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# VACUETTE® Preanalytics Manual



Preanalytics  
Handling  
Recommendations

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## Foreword

As far as healthcare is concerned, medical laboratory tests for diagnostics, patient monitoring, drug monitoring and prognoses are of utmost importance. According to studies in Germany, laboratory results contribute to a diagnosis in two thirds of cases, and in the USA, the total is around 80%. Furthermore, certain diagnoses can only be made on the basis of a laboratory result.

Laboratory results are sensitive to even the slightest of deviations to a normal condition or to changes in the disease progress, in some cases more specifically, and thus more effectively than the doctor's perception or the patient's subjective opinion. Therefore, important decisions on beginning therapy or medication are often made on the basis of laboratory results.

It is essential, that the laboratory results are accurate, and that even minimal changes to measurements are recorded exactly. Modern technology and sensitive procedures together with a sophisticated quality assurance allow us to fulfil both of these conditions. The prerequisite is that the specimens for analysis arrive at the laboratory corresponding to the in vivo state. Various influences and interference factors which can take effect between the patient and laboratory – i.e. prior to analysis in the preanalytical phase – can falsify the laboratory results considerably, thus leading to incorrect evaluations and in the worst case, even to false diagnoses or the wrong therapy.

The preanalytical phase covers all stages from preparing the patient for specimen collection to introducing the specimen into the analytical process. This includes recording all facts and data, which influence the laboratory values, and should be taken into consideration when judging the laboratory results. Clearly, there are several persons involved in the preanalytic process, whereby each is responsible for his/her part in the process.

Each person involved at this time, must be aware of the significance of preanalytics, and that if errors are made during this phase, the laboratory result could become meaningless. The intention of this brochure is to increase awareness of the possibilities for making errors and point out how errors can be avoided in preanalytics and is aimed at personnel involved with requesting and evaluating laboratory results as well as specimen collection, preparation, storage and transport of specimen material.

**Prof. Dr. Dieter Meißner; Dresden**

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# 1. What Is Preanalytics?

The term “Preanalytics” refers to the entire administrative and practical process of collecting, processing, storing and transporting diagnostic examination material prior to carrying out laboratory tests.

This covers preparation of the patient, sample collection, pre-processing, storage and transport of specimen material as well as handling in the laboratory prior to analysis.

We differentiate here between patient-related influencing factors and errors.

Patient-related influencing factors affect the concentration of a parameter, and are taken into account in the reference values.

These influences always come from the patients, from the physical condition or from his/her behaviour, and can be taken into consideration when interpreting the results, provided that the appropriate information has been made available to the laboratory.

Errors are often made due to not knowing the correlation, and thus, even errors made during the preanalytical phase can have an effect on the final analysis results, or can cause implausible laboratory values or under certain circumstances even false diagnoses.

The following should describe the handling basics, which serve to take patient-related influences into consideration. Furthermore, the most frequent errors made in the varying activities of the preanalytical field are represented together with their consequences.

# 2. Who Is Involved in Preanalytics?

## There are always several persons involved in Preanalytics:

The patient, the treating doctor, the nurse, the attendant, the transport service, the medical technical assistant and the laboratory doctor; They all share the responsibility for the quality of the specimen material and should understand the significance of the preanalytical phase, as well as recognise possible causes of error and their consequence for the examination results.

Activities	Persons involved
Requisition for analysis	Treating doctor
Preparation of patient	Treating doctor, nursing staff, doctor's assistant, patient, lab staff
Identification of patients and specimens	Treating doctor, nursing staff, doctor's assistant, patient, lab staff
Blood collection	Doctor, nursing staff, doctor's assistant
Mixing blood sample	Doctor, nursing staff, doctor's assistant
Storage until transportation	Nursing staff, doctor's assistant
Transportation	Collection or courier service
Acceptance, storage and preparation of samples	Laboratory staff, medical technical assistants, laboratory doctors

Table 1: Activities during and after the preanalytical phase and responsible personnel

The time required for the preanalytical phase is often underestimated. In fact, it takes up more than 58% of total time required, which is more than required for the laboratory analysis.

Thanks to modern technology, the actual analysis only takes up around 25% of the time.

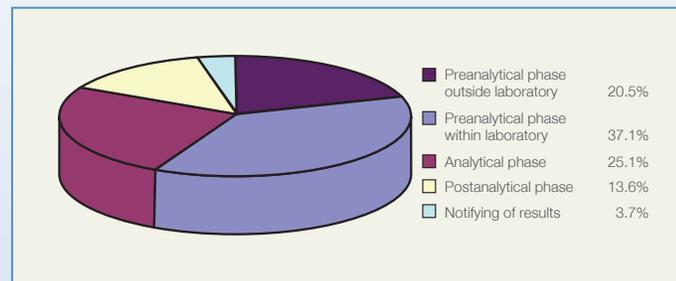


Fig. 1: Total time for the different preanalytical phases

### 3. Patient-Related Influencing Factors

Patient-related influencing factors can differ from patient to patient, and can even remain the same for a lifetime. However, they could also change for the same patient either long-term or short-term, from one day to the next or even during one day.

#### 3.1 Non-changeable influencing factors

Gender, geographical origin and ethnic differences are included here.

##### 3.1.1 Gender

The differences between genders can account for up to 80%. In addition to gender specific hormones, clinical chemistry and haematological parameters such as triglyceride, creatinine, HDL-cholesterol, iron and others can differ significantly.

Parameters	Man	Woman	Unit
Alanine aminotransferase	< 50	< 35	U/l
Iron	6,3 - 30,1	4,1 - 24	µmol/l
Ferritin	18 - 360	9 - 140	µg/l
Uric acid	3,6 - 7	2,3 - 6,1	mg/dl
Creatinine, Jaffé reaction kinetic	0,81 - 1,44	0,66 - 1,09	mg/dl
Haematocrit	40 - 53	36 - 48	%
Haemoglobin	13,5 - 17,5	12 - 16	g/dl
Erythrocyte sedimentation rate	< 15	< 20	mm/1h

Table 2: Gender specific differences  
Source: Thomas L.: Labor und Diagnose 6. Edition

##### 3.1.2 Geographical origin and ethnic differences

Leukocyte counts are significantly lower in black populations than in fair-skinned populations, whilst Europeans have higher concentrations of granulocytes and monocytes.

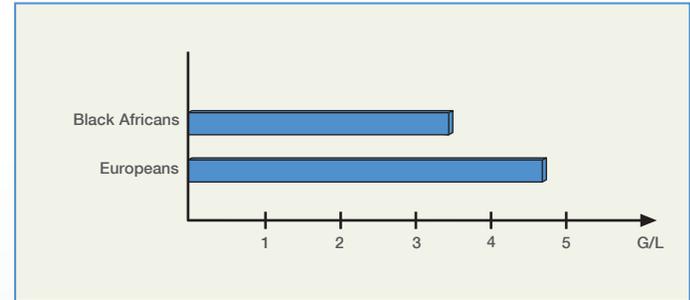


Fig. 2: Influence of origin on concentration of granulocytes

The alpha-amylase concentration for North-West Europeans is significantly different to that of the inhabitants of the Antilles and Asia. Around 50% of the values, taken from Antilles inhabitants, were pathologically evaluated, compared with British normal values.

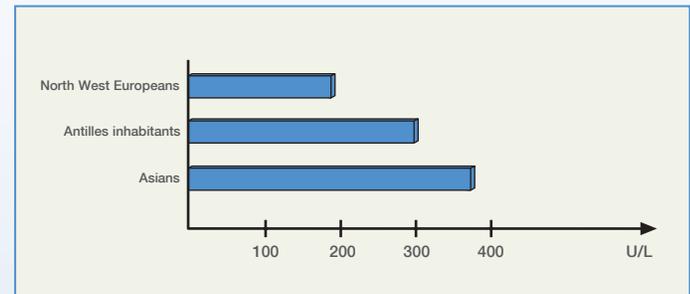


Fig. 3: Influence of origin on alpha-amylase concentration

### 3.2 Long-term changeable influencing factors

#### 3.2.1 Age

The total of erythrocytes and thus the haemoglobin and bilirubin concentrations are significantly higher for neonates than for adults. Alkaline phosphatase is considerably higher during a young person's growth period. The cholesterol value, in particular LDL-cholesterol, increases with age.

Decreases with age	Increases with age
Albumin	Cholesterol
Calcium	Erythrocyte sedimentation rate
Creatinine clearance	Ferritin
Anorg. phosphate	Glucose
pO <sub>2</sub>	
Quick	

Table 3: Influence of age on selected parameters

### 3.2.2 Body weight

With increasing body weight, the following factors also increase: cholesterol, triglyceride, uric acid, cortisol and insulin, amongst others.

### 3.2.3 Lifestyle

Particular lifestyle habits such as occupational stress or sport have an influence on different laboratory values. Regardless of fitness level, athletes have, for example, increased creatine kinase.

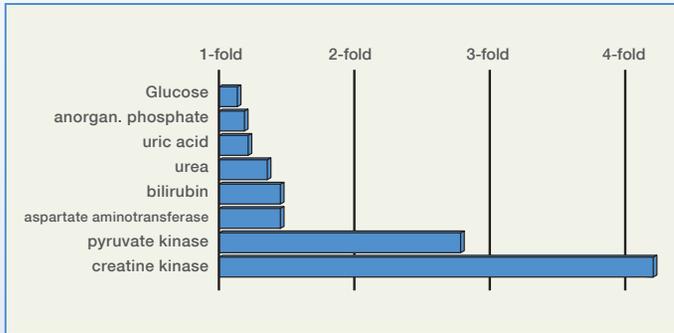


Fig. 4: Change of various serum concentrations after extreme physical activity – marathon run

### 3.2.4 Pregnancy

During pregnancy, the plasma volume increases by around 50%. Concentration changes can be seen in a range of parameters; important electrolytes are reduced, blood lipids are elevated, copper is doubled.

For patient-related influencing factors such as gender, age and pregnancy, different reference ranges for men, women, pregnant women are taken into consideration as well as different age groups. Under certain circumstances for foreign geographical origin and ethnic differences, other reference ranges that are not typical of the region must be taken as a basis.

An essential condition for correct classification of the reference ranges is correct patient data on the requisition form.

## 3.3 Short-term changeable influencing factors

### 3.3.1 Daily rhythms and biorhythms

Various parameters change over the daily rhythm. Some parameters will have their maximum in the morning, some at midday or in the evening.

Maximum fluctuations in course of day as %			
Maximum in the morning			
Adrenocorticotropic (ACTH)	200 %	Adrenaline	20 %
Renin	140 %	Haemoglobin	20 %
Noradrenaline	120 %	Haematocrit	20 %
Prolactin	100 %	Leukocytes	20 %
Aldosterone	80 %	Protein	20 %
Cortisol	50 %	Thyroxin (T4)	20 %
Testosterone	50 %	Bilirubin	20 %
Maximum at midday			
Iron	100 %		
Eosinophil granulocytes	30 %		
Potassium	15 %		
Maximum in the evening			
Creatinine	100 %		
Uric acid	50 %		
Thyretropine (TSH)	50 %		
Acid phosphatase	200 %		

Table 4: Fluctuation over course of day in the daily rhythm

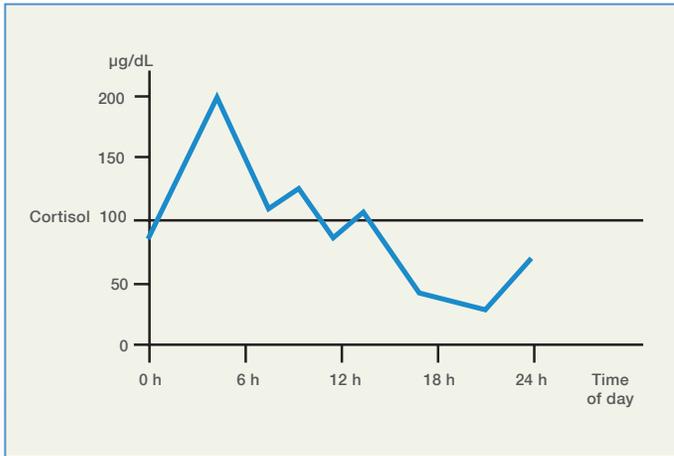


Fig. 5: Fluctuation of cortisol in daily rhythm

The influence of fluctuations in the daily rhythm is minimised by adhering to the recommended specimen collection time of between 7 am and 9 am.

With regard to the bio-rhythm, not just fluctuations due to different times of year should be taken into consideration, but also, for example, fertility hormones in the menstrual cycle and vitamin D concentrations, for which the values are at their highest in the summer.

Besides fluctuations in daily rhythms and bio-rhythms, there are considerable intra-individual fluctuations for different parameters from day to day.

### 3.3.2 Physical strain

When under physical strain, water and small molecules from vessels leak into the extravascular space. This increases the concentration of high-molecular structures like proteins or substances bound to protein in vessels.

This also happens when sitting up after lying down and during stasis cf. chapter 6.3 and 6.4.

-  Before giving a blood specimen as an outpatient, the patient should rest for around 5 minutes.
-  A blood sample should never be taken after physical exertion, e.g. after a morning jog.
-  During the 3 days prior to giving a blood sample, there should be no exhausting physical activities.

### 3.3.3 Stress

Fear of blood collection or the situation prior to an operation can lead to extreme mental stress.

This causes various hormones to be released, e.g. aldosterone, catecholamine, cortisol, prolactin and renin.

Increased concentrations of albumin, fibrinogen, glucose and insulin can also be observed.

A calm atmosphere and encouragement prior to blood collection can have a very positive effect.

### 3.3.4 Food intake

After food intake, depending on composition of the meal and the time passed since eating and taking the specimen, various parameters can be altered.

After a meal rich in fat the effects are visible due to cloudiness in the plasma – lipaemia. Lipaemic samples only have limited use in a laboratory.



Fig. 6: Samples with varying degrees of cloudiness

Alkaline phosphate	Insulin
Cholesterol (total, HDL, LDL)	Potassium
Dopamine	Cortisol
Iron	Corticotropin stimulation test
Glucose	Anorg. phosphate
Uric acid	Triglyceride

Fig. 7: Parameters for which 12-hour food abstinence is required prior to taking the specimen

Prior to blood collection, a 12-hour food abstinence is recommendable, in particular for a lipometabolism diagnosis.

For glucose tolerance tests, a high-carb diet should be adhered to during the 3 days prior to the test, i.e. > 150g carbohydrates per day.

Long-term fasting can also influence laboratory results.

### 3.3.5 Stimulants: Coffee, nicotine, alcohol

Coffee drinking can lead to a strong increase in cortisol – up to 40% after 200mg of caffeine (contained in two cups of coffee).

Heavy smoking leads to changes in leukocytes, lipoproteins, enzyme activities, hormones, vitamins, tumour markers and heavy metals.

Just one cigarette can lead to very significant changes in the serum concentration of different measurements within an hour.

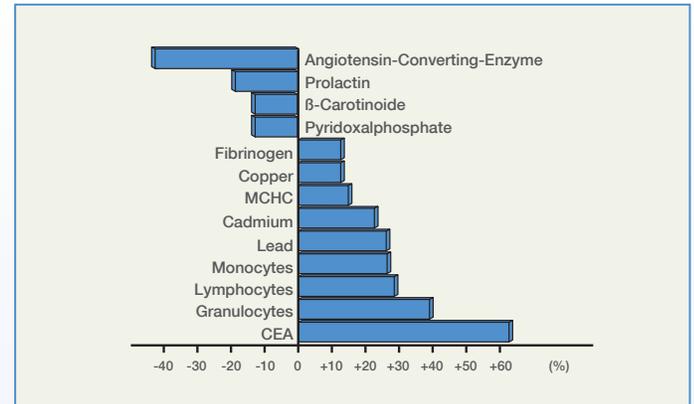


Fig. 8: Differences of more than 10% for smokers compared to non-smokers

In case of alcohol consumption, there is a difference between acute and chronic effects. The increased activity of liver enzymes is most well-known.

It is recommended to neither smoke nor drink before blood collection. Furthermore, alcohol should be abstained from for 24 hours.

Alcohol excesses must be avoided, in the days leading up to blood collection.

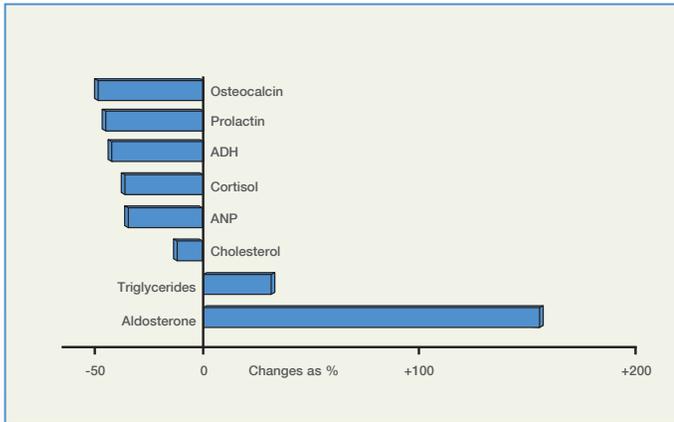


Fig. 9: Acute changes in case of alcohol consumption

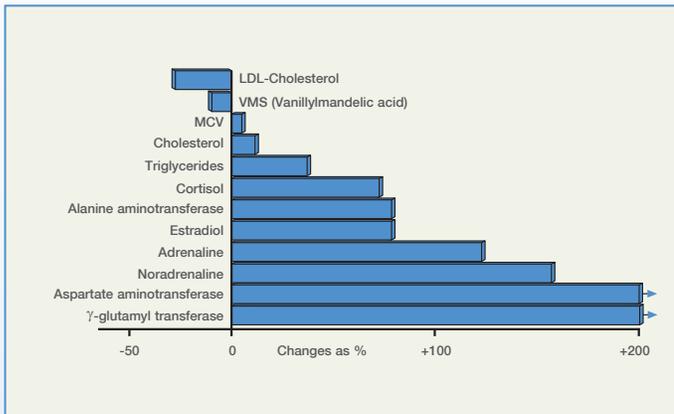


Fig. 10: Chronic changes in case of alcohol consumption

### 3.3.6 Drugs

Drug consumption has biological effects, which can influence laboratory examinations, whereby every drug has its own effects.

Cannabis, for example can lead to an increase in sodium, potassium, urea, insulin and chloride, whilst leading to a decrease in creatinine, glucose and uric acid.

### 3.3.7 Medication

Similar effects can be seen if medication is being taken. This is a common cause of interference to laboratory analyses in a hospital.

To prevent misinterpretations of laboratory results, the patient should always be asked if medication is taken regularly, and if medication has been taken before blood collection.

Consumption of vitamins and hormones should be referred to specifically, as these substances are not automatically viewed by patients as medication. The substance, the amount taken and the time of consuming should be reported to the laboratory.

For therapeutic drug monitoring, blood collection should be as soon as possible prior to taking the medication (measurement at trough level). The collection is not to be carried out when plasma is at maximum concentration.

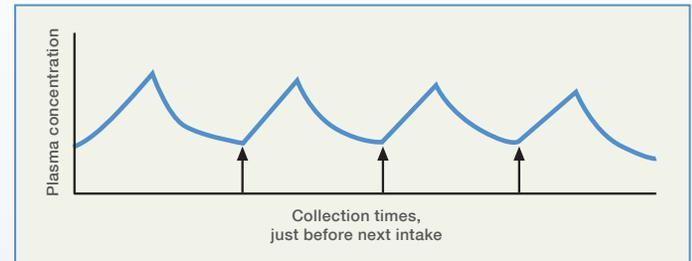


Fig. 11: Ideal time for measuring medication levels

However, blood collection must be carried out immediately if there is suspicion of an overdose or intoxication.

### 3.3.8 Patient's Behaviour

The patient is not always aware of many of the influencing factors, and he/she can only behave appropriately, if the factors are made known.

- Thoroughly preparing the patient can help avoid errors.
- Asking questions prior to blood collection can help to expose incorrect behaviour.
- Under certain circumstances, the blood collection procedure should be postponed due to incorrect behaviour.

## 4. Widespread Errors Made With Identification

Errors in identification do not impair the quality of a sample, but they do complicate laboratory work considerably.

Misunderstandings and late results can occur, or it could even become impossible for the laboratory results to be traced back to the patient. Missing samples or requisition papers, or illegible labelling come under this category. These potential sources of error can be counteracted by using pre-barcoded specimen containers.

Errors made with identification can often be put down to carelessness, rushing or being distracted.

Incorrect assignment of a sample and the test requisition leads to mistakes that will only be discovered at the plausibility check or by the treating doctor, if at all.

Prior to blood collection, the patient should identify him/herself by stating his/her name.

### 4.1 Patient identification / requisition papers

Missing patient identification data on the requisition papers occurs again and again. The additional identification provided by scanning the patient's wristband can increase safety.

The following data is obligatory:

- Surname, first name, date of birth
- Patient number, ward, room number, name or number of doctor's office
- Date and collection time
- Gender
- If required week of gestation

For different analytes of tests, the following data is also required:

- Collection time for day's profiles or function tests.
- Intake of medication including vitamins and hormones.
- Body size and weight.
- For 24h urine: total collected volume.

For small amounts of specimen material, only the most important parameters should be entered.

### 4.2 Sample identification

Frequent errors made here are: incorrectly attached, dirty, illegible or incorrect labelling.

An incorrectly attached label prevents optical control of the specimen. Visual control of the fill line and sample quality is prevented.

The fill level and specimen consistency cannot be judged. For barcode labels, it is difficult or even impossible to scan in data. An illegible or incomplete label may be refused for analysis.



Fig. 12: Incorrectly attached labels, compared to correctly attached label (left)

To avoid errors, the following must be observed:

If labels for automatic reading are used, special attention should be paid to the preparation of whole tube series to avoid mixing up tubes.

- Fill in label carefully and legibly.
- Only use waterproof pens.
- Label STAT samples specially.
- Label infectious material on the sample tube and requisition papers.
- NOTE: Timing of labelling varies from country to country.
- Always position the label correctly.
- Stick the label onto the collection tube, never on the transport tube.

Pre-barcoded sample tubes guarantee consistent high quality of barcodes, which are already in the correct position on the tube.



Fig. 13: Pre-barcoded tubes

## 5. The Special Meaning of Haemolysis

Various errors can lead to haemolysis, which makes it a very important topic for preanalytics. Therefore a separate chapter has been dedicated to it. Individual activities, the formation of haemolysis will be covered in detail, as well as how it can be avoided.

Haemolysis occurs, when the cell membrane of the red blood cells is destroyed. Intracellular components get into serum or plasma. Even just a slight haemolysis can cause increased serum or plasma values, in parameters with a high concentration difference between erythrocyte and in serum.

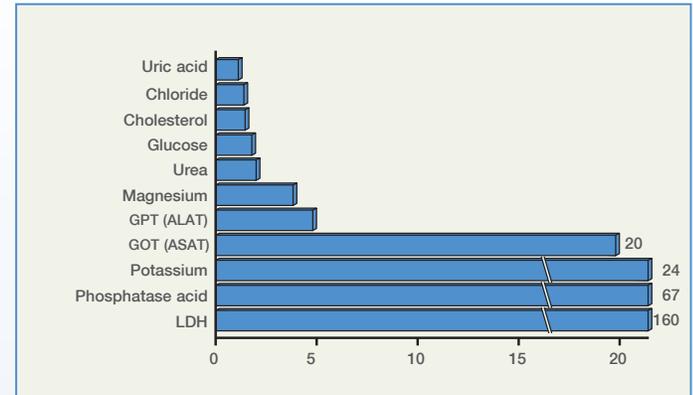


Fig. 14: Concentration ratio of various parameters in erythrocytes and serum  
e.g. the concentration of LDH in erythrocytes is 160 times higher as serum

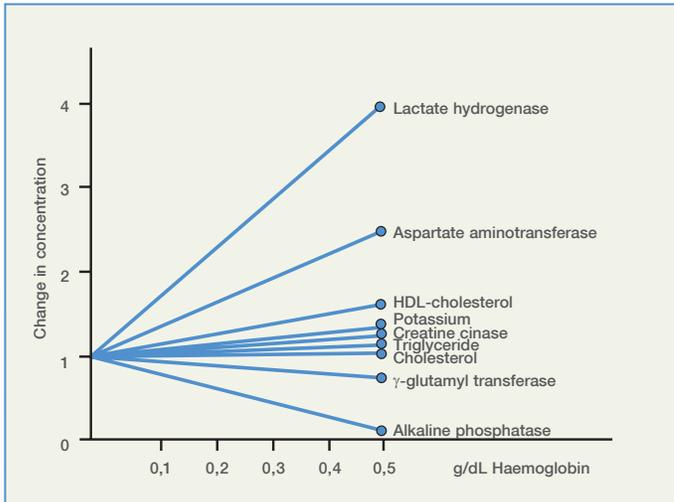


Fig. 15: Changes in different parameters at a haemoglobin concentration of 0.5 g/dL

The serum or plasma, turns red due to the haemoglobin from the erythrocytes. As of a haemoglobin concentration of around 0,03 g/dL it is possible to see the discolouration with the naked eye. The intensity of the haemolysis is indicated by the intensity of the red colouring.



Fig. 16: Samples in various strengths of haemolysis

The following errors lead to haemolysis, and should be avoided in any case:

- Tourniquet applied too tightly.
- Needles with too small diameter.
- Aspiration of tissue fluid after puncturing the vein.
- Transfer of blood into other containers with a syringe.
- Shaking the sample instead of gently mixing.
- Delayed separation of cells from serum or plasma > 3 hours.
- Too long or too high centrifugation.
- Influence of temperature, heat or cold, e.g. during transport or if samples touch cooling elements.
- Freezing whole blood.

**Haemolysis has a three-fold effect:**

1. The above-described release of components from cells changes the concentration in serum or plasma.
2. The red discolouration due to haemoglobin interferes with the photometric measuring.
3. Chemical reactions during analysis can be influenced by cell substances.

## 6. Widespread Errors Made During Blood Collection

### 6.1 Patient preparation

The patient's family doctor must emphasise the significance of his/her behaviour prior to a blood collection.

Patients are not always aware of the short-term changing influencing factors due to diet, stimulants, stress, physical activity etc., see chapter 3.3. Correct behaviour is only possible, if there is awareness of possible problems.

Behaviour recommendations are often just forgotten. It can be helpful to ask prior to blood collection, in order to find out if there has been unsuitable behaviour. If circumstances require, then the blood collection may have to be postponed.

### 6.2 Time of blood collection

The influence of fluctuations due to daily rhythm, see chapter 3.3.1, can be minimised if the time of blood collection is kept to between 7 am and 9 am. Collection at any other time of day may cause incomparable results.

### 6.3 Body position

Changing from a lying position to a sitting position causes displacement of plasma volume and various small volume blood components from the vessels to the extra vascular space by around 12%.

This also involves a change in concentration of a number of parameters, in particular blood cells and high molecular substances.

If possible, blood collection for outpatients should be carried out in a lying position rather than sitting. If this is not possible then the sitting position can be used.  
It is important that blood collection is always carried out in the same body position. In this way the results remain comparable.

Increase when changing from lying down to sitting up	Parameter
Up to 10%	Haemoglobin Leukocytes Total calcium Aspartate aminotransferase Alkaline phosphatase Thyroxin Immunoglobulin G und A Albumin Total protein Cholesterol Triglycerides
Between 10% and 20%	Haematocrit Apolipoprotein Erythrocytes
More than 50%	Adrenaline Renin Noradrenaline

Table 5: Influence of body position during sample collection

### 6.4 Duration and intensity of the stasis

A tourniquet is applied to assist in locating the vein and make the venipuncture easier.

This creates a filtration pressure in the vein, which results in haemoconcentration. The effects are similar to those as described in chapter 4.1, "Body position". The change in concentration depends on length and intensity of the stasis.

**A stasis of up to 60 seconds is acceptable and has no significant effect on the sample.**

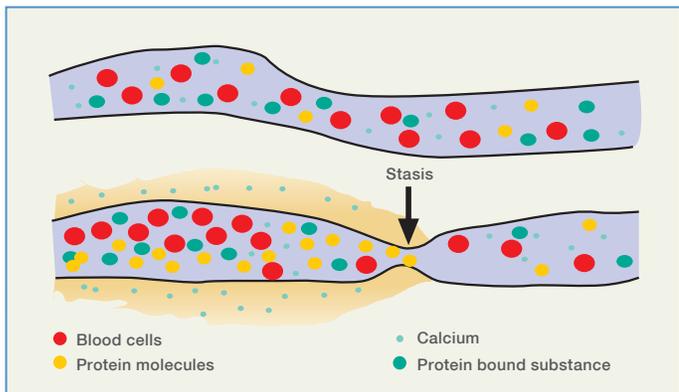


Fig. 17: Haemoconcentration due to transfer of plasma and small molecules from intravascular to interstitial space

Increased Between 6% und 12%	Decreased Up to 4%
Alanine aminotransferase	Glucose
Creatine kinase	Anorg. phosphatase
Bilirubin	Leucocytes
Lactate dehydrogenase (LDH)	Urea
Albumin	Creatinine
γ-glutamyl transferase	Chloride
Alkaline phosphatase	
Total protein	
Cholesterol	
Triglyceride	
Aspartate aminotransferase	
<b>Changes can occur even in case of shorter stases</b>	

Table 6: Change in different parameters as percent

The tourniquet pressure should be 40mmHg. The purpose of the tourniquet is to reduce venous outflow without affecting the arterial inflow. In this way, intravenous pressure is increased, the vein fills well and is thus easier to palpate. In addition to this, a properly applied tourniquet makes it easier to differentiate between a vein and pulsating artery.

The tourniquet should not be applied too tightly – it should still be possible to feel the pulse.

Furthermore, if the tourniquet remains applied for the entire blood collection, haemolysis can occur, in particular for patients with good vein conditions and high blood pressure.

In case of good veins, the tourniquet should be loosened immediately on successful puncture of the vein, before the blood collection is started.

## 6.5 Techniques for finding the vein

For easy location of the vein, there are various techniques that are often applied, which have an effect on the specimen quality and should therefore be avoided:

### Improper techniques for finding the vein more easily:

- The patient opens and closes his fist. This technique is also known as "pumping". This can cause a considerable increase in potassium.
- Tapping the puncture site can also lead to distortion of the sample.

Opening and closing the fist as well as tapping of the puncture site are improper techniques.

### Proper techniques for finding the vein more easily:

- Make a fist, do not pump
- Apply warmth, by using a warm arm bath, a heating pad or a local anaesthetic patch.

In the booklet "**VACUETTE**® Blood Collection Techniques", the procedure for successful venipuncture is described. Please also refer to the "**VACUETTE**® Collection System Handling Recommendations" from Greiner Bio-One.

## 6.6 Disinfecting the puncture site

If disinfection is carried out incorrectly, disinfectant can also get into the blood sample and corrupt the analysis results.

Disinfectant should be used in accordance with the manufacturer's instructions. Before carrying out the skin puncture, the disinfected site should be allowed to dry completely.

## 6.7 Venipuncture

Repeated attempts during venipuncture to locate the vein or probing the tissue can lead to contamination due to tissue thromboplastin, which can, for example, have a considerable influence on coagulation determinations.

Do not probe when locating the vein. If necessary, puncture the other arm.

## 6.8 Collection from catheter

If possible do not take blood from a horizontal catheter.

If collection from a horizontal catheter is unavoidable, great care should be taken to avoid contaminating the sample with remains of infusion solution.

The first 10ml of blood from a catheter are not to be used as specimen material and should be discarded.

## 6.9 Order of draw

Filling blood collection tubes in the wrong order can also lead to sample contamination. The outside of a cap can be contaminated, meaning that bacteria can get into the sample.

For this reason, a blood culture sample should always be taken first. Anticoagulants or coagulation activators can be carried over into the next tube, or tissue fluids get into the tube due to problems.

**This is the recommended order of draw:**

1. No additive tube or blood culture
2. Citrate blood for coagulation diagnostics
3. Whole blood for serum
4. Heparin blood for plasma
5. EDTA blood for haematology
6. Na-fluoride blood for glucose determination
7. Other tubes

**Always adhere to the blood collection guidelines applicable within your institution or facility.**

-  If a blood culture sample is not required, a no-additive discard tube should be filled and discarded first.
-  If a citrate tube for coagulation diagnostics is used as the first or only tube, a 5ml no additive should be filled and discarded beforehand. This prevents impurities due to tissue thromboplastin.

## 6.10 Wrong anticoagulant

Thanks to the ISO 6710 international coding system for blood collection tubes, confusion is by and large avoided.

Nevertheless, carelessness or lack of knowledge can lead to taking the wrong anticoagulant or tube. Such samples can then no longer be used in the laboratory.

Use the correct anticoagulant or tube.

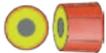
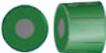
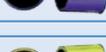
VACUETTE® tube type	Colour coding of cap	Additive	Intended purpose
Serum		Clot activator	Determinations in serum for clinical chemistry, microbiological serology, immunology, TDM
Serum Sep		Clot activator and separator	Determinations in serum for clinical chemistry, microbiological serology, immunology, TDM
Serum Crossmatch		Clot activator	Determinations in serum for crossmatch testing
Plasma		Sodium heparin	Determinations in heparinised plasma for clinical chemistry
Plasma		Lithium heparin	Determinations in heparinised plasma for clinical chemistry
Plasma Sep		Lithium heparin and separator	Determinations in heparinised plasma for clinical chemistry
EDTA		K2 EDTA K3 EDTA	Determinations in EDTA whole blood for haematology
EDTA Crossmatch		K3 EDTA	Determinations in EDTA whole blood for crossmatch testing
EDTA Sep		K2 EDTA and separator	Determinations in EDTA plasma for molecular biological identification of viruses, parasites and bacteria
Coagulation		Citrate solution (3.2%) Citrate solution (3.8%)	Determinations in citrated plasma for coagulation testing
CTAD		CTAD (3.2%)	Determinations in citrated plasma for coagulation testing where the artificial entry of platelet factors into the plasma is avoided
Glucose		Anticoagulant glycolysis inhibitor	Determinations in stabilised anti-coagulated whole blood or plasma for glucose and lactate testing
Trace Elements		Sodium Heparin	Determinations in heparinised plasma for trace elements testing
Blood Grouping		ACD-A ACD-B CPDA	Determinations in ACD / CPDA whole blood for blood grouping

Table 7: International Colour Coding according to ISO 6710

Parameter	Interfering Anticoagulants
Acid phosphatase	Citrate, EDTA, fluoride, heparin, oxalate
Albumin	Heparin
Alkaline phosphatase	Citrate, EDTA, fluoride, oxalate
Alpha-amylase	Citrate, EDTA, fluoride
Alpha-1-antitrypsin	Citrate, EDTA, oxalate
ALAT (GPT)	Oxalate
ASAT (GOT)	Oxalate
Bilirubin	Citrate, fluoride, oxalate
Calcium	Citrate, EDTA, oxalate
Cholesterol	Citrate, fluoride
Cholinesterase	EDTA, fluoride, heparin
CK-MB	Citrate, EDTA, fluoride, heparin, oxalate
Coeruloplasmin	EDTA
Copper	Citrate, EDTA, fluoride, oxalate
Creatine kinase (CK)	Citrate, fluoride, oxalate
Creatinine	Citrate, EDTA, fluoride
Erythrocyte sedimentation rate (ESR)	Heparin
γ-Glutamyltransferase	Citrate, fluoride, heparin, oxalate
GLDH	Fluoride
Glucose	Citrate, oxalate
HBDH	Oxalate
HDL-cholesterol	Citrate, fluoride
Insulin	Oxalate
Iron	Citrate, EDTA, heparin, oxalate
Iron-binding capacity	EDTA
Lactate dehydrogenase (LDH)	Fluoride, oxalate
LDL-cholesterol	Oxalate
Leucyl aminopeptidase (LAP)	Citrate, EDTA, fluoride, heparin, oxalate
Lipase	EDTA
Lipids	EDTA
Lipid electrophoresis	Oxalate
Lithium	Oxalate
Phosphate	Citrate
Potassium	Oxalate
Protein electrophoresis	Oxalate
Quick (Thromboplastin time)	Oxalate
Sodium	Citrate, EDTA, oxalate
T3 (triiodothyronine)	Oxalate
Triglycerides	Citrate, fluoride, oxalate
Urea	Fluoride
Uric acid	EDTA, citrate, fluoride
Vitamin B12	Oxalate

Table 8: Influence of anticoagulants on selected parameters

## 6.11 Expiry date

The vacuum in the tubes can only fulfil its function if used prior to the expiry date printed on the label. The tube should no longer be used after this date.

-  Always use up all tubes before opening a new carton.
-  First use products with the earliest expiry date.

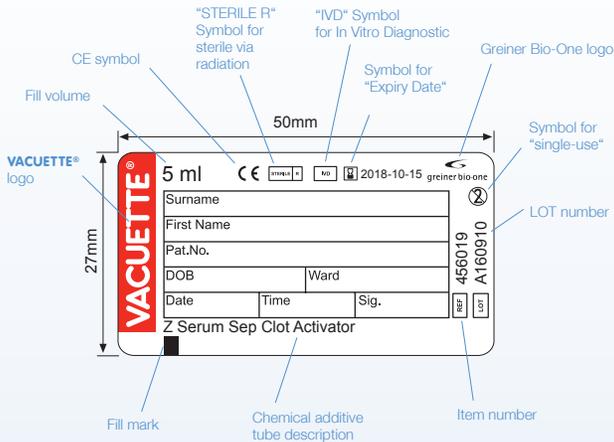


Fig. 18: Colour-coded label with expiry date according to ISO 6710

## 6.12 Mixing ratios and specimen volumes

It is absolutely essential, that tubes (in particular those with anticoagulant ratios) are filled exactly, taking fill tolerances into account.

Particularly serious errors can occur when citrate tubes for coagulation diagnostics are either over-filled or under-filled.

Fill tubes exactly and ensure correct mixing ratio.



Fig. 19: Fill tolerances for coagulation tubes

However, even tubes not containing anticoagulants arrive in the laboratory with incorrect fill levels. These samples are not false, but the specimen volume may be insufficient to measure all required parameters.

## 6.13 Mixing blood and tube additives

Nowadays, there are additives in nearly all specimen tubes. Even the supposedly "empty" tubes for serum contain additives for accelerating the blood coagulation. Tube contents must be mixed thoroughly and slowly immediately after blood collection, so that the additive can mix with the blood. Coagulation tubes are inverted 4-5 times, all other tubes 5-10 times.

All tubes should be **fully inverted 5 times** immediately after sample collection - **do not shake**. Even serum tubes contain additions and need to be inverted.

Particular care must be taken when mixing tubes with a high fill level and not much space.

An indicator for good mixing is the air bubble which moves through the tube from top to bottom during inversion.



Fig. 20: Air bubble as indicator for mixing

## 7. Frequent Errors When Storing and Transporting Samples

### 7.1 Storage temperatures and storage periods

The shelf-life of a sample is limited. Many samples can be kept at room temperature for a lengthy period, whilst others must be stored in the refrigerator or frozen.

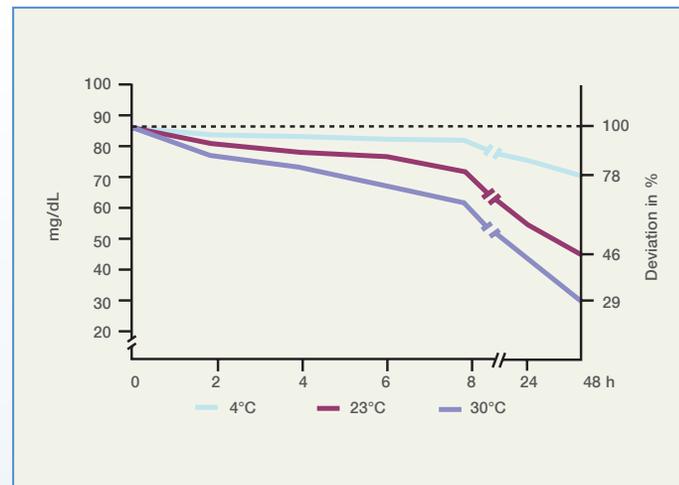


Fig. 21: Influence of time and temperature on, e.g. glucose

#### As a general guideline:

EDTA blood for haematology should be stored at room temperature. For blood cell counting it can be kept for 24 hours at room temperature, for differential blood counts 2 – 3 hours.

With a few exceptions, serum or plasma samples must be stored in the refrigerator at 4°C after cell separation.

Since blood cells are destroyed during the freezing process, only serum or plasma may be frozen.

For long-term storage of serum or plasma, temperatures of  $< -20^{\circ}\text{C}$  are necessary. The defrosting process must be slow, either overnight in the refrigerator or in a water bath, with continual mixing.

Your laboratory can tell you which samples require particular storage temperatures or must be deep frozen.

## 7.2 Storage conditions

If samples are not closed firmly during storage, evaporation can occur, which can change the concentration.

If serum or plasma is not separated from the cells, either via separator gel or after centrifugation by decanting, substances can seep from cells via diffusion into plasma or serum.

The cell wall is not destroyed during this process, as in haemolysis. However, the effects on the sample are similar, resulting in increased LDH and potassium values, for example.

Blood sugar is broken down via glycolysis. During this, the cells also absorb in vitro glucose from the serum or plasma, changing the blood sugar level continually over time.

If serum or plasma is not separated from cells, the process can lead to significant changes after just 2 – 3 hours.

-  Only store samples in closed containers.
-  Serum or plasma must be separated from cells immediately after centrifugation, either via separator gel or decanting.

## 7.3 Specimen transport

Due to the partly very short stability, samples should be brought to the laboratory as soon as possible.

If light-sensitive parameters are to be determined, e.g. bilirubin, the samples must be protected from light during transport and storage.

Extreme fluctuations of temperature during transport can have negative effects. When the temperatures are especially high, temperature stability with suitable isolating containers is essential.

It is advisable, to transport centrifuged tubes and tubes that are to be centrifuged later in an upright position.

-  Transport the samples as quickly as possible to the laboratory.
-  If necessary, protect from light.
-  Avoid extreme fluctuations of temperature.
-  Transport serum and plasma tubes in an upright position as far as possible.
-  Avoid spillages.

## 7.4 Specimen mailing

The ADR (Accord Européen Relatif au Transport International des Marchandises Dangereuses par Route) regulations are applicable for sample transport. The purpose is safe transport as well as protection of the sample and personnel.

With reference to infection risk, there are two categories for differentiation, category A and category B. Category A corresponds to the WHO risk classifications 3 and 4. Category B corresponds to the WHO risk classification 2. Samples in the WHO risk classification 1 are not affected by the ADR.



Fig. 22: Suitable transport containers

If category A specimens are to be transported, it is sufficient to pack according to the Instruction P620. P620 is basically comparable to P650, but must be design approved and licensed.

Category B specimens are subject to the instruction P650. The specimen is in a primary container, the secondary container is a liquid-proof, unbreakable container with an absorbent pad inside, sufficient to absorb entire contents. The outer packaging is a carton or padded envelope, labelled with "Diagnostic Specimen" or "Clinical Specimens". The UN symbol 3373 must also be printed on the outer packaging. (The number 3373 is a diamond shape).

As a general rule, when sending blood specimens for diagnostic purposes, the packaging instruction P650 according to ADR should be observed. Mailing of category B samples by post is only permissible as a small packet and if packed in accordance with the instruction P650.

If there is any suspicion, that the diagnostic specimen could contain category A pathogens, the laboratory must be contacted, in order to clarify the method of dispatch.

The sender is responsible for observing the legal requirements. Any members of staff involved in packing, sending and transporting samples must be trained accordingly.

Observe specimen shipping regulations.

## 8. Widespread Errors in Sample Preparation

### 8.1 Errors when centrifuging

Waiting too long before centrifugation can cause changes in serum / plasma above the cells (see chapter 7.2).

Specimen coagulation in upright tubes means better separation during centrifugation, in particular for tubes with separator gel.



Fig. 23: Samples that have coagulated in horizontal and upright positions.

If the waiting time for serum tubes before centrifugation is too short, and the blood has not been able to fully coagulate, post-clotting could occur in serum. This results in fibrin fibres in serum which may cause blockages in the tubing of the analyser. Furthermore, the gel in the separator gel tubes will not be able to form a sufficient barrier. Serum tubes should not be centrifuged within 30 minutes after blood collection.



Fig. 24: Serum sample after being centrifuged straight after blood collection. The fibrin thrombus in the serum can be seen.

For patients taking anticoagulation therapy, the clotting will be delayed. These samples should only be centrifuged when retraction (i.e. the blood clot has contracted) is completely finished.

It is not necessary to wait with plasma samples.

Extreme cooling down or warming up in the centrifuge can lead to haemolysis. The temperature inside the centrifuge should be 20°C – 22°C. (CLSI recommendation).

Centrifugation in open containers leads to evaporation of the sample, especially in case of smaller specimen volumes.

Therefore always ensure that specimen containers are firmly closed for centrifugation, and hygiene.

	Centrifugation speed	Duration
Coagulation tubes		
Platelet rich citrate plasma (PRP)	150 g	5 minutes
Platelet poor citrate plasma (PPP)	1500 – 2000 g	10 minutes
Platelet free citrate plasma	2500 – 3000 g	20 minutes
Serum tubes	1800 – 2200 g	10-15 minutes
Serum sep tubes	1800 – 2200 g	10-15 minutes
Heparin tubes	1800 – 2200 g	10-15 minutes
Heparin sep tubes	1800 – 2200 g	10-15 minutes
EDTA sep tubes	1800 – 2200 g	10-15 minutes
Glucose tubes	1800 – 2200 g	10-15 minutes

Table 9: Centrifugation recommendations of VACUETTE® tubes

The English terms g-force or RCF stand for relative centrifugal force and should not be confused with rotations per minute.

This formula is used for the calculation:

$$g = RCF = 1,118 \times 10^{-5} \times r \times (rpm)^2$$

g = RCF = relative centrifugal force

r = radius in cm

rpm = revolutions per minute



Fig. 25: Incorrectly centrifuged serum sep tubes: the wrong centrifugation speed has been applied. From left to right, the effects of an increasingly higher g-force. On the right, a correctly centrifuged serum sep tube.

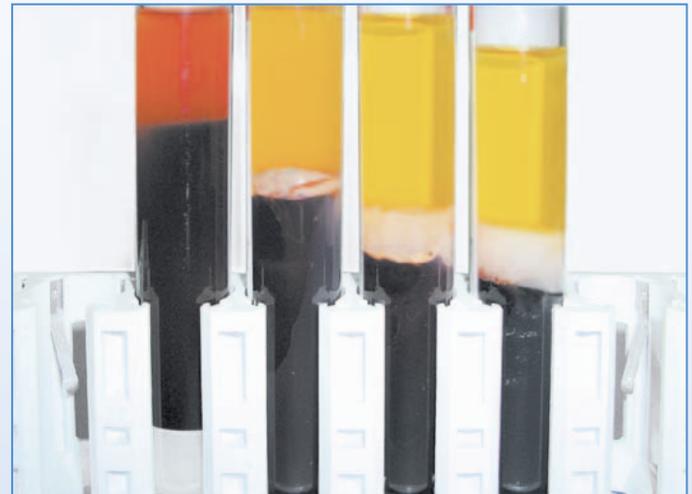


Fig. 26: Incorrectly centrifuged serum sep tubes: the tubes have been centrifuged for too long / insufficiently. From left to right, an increasing centrifugation length. On the right, a correctly centrifuged serum sep tube.

During centrifugation, the following should be observed:

- ☞ The serum specimen should be allowed to coagulate in an upright tube.
- ☞ Centrifuge as soon as possible, taking note of required waiting times.
- ☞ Select correct temperature in the centrifuge.
- ☞ Only centrifuge firmly closed specimens.
- ☞ Apply recommended duration and speed of centrifugation.

For an optimal gel barrier in serum sep tubes a swing-out centrifuge should be used.

If tubes that have been centrifuged in this manner are transported upright, the gel barrier remains stable even when shaken during transport from the doctor's surgery into the laboratory.

In a fixed-angle centrifuge, a sloping gel barrier will be formed. In this position, the gel barrier is less stable, and even the slightest of shakes during road transportation could cause the barrier to break down, especially if horizontal.

- ☞ Where possible, do not use a fixed-angle centrifuge, but rather a swing-out centrifuge.
- ☞ Transport centrifuged gel separator tubes in an upright position whenever possible.

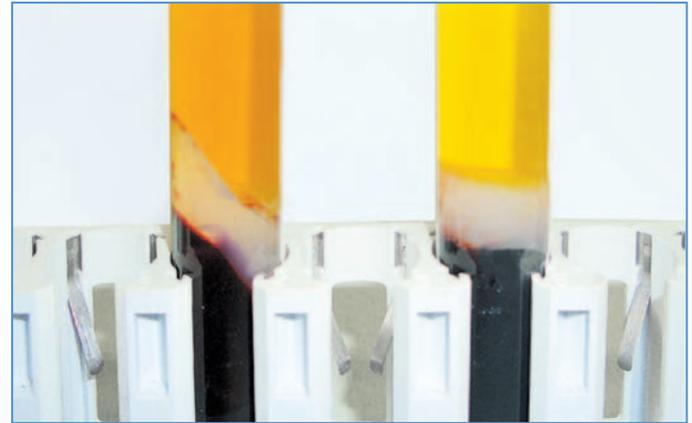


Fig. 27: Left: Separator gel tubes centrifuged in a fixed-angle centrifuge. Right: Separator gel tubes centrifuged in a swing-out centrifuge.

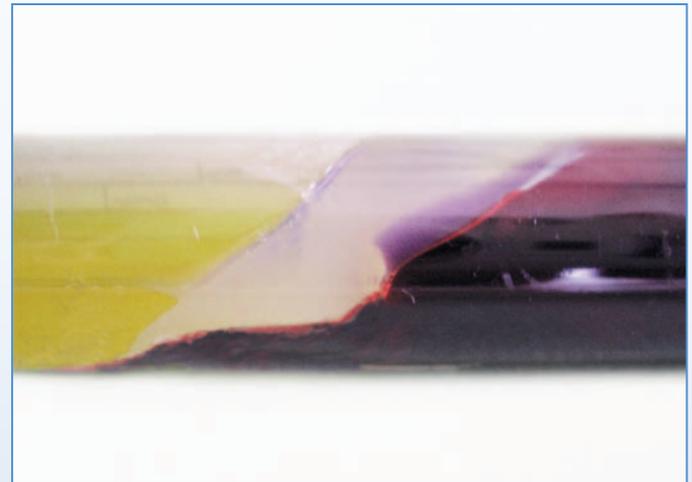


Fig. 28: Separator gel tubes centrifuged in fixed-angle centrifuges and transported horizontally. Jolts could break the gel barrier.

## 8.2 Insufficiently homogenised samples

Whole blood must be homogenous before being inserted into the analyser. EDTA whole blood, for example, must be mixed thoroughly before being used. Mechanical mixers are preferable.

A particular problem is when using blood collection tubes with a small diameter, for example erythrocyte sedimentation rate (ESR) tubes can occur, when samples are not sufficiently homogenised, resulting in increased sedimentation rates.

Special care must therefore be taken to mix ESR tubes thoroughly before placing them in the sedimentation stand, if the blood collection was some time ago, and the erythrocyte sedimentation has already started (see chapter 6.13).

Thawing causes serum or plasma specimens to have different concentration gradients. The substances dissolved in the specimen are no longer evenly distributed. These samples must of course be sufficiently mixed before being processed further.

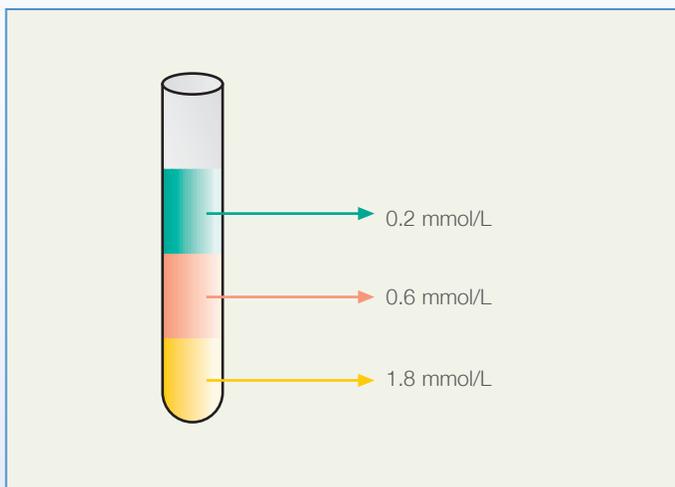


Fig. 29: Calcium concentration after thawing and before sufficient mixing

Always mix carefully prior to analysis, not just thawed samples but newly arrived samples.

## 9. Special Features of Blood Culture for Microbiological Diagnostics

Contaminants are a particularly frequent cause of interference in microbiological examinations of blood samples. Contaminating skin germs often get into blood culture bottles.

If the specimen is handled incorrectly, these germs can reproduce faster than the pathogen, resulting in an overgrowth of germs, which makes it difficult for the laboratory to find the actual pathogen.

Usually only a few pathogens are to be found in blood. The total of pathogens is highest when the fever is on the increase. This criteria must be taken into consideration when deciding on the time for collecting the sample.

Cooling the sample as well as changes to the pH value impair the survival chances of various pathogens. It is essential that the ideal transport conditions are provided.

Short transport times are important, as sensitive germs which can furthermore be weakened due to treatment with antibiotics, can quickly die off, whilst the contaminants can increase over a longer transport time. Prolonged transport times can therefore often cause inaccurate results.

### The following basics should be observed when collecting samples for blood cultures:

- Use blood culture bottles with the appropriate culture medium.
- Store blood culture bottles according to manufacturer's instructions.
- Allow to warm to room temperature before use.
- Always collect blood sample prior to beginning with antibiotic treatment.
- The sample should be collected during the shivers stage, when the patient has a fever. The germ density is at its highest during this phase. If antibiotic treatment has been started, the sample should be collected at the end of the dosage interval.
- It is very important to thoroughly disinfect the skin prior to sample collection. Apply the disinfectant, and allow at least 30 seconds

(or follow manufacturer's instructions) for it to take effect.  
Do not wipe off. After disinfection, do not touch the skin again.

-  The rubber stoppers to blood culture bottles must also be disinfected after the protective cap has been removed.
-  If several samples are to be taken, blood culture samples should be taken first.
-  Do not take samples using a horizontal catheter.
-  For collection, a closed system is used without decanting, whereby the blood culture bottle should be held below the venipuncture site.
-  To prevent air from getting into the anaerobic bottle, the aerobic bottle should be filled first.
-  Details on the suspected clinical diagnosis are to be given on the accompanying report.
-  Immediate transport to the laboratory.
-  Never store in the refrigerator.

If the in vitro multiplication of a fastidious pathogen is difficult or too time consuming, it is preferable to use molecular biological detection methods, e.g. PCR.

**When collecting samples for PCR analytics, particular care should be taken in the preanalytical stage:**

-  Always collect samples wearing disposable gloves.
-  Do not touch the puncture site again once disinfected, even if wearing gloves.
-  Always use a separate tube for samples.
-  **VACUETTE®** K2E K2EDTA Separator tubes are recommended.
-  Never decant samples.
-  Do not use heparin tubes.
-  Refer to the package insert of the Test Kit and treat specimen material as instructed.

## 10. Preanalytical Special Features in Urinology

In urine, substances usually eliminated in urine are detected and in pathological cases, even substances that do not normally occur in urine, for example metabolites, exogenic substances and cells in urine sediment.

Only a clean and correctly collected urine sample can provide accurate results.

### 10.1 When should a urine sample be taken

There is a difference between random urine, morning urine and collection urine.

#### 10.1.1 Random urine

Random urine is taken at any time. This is the simplest form of urine collection, and is generally only useful if the clinical symptoms indicate that an immediate analysis is necessary, for example, if there is a suspicion of urinary tract infection or intoxication.

#### 10.1.2 Morning urine

There is a further differentiation to be made between first morning urine and second morning urine.

First morning urine is often sour and concentrated, making it suitable for detection of bacteria. The second morning urine is after a time interval has elapsed following emptying of the bladder in the morning.

This kind of sample is recommended for determining hyperglycosuria and for examination of the urine sediment.

**Ensure the following when collecting second morning urine:**

-  If required, patient has empty stomach.
-  No sport participation prior to sample.

### 10.1.3 24 hour urine collection

The urine is collected over a 24 hour period. This balances out fluctuations occurring throughout the day. Collection errors occur frequently and can be avoided by giving careful, exact instructions to the patient.

#### Ensure the following when collecting 24-hour urine:

- ☞ Intake of 1.5 - 2 litres liquid throughout the 24 hours.
- ☞ If the urine must be stabilised, add appropriate preservatives.
- ☞ Begin at 7am in the morning.
- ☞ Discard first morning urine.
- ☞ Collect all urine throughout the 24-hour period until the first morning urine of the next day.
- ☞ Hygienic conditions.
- ☞ Cool storage conditions, protected from light.
- ☞ Exact measurement of the collection volume.
- ☞ Mix the urine thoroughly.
- ☞ Transfer required amount into specimen tube.
- ☞ Give the patient exact instructions on urine collection, as the completeness of collection and quality of sample is dependent on the patient's cooperation.

## 10.2 Techniques for collecting and preparing urine

### 10.2.1 Mid-stream urine

If possible, mid-stream urine should be used for all urine examinations. By collecting mid-stream urine, contamination by foreign bacteria is effectively prevented

#### Take note of the following when collecting mid-stream urine:

- ☞ Thorough cleansing of genital area.
- ☞ Do not use cleaning substances or disinfectants.
- ☞ If cleansing is too intensive, slight bleeding can occur, causing addition of erythrocytes.
- ☞ The first amount of urine contains contaminants and is discarded.
- ☞ The second amount of urine is collected in a sterile beaker without interrupting the stream.
- ☞ The end stream is discarded.
- ☞ Urine is mixed well in the beaker and is transferred to a urine tube.
- ☞ The urine sample should be brought to the laboratory within 2 hours.



Fig. 30: Sample is transferred from urine beaker to urine tube

Bladder catheters and bladder puncture are reserved for special cases.

### 10.2.2 Urinary sediment

To make urine sediment, a defined part of the urine specimen is first centrifuged. The supernatant is decanted. The sediment is homogenised and finally microscopied.

The sample should not be more than 2 hours old, otherwise sedimenting uric acid crystals, lysis and morphological changes of cylinders and cells could influence the analytics.

**To obtain a standardised sediment, the following should be observed:**

-  Use at least 10ml mid-stream urine, already mixed.
-  Centrifuge for 5 minutes at 400g.
-  Discard 9.5ml of the supernatant.
-  Add the remaining 0.5ml to the analysis.
-  The sample may not be more than 2 hours old.

### 10.3 Microbiological urine examinations

For microbiological urine examinations, a mid-stream from first morning urine is preferable.

**The following should be ensured during collection:**

-  Take urine prior to beginning antibiotics treatment.
-  Use the first morning urine – the patient should not pass water after 2 am.
-  Use mid-stream urine, see chapter 10.2.1
-  After mixing, transfer urine from the sterile beaker to a sterile specimen tube, and close the tube firmly.
-  If using an immersion culture medium, please observe the instructions for use.
-  Transport quickly to laboratory.
-  For long-term catheter users, do not use the urine from the collection bag. Instead, puncture the intended site after careful disinfection.

### 10.4 Drug screening

During drug screening, it is not unusual for a drug user to attempt to manipulate urine samples in order to cause falsely negative results.

This can be brought about by diluting, excessive drinking or even by providing foreign urine or by adding substances which could distort the analysis (e.g. washing powder or similar).

To a large extent, this can be prevented, for example, by checking identity and supervising the urine collection, as well as determining the creatinine concentration as control value.

With supervised saliva tests, this problem can be completely avoided.

## 11. Drug detection from saliva

Substances can be detected in saliva, which are produced either by the saliva glands or get into saliva from the blood by means of passive diffusion, active transport or ultrafiltration. This is made possible, mainly because in comparison to blood, saliva has a slightly acidic pH-value and behaves hypotonically.

Only a clean, correctly collected saliva sample ensures a correct analysis result.



Fig. 31: Sample is transferred from the saliva collection beaker to the saliva transfer tube.

Please observe the following when collecting saliva:

- Wait 10 minutes to ensure an empty oral cavity.
- Monitor the collection with the patient.
- Keep to the recommended collection time.

Drug analysis using saliva is becoming increasingly common. It is significant, that the saliva is collected on an acidic basis, as then it is easier for drugs, which are mainly alkaline, to diffuse in saliva.

Detecting falsification of a sample or sabotage during saliva collection is the big challenge in the area of drug testing.

The simplest way of doing this is with water in the oral cavity. The authenticity of the sample can be checked by determining endogenous biomarkers, for example saliva amylase or cortisol.

## 12. Summary of Tips for Avoiding Errors

### Patient preparation:

- ☞ Inform patient of abstinence from food and give dietary instructions.
- ☞ Remind the patient that physical activity, e.g. jogging is not allowed.
- ☞ Indicate to patient that smoking, coffee and alcohol should be abstained from.
- ☞ Establish medication intake and dose.
- ☞ Get the doctor's order and request permission from the patient.

### Identification:

- ☞ Clearly identify the patient.
- ☞ Enter patient data fully and accurately.
- ☞ Write clearly.
- ☞ Label STAT samples.
- ☞ Label infectious material.
- ☞ Write on label legibly with a waterproof pen.
- ☞ Position label correctly.
- ☞ Write on label prior to blood collection, then attach (if applicable). NOTE: Timing of labelling varies from country to country.
- ☞ Never attach label to transport tube!  
Label the specimen tube.

### Blood collection:

- ☞ Select correct anticoagulant and tubes.
- ☞ Take the blood sample between 7 am and 9 am.

- ☞ Reduce fear and stress, especially for children.
- ☞ Create a calm atmosphere.
- ☞ Outpatients should sit quietly for 5 minutes prior to blood collection.
- ☞ Take the sample from the patient lying down, wherever possible (outpatients sitting).
- ☞ No opening and closing of fist for patient.
- ☞ No hard tapping on vein.
- ☞ Do not apply tourniquet for longer than 60 seconds. Arterial blood flow may not be interrupted.
- ☞ Do not apply tourniquet too tightly (40mmHg) - it should still be possible to feel pulse.
- ☞ Allow the disinfectant to dry according to instructions.
- ☞ Carry out the venipuncture correctly, refer to the "VACUETTE® Blood Collection Techniques" booklet.
- ☞ Do not probe tissue to locate the vein.
- ☞ If possible, do not collect from a catheter.
- ☞ Release the tourniquet upon successful venipuncture as soon as blood flows into the first tube.
- ☞ Follow the recommended order of draw for blood collection tubes.
- ☞ Check the fill mark.
- ☞ Fill tube completely.
- ☞ After blood collection, mix the tube contents thoroughly.
- ☞ Mix tube contents gently, do not shake.
- ☞ Avoid transferring blood from syringes into other containers.

### Storage and transport:

- ☞ Avoid temperature fluctuations, e.g. direct sunlight.
- ☞ Ensure that tubes are closed firmly for storage and transport.
- ☞ Keep serum and plasma cool at 4°C.

- ☞ Thaw out frozen samples slowly in a refrigerator or in water bath, mixing constantly.
- ☞ Do not re-freeze thawed samples.
- ☞ Samples should be transported to the laboratory as quickly and smoothly as possible, if necessary, cooled.
- ☞ Transport serum and plasma samples in an upright position where possible.
- ☞ Pay attention to light protection in case of light-sensitive parameters.
- ☞ Pay attention to sample transport regulations.

### Sample preparation:

- ☞ Let serum samples coagulate fully for around 30 minutes in upright tubes, then centrifuge.
- ☞ For serum samples from patients having anticoagulant treatment, wait at least 60 minutes or until clot retraction is complete.
- ☞ Plasma samples can be centrifuged immediately.
- ☞ Set the correct temperature in a cooling centrifuge.
- ☞ Observe the specified length and speed of centrifugation.
- ☞ Differentiate between g-force and rotations per minute.
- ☞ Always ensure tubes are closed before centrifuging.
- ☞ Use serum or plasma soon after centrifugation of cells, or use separator gel tubes.
- ☞ Mix carefully prior to analysis – even thawed samples.
- ☞ Mix ESR tubes thoroughly before placing in sedimentation stand or ESR analyser.

### Blood culture:

- ☞ Use blood culture bottles with appropriate culture medium.
- ☞ Store blood culture bottles according to manufacturer's instructions.

- ☞ Warm to room temperature before use.
- ☞ Always take a first blood culture before beginning antibiotic treatment.
- ☞ Collect blood during the shivers stage when the patient has a fever, and if antibiotic treatment has already started, at the end of a dosage interval.
- ☞ Rub in disinfectant and allow to take effect for at least 30 seconds, according to manufacturer's instructions. Do not wipe.
- ☞ Do not touch the puncture site after disinfection.
- ☞ Also disinfect the stopper of the blood culture bottle when the protective cap is removed.
- ☞ Take the sample for blood culture first.
- ☞ Do not take samples from horizontal catheters.
- ☞ Use a closed collection system for taking samples. No decanting.
- ☞ Observe the manufacturer's instructions when filling aerobic or anaerobic blood culture bottles.
- ☞ Enter data regarding suspected clinical diagnosis in accompanying report.
- ☞ During blood collection, hold blood culture bottle below puncture site.
- ☞ Transport immediately to laboratory.
- ☞ Never store in the refrigerator.

### PCR diagnostics:

- ☞ Always wear fresh disposable gloves when taking samples.
- ☞ Always use separate tubes.
- ☞ Never decant samples.
- ☞ Do not use heparin tubes.

### Morning urine:

- ☞ If required, food abstinence for patient.
- ☞ No early morning sport prior to sample.

### 24 hour collection urine:

- ☞ Liquid intake of 1.5 – 2 litres over the 24 hours.
- ☞ If the urine is to be stabilised, add appropriate preservative.
- ☞ Begin at 7 am.
- ☞ Discard first morning urine.
- ☞ Collect all amounts of urine up until the next morning, including the first morning urine of the next day.
- ☞ Ensure hygienic conditions.
- ☞ Keep urine cool and protected from light.
- ☞ Measure collection volume exactly.
- ☞ Mix urine well.
- ☞ Transfer required amount into specimen tube.
- ☞ Give the patient precise instructions on collecting urine.

### Mid-stream urine:

- ☞ Thorough cleansing of genital area.
- ☞ Do not use cleansing substances or disinfectants.
- ☞ Intensive cleaning can cause slight bleeding and admixture of erythrocytes.
- ☞ The first urine amount contains contamination germs and is discarded.
- ☞ The second amount is collected in a sterile beaker without interrupting the stream. The end stream is discarded.
- ☞ Mix urine in beaker well and transfer to a urine tube.
- ☞ Transport the sample immediately into laboratory.

### Urinary sediment:

- ☞ Use 10ml mid-stream urine, already mixed.
- ☞ Centrifuge for 5 minutes at 400g.
- ☞ Discard 9.5ml supernatant.
- ☞ Use the remaining 0.5ml for the analysis.
- ☞ The sample may not be more than 2 hours old.

### Urine culture:

- ☞ Take urine before beginning antibiotics treatment.
- ☞ Use the first morning urine – the patient should no longer pass water after 2am.
- ☞ Use mid-stream urine.
- ☞ After mixing the urine in the sterile beaker, transfer to a sterile specimen tube and close tube firmly.
- ☞ If using immersion culture media, pay attention to application instructions.
- ☞ Quickly transport to laboratory.
- ☞ For long-term catheter users, never take urine from collection bag.

### Saliva Collection:

- ☞ Wait 10 minutes to guarantee empty oral cavity.
- ☞ Monitor sample collection.
- ☞ Keep to the recommended collection time.

## 13. Literature

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