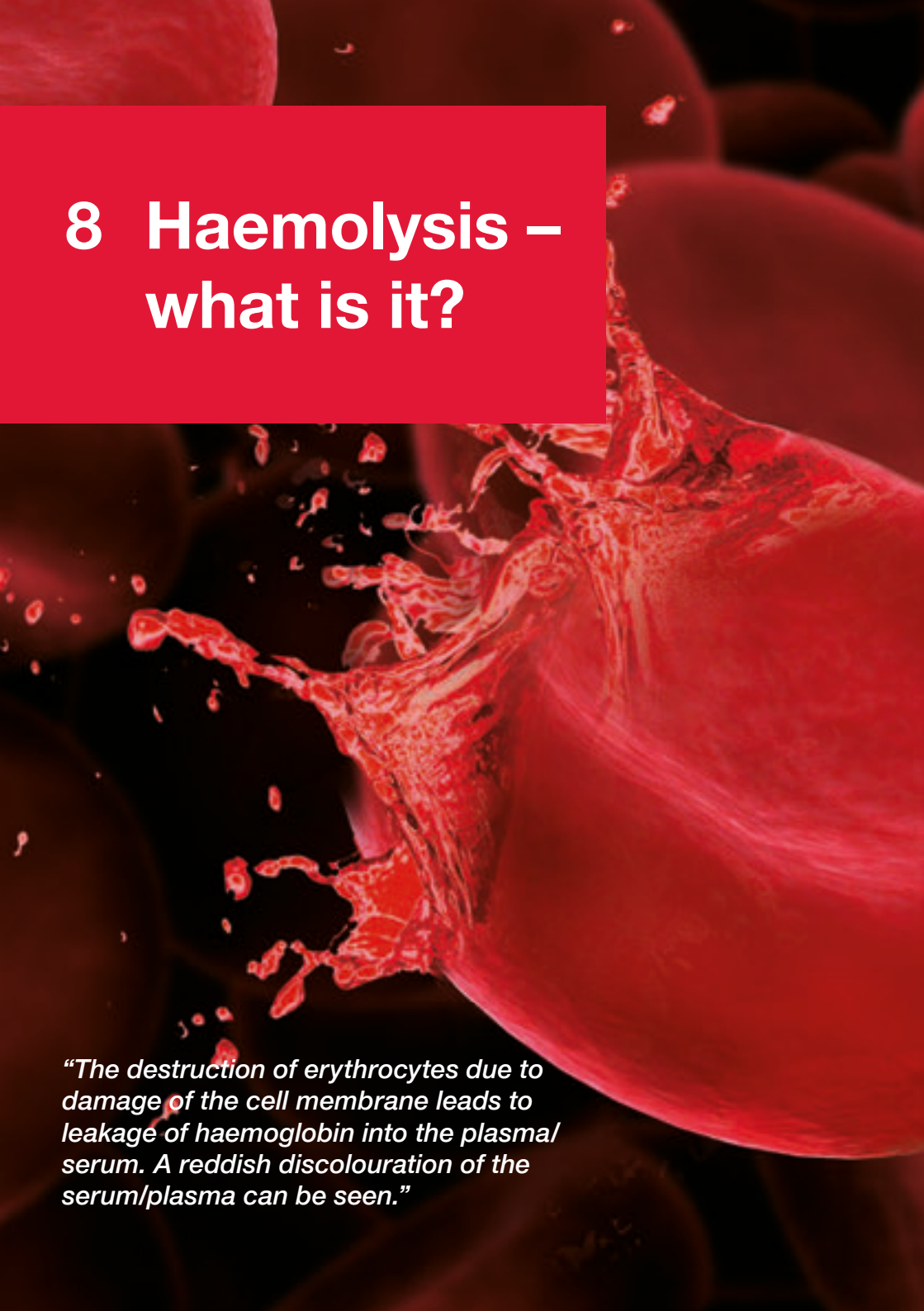
The S-Monovette® lithium heparin gel contains anticoagulated whole blood before centrifugation. The corpuscular components of the blood are distributed widely in the plasma in this process. During centrifugation, a fractionated ascent of the gel around the corpuscular components occurs. The optimally formed gel barrier ensures a safe separation between plasma and corpuscular components.

Re-centrifugation

Repeated centrifugation of sample tubes is not recommended.[29]
This may cause lysed blood components to diffuse back from the centrifuged blood cells into the serum/plasma. As a consequence, parameters, including cell-sensitive parameters such as potassium, phosphate, glucose or LDH, are changed.[30]

[29] CLSI, GP44-A4 2010; § 5.4.3
[30] Hue et al.; Observed changes in serum potassium concentration following repeat centrifugation of Sarstedt Serum Gel Safety Monovettes after storage; Ann Clin Biochem 1991; 28: 309-10
Shafi et al.; The Effect of Recentrifugation of Serum Separator Tubes on Concentration of Serum Analytes; Ann Clin Lab Sci 2012 42 (3):318-319
Hira et al.; Pseudohyperkalaemia caused by recentrifugation of blood samples after storage in gel separator tubes; Ann Clin Biochem 2001 38(Pt 4):386-90 Hira et al.; High Serum Potassium Concentrations after Recentrifugation of Stored Blood Specimens; NEJM 2000 343(2):153-154

8 Haemolysis – what is it?



“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.”

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.

[31] CLSI; Hemolysis, Icterus, and Lipemia/Turbidity Indices as Indicators of Interference in Clinical Laboratory Analysis; Approved Guideline; 2012; C56-A

8.1 *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

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

8.2 *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 tourniquet application
- Physical shearing forces (needle too thin, bent needle)
- Traumatic venous puncture (poking)
- Blood collection from catheters using the vacuum technique[15]
- Intravenous catheter combined with too large vacuum force[17, 33–39]
- Infusion solutions (dilution, distortion)

[15] Lippi et al.; Prevention of hemolysis in blood samples collected from intravenous catheters Clin Biochem 2013; 46(7-8): 561–64
[17] Millius et al.; The „EPiQ"-Study (Evaluation of preanalytical quality): S-Monovette® in manual aspiration mode drastically reduces hemolytic samples in head-to-head study; 2021 Pract Lab Med 27 e00252
[33] Omar et al.; Reducing blood sample hemolysis in the emergency department using S-Monovette® in aspiration mode; 2023; Pract Lab Med 35 e00315
[34] Halm et al.; Obtaining blood samples from peripheral intravenous catheters: best practice? Am J Crit Care 2009;18(5): 474–78
[35] 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(11): 1151–55.
[36] ENA's Translation Into Practice. Reducing Hemolysis of Peripherally Drawn Blood Samples. 2012 (Emergency Nursing Association)
[37] 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 2012; 45(13-14): 1012-32
[38] Straszewski et al. J; Use of seprate venipunctures for IV access and laboratory studies decreases hemolysis rates; Intern Emerg Med 2011; 6(4): 357-59
[39] Dugan et al.; Factors Affecting Hemolysis Rates in Blood Samples Drawn from Newly Placed IV Sites in the Emergency Department; J Emerg Nurs 2005; 31(4): 338-45

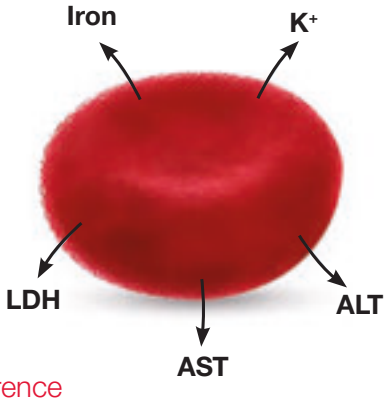
Common causes after blood collection

- 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

8.3 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.

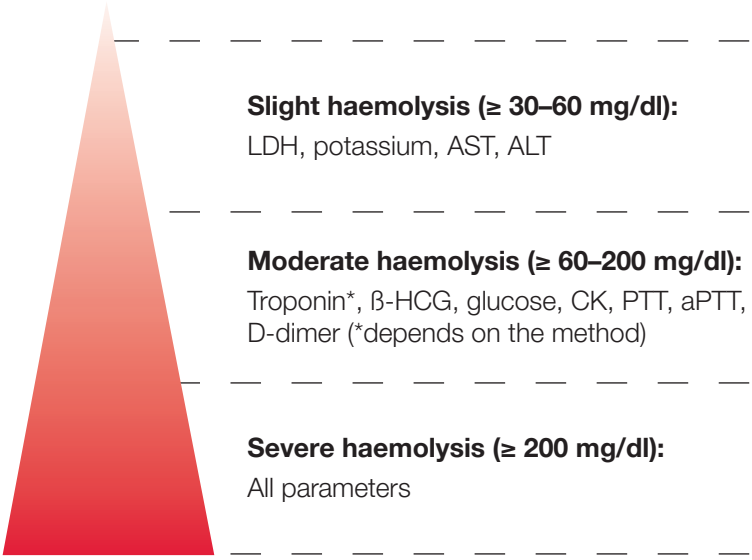
Released cell content	Affects analysis
Free haemoglobin	Bilirubin
Adenylate kinase	CK, CK-MB
Hydrolase	Coagulation

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.

8.4 Clinical relevance

The following parameters are affected:



[40] Lippi et al.; Hemolyzed specimens: a major challenge for emergency department and clinical laboratories, Crit Rev Clin Lab Sci 2011; 48(3): 143-53

Note: ***The analytical results are altered by haemolysis and do not reflect the conditions in the patient. This can lead to misdiagnoses, or have incorrect, missing or unnecessary diagnostic consequences.***

In many cases, repeat blood collection to determine the correct analytical values is necessary.
This causes avoidable patient stress, time wasting and additional costs.[33,41,42,43]

[33] Omar et al.; Reducing blood sample hemolysis in the emergency department using S-Monovette® in aspiration mode; 2023; Pract Lab Med 35 e00315
[41] Cadamuro et al.; The economic burden of hemolysis; CCLM 2015
[42] Jacobs et al.; Cost of hemolysis; AnnClinBiochem 2012; 49(Pt 4): 412
[43] Jacobs et al.; Haemolysis Analysis; An Audit of Haemolysed Medical Admission Blood Results; AcuteMed 2010; 9(1): 46-47

9 Storage and transport

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

9.1 Sample transport

To ensure correct storage, transport conditions and sample shipping, the relevant shipping regulations[44,45] and the stability of the individual parameters must be taken into account. This requires optimal organisation.

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

[44] P650 IATA/ADR
[45] 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 find out whether the samples will be shipped via a land, rail or air transport route.

The P650 Packaging Instruction, which is also incorporated into the ADR (European Agreement concerning the International Carriage of Dangerous Goods by Road – road and rail transport) and in the IATA (International Air Transport Association – air transport), 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 minimum dimensions of 100 × 100 mm; labelled ‘BIOLOGICAL SUBSTANCE, CATEGORY B’ with the UN code ‘UN3373’ printed in a diamond with minimum dimensions 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.

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 dimensions 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.

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.[45]

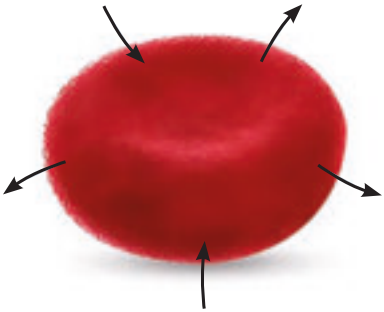
[45] TRBA 100

9.2 Influence of temperature, time and cellular metabolism

Concentrations that are measured change due to the stability of the individual parameter 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.



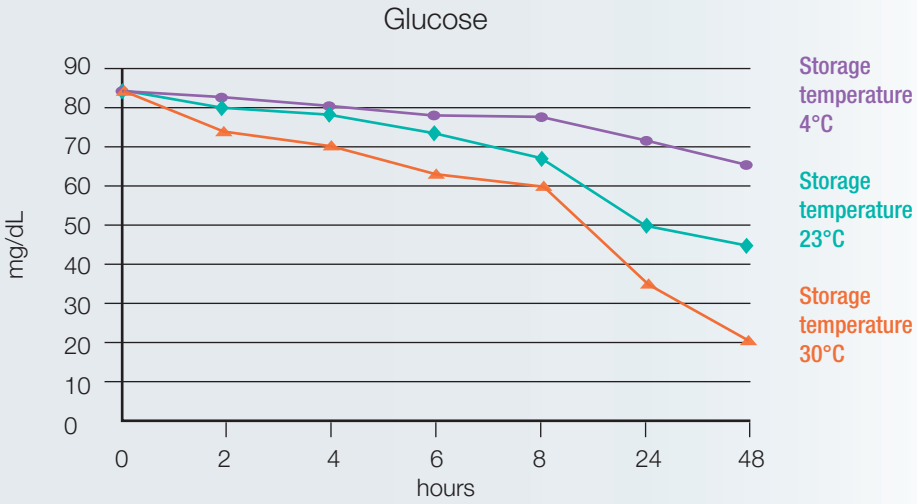
Note: Blood is alive!

Effect of storage on various parameters

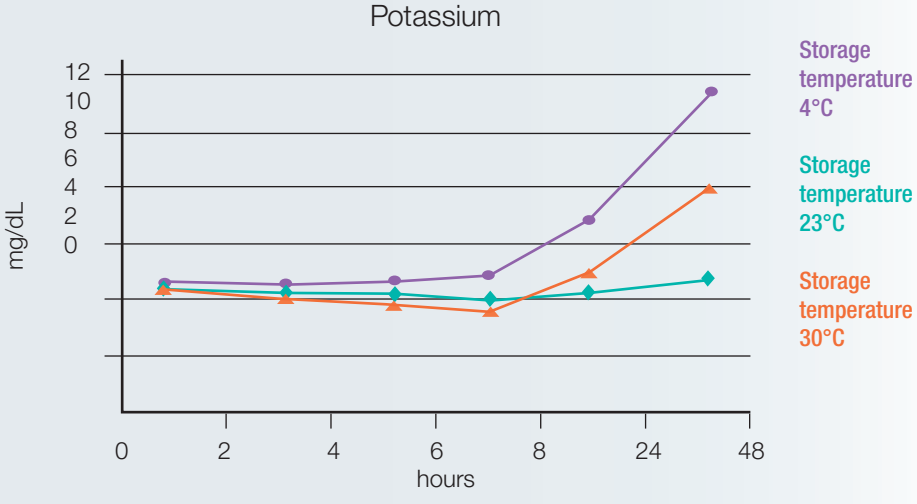
Parameter	Value
Lactate	Increases
Ammonia	Increases
Potassium	Increases
Glucose	Decreases
pCO_2	Decreases

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] Guder, Narayanan; Pre-Examination Procedures in Laboratory Diagnostics: Preanalytical Aspects and their Impact on the Quality of Medical Laboratory Results 2015 DOI:10.1515/9783110334043



[5] Guder, Narayanan; Pre-Examination Procedures in Laboratory Diagnostics: Preanalytical Aspects and their Impact on the Quality of Medical Laboratory Results 2015 DOI:10.1515/9783110334043

Note: ***There is no ideal temperature for all analytes. Correctly collected, fresh samples allow for correct results.***

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 longer-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).

Coagulation diagnostics:

- For coagulation diagnostics, the sample should be transported at room temperature (18–25°C) as a rule.[6]
  Most guidelines[3, 35] recommend that coagulation samples are centrifuged within an hour after collection and analysed within four hours. During this period, storage can be at room temperature.

Haematology:

- EDTA blood for a blood count can be stored at room temperature (18–25°C) for up to 24 hours.[46]

[6] Endler et al.; The importance of preanalytics for the coagulation laboratory; Hämostaseologie 2010; 30(2): 63-70
[46] Tatsumi et al.; Specimen Collection, Storage, and Transmission to the Laboratory for Hematological Tests; International Journal of Hematology 2002; 75(3); 261-68

Checklist for transport

- Seal sample (evaporation)
- Store serum/plasma at 4–8°C
- Store upright
- Store EDTA for blood count at room temperature
- Avoid repeated freezing and 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)

Pneumatic tube transport systems

Pneumatic tube transport systems can considerably shorten the time between blood collection and the analytical result.[47] 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.[48,49,50]

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

By complying with the following tips, samples can be transported using a pneumatic tube system without significant effects on the values.[51,52]

- 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

[47] Koessler et al.; The preanalytical influence of two different mechanical transport systems on laboratory analysis; Clin Chem Lab Med; 2011; 49(8): 1379-82
[48] Kratz et al.; Effects of a pneumatic tube system on routine and novel hematology and coagulation parameters in healthy volunteers; Arch Lab Med; 2007; 131(2): 293-96
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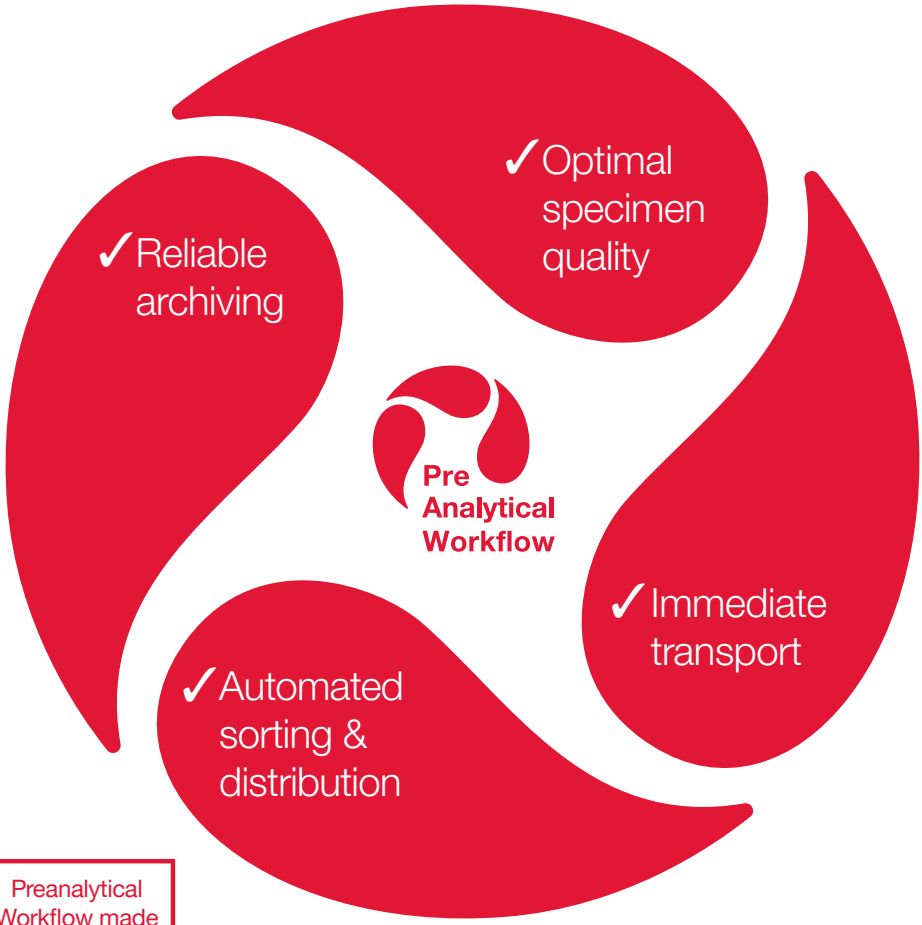

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