

making a difference



PREANALYTICS MANUAL

Preanalytics
Handling
Recommendations


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PREANALYTICS MANUAL

Preanalytics Handling Recommendations

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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

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INTRODUCTION

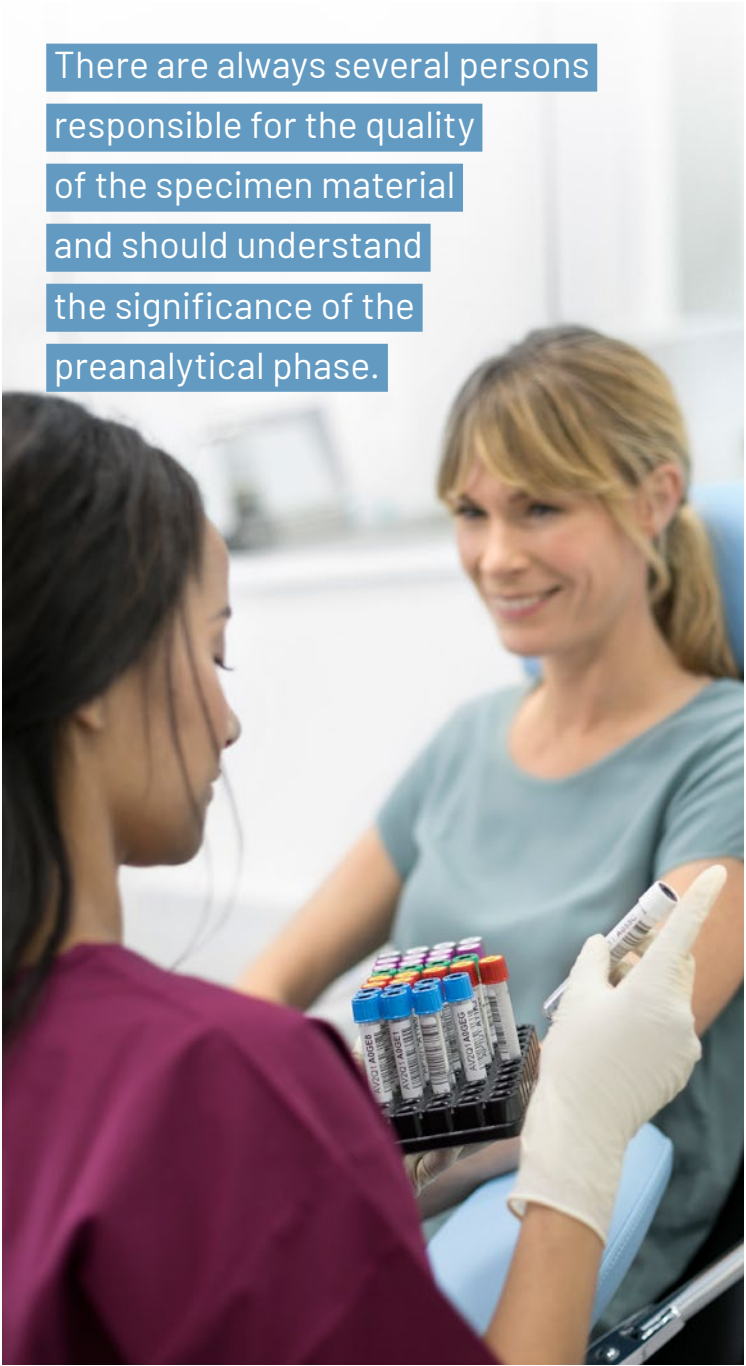
THE TERM “PREANALYTICS” REFERS TO THE ENTIRE ADMINISTRATIVE AND PRACTICAL PROCESS OF COLLECTING, PROCESSING, STORING AND TRANSPORTING DIAGNOSTIC EXAMINATION MATERIAL PRIOR TO CARRYING OUT LABORATORY TESTS.

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.



There are always several persons responsible for the quality of the specimen material and should understand the significance of the preanalytical phase.

PERSONS INVOLVED IN PREANALYTICS:

- / Patient
- / Treating doctor
- / Nurse
- / Transport service
- / Medical technical assistant
- / 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 during and after the preanalytical phase and responsible personnel	
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, lab staff
Mixing blood sample:	Doctor, nursing staff, doctor's assistant, lab staff
Storage until transportation:	Nursing staff, doctor's assistant, lab staff
Transportation:	Collection or courier service
Acceptance, storage and preparation of samples:	Laboratory staff, medical technical assistants, laboratory doctors

The time required for the preanalytical phase is often underestimated and yet it takes up more time in the diagnostic process than the time required for laboratory analysis. Thanks to modern technology, the actual analysis takes little time.

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.

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.



NON-CHANGEABLE INFLUENCING FACTORS

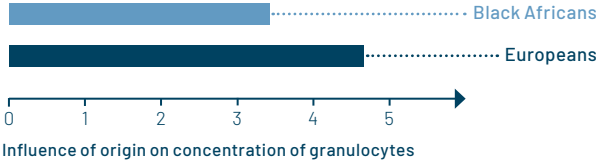
GENDER

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.

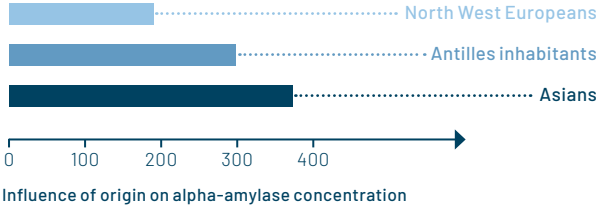
Parameters	Man	Woman	Unit
Alanine aminotransferase	< 50	< 35	U/l
Iron	6.3 - 30.1	4.1 - 24	µmol/l
Ferritin	18 - 360	9 - 140	µg/l
Uric acid	3.6 - 7	2.3 - 6.1	mg/dl
Creatinine, Jaffé reaction kinetic	0.81 - 1.44	0.66 - 1.09	mg/dl
Haematocrit	40 - 53	36 - 48	%
Haemoglobin	13.5 - 17.5	12 - 16 g	g/dl
Erythrocyte sedimentation rate	< 15	< 20	mm/1h

Gender specific differences of selected parameters
Source: Thomas L.: Labor und Diagnose 6th Edition

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.

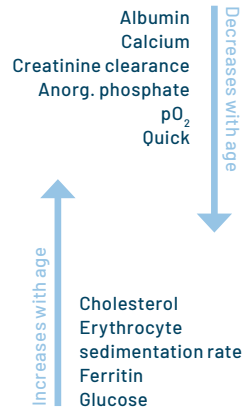


The **alpha-amylase concentration** for North-West Europeans is significantly different to that of the inhabitants of the Antilles and Asia. Around 50% of the values, taken from Antilles inhabitants, were pathologically evaluated, compared with British normal values.

LONG-TERM
CHANGEABLE
INFLUENCING FACTORS

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.



BODY WEIGHT

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

PREGNANCY

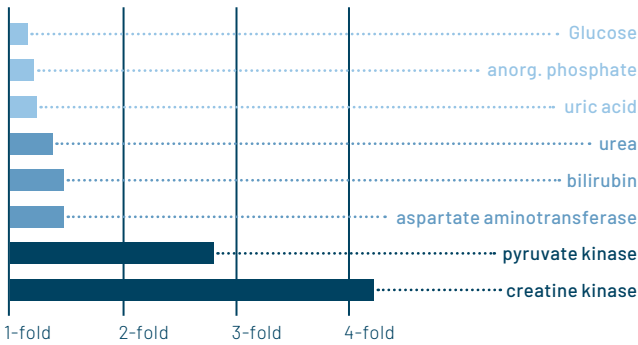
During pregnancy, the plasma volume increases by around 50%. Concentration changes can be seen in a range of parameters; important electrolytes are reduced, blood lipids are elevated, copper is doubled.

FOR CORRECT CLASSIFICATION
OF THE REFERENCE RANGES

correct patient data on the requisition
form is an essential condition.

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.



Change of various serum concentrations after
extreme physical activity – marathon run

SHORT-TERM
CHANGEABLE
INFLUENCING FACTORS

DAILY RHYTHMS AND BIORHYTHMS

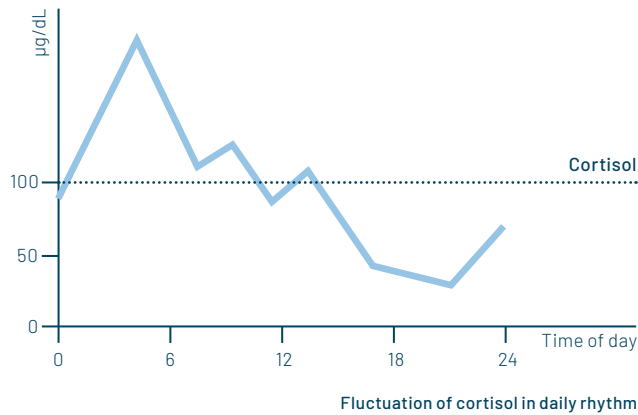
Various parameters change over the daily rhythm.
Some parameters will have their maximum in the morning,
some at midday or in the evening.

Maximum fluctuations in course of day as %

Maximum in the morning			
Adrenocorticotrophic (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 %	Potassium	15 %
Eosinophil granulocytes	30 %		
Maximum in the evening			
Acid phosphatase	200 %	Uric acid	50 %
Creatinine	100 %	Thyretropine(TSH)	50 %

Fluctuation over course of day in the daily rhythm

BY ADHERING TO THE
RECOMMENDED SPECIMEN
COLLECTION TIME OF
BETWEEN 7 AM AND 9 AM
the influence of fluctuations in
the daily rhythm is minimised.



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.

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. (see chapter „Body position“ on page 38 and „Intensity and Duration of the stasis“ on page 40)

- / 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.

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.

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. Long-term fasting can also influence laboratory results.

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.



Samples with varying degrees of cloudiness

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

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

- / 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.

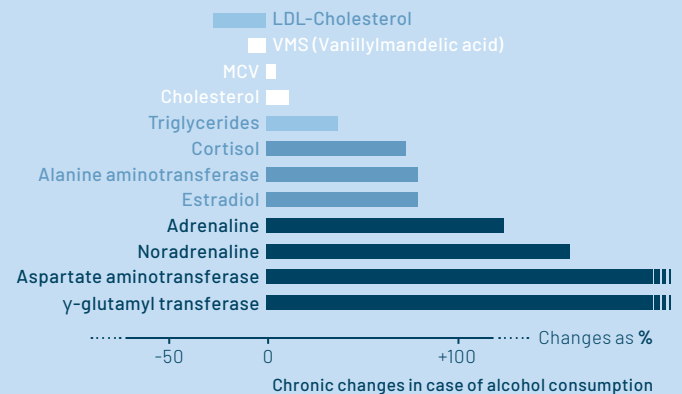
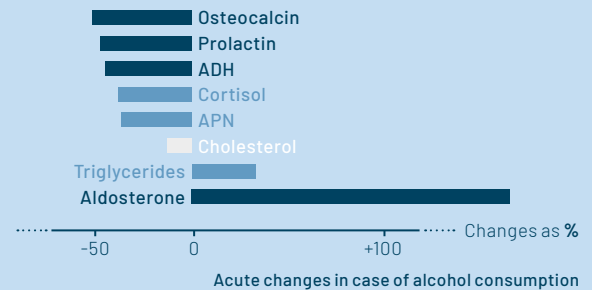
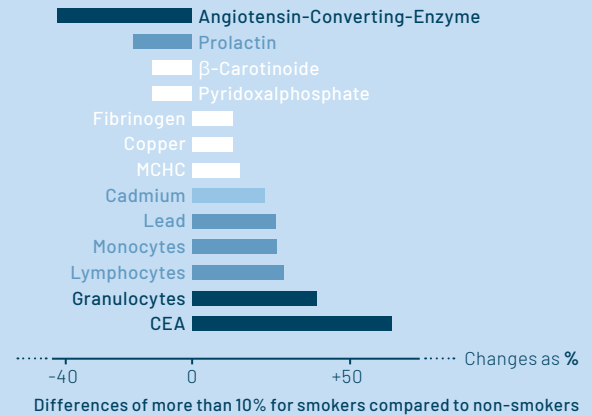
STIMULANTS: COFFEE, NICOTINE, ALCOHOL

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.

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.



DRUGS

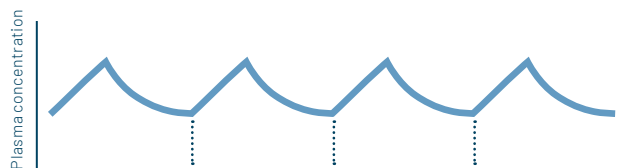
Drug consumption has biological effects, which can influence laboratory examinations, whereby every drug has its own effects.

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



The ideal collection times for measuring medication levels are just before next intake

**THOROUGHLY
PREPARING THE
PATIENT CAN HELP
AVOID ERRORS.**

**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.**

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.

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.

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.

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

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.



Incorrectly attached labels, compared to correctly attached label (left)

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



**TO AVOID ERRORS
IF LABELS FOR
AUTOMATIC READING
ARE USED, SPECIAL
ATTENTION SHOULD
BE PAID TO THE
PREPARATION OF
WHOLE TUBE SERIES
TO AVOID MIXING
UP TUBES.**

TO OBSERVE:

- / Fill in label carefully and legibly.
- / Only use waterproof pens.
- / Label STAT samples specially.
- / Always position the label correctly.
- / Stick the label onto the collection tube, never on the transport tube.

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.

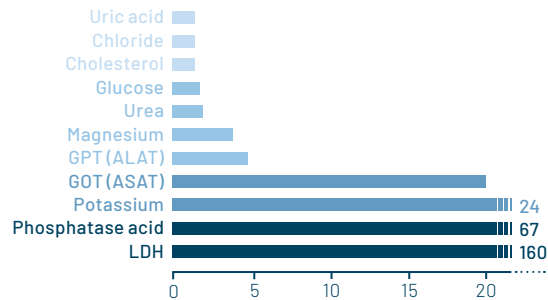
Individual activities, the formation of haemolysis will be covered in detail, as well as how it can be avoided.

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.

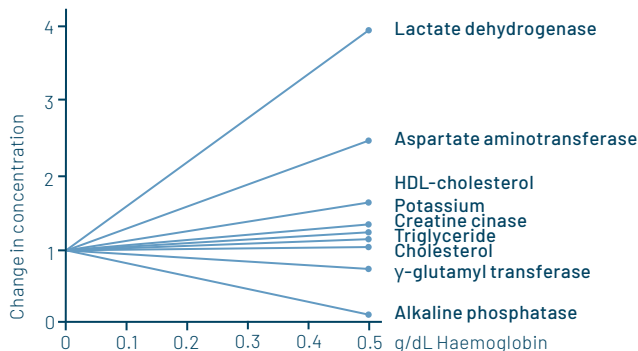
The serum or plasma, turns red due to the haemoglobin from the erythrocytes. As of a haemoglobin concentration of around 0.03 g/dL it is possible to see the discolouration with the naked eye. The intensity of the haemolysis is indicated by the intensity of the red colouring.

HAEMOLYSIS HAS A THREE-FOLD EFFECT:

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



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



Changes in different parameters at a
haemoglobin concentration of 0.5 g/dL



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.

WIDESPREAD ERRORS MADE DURING BLOOD COLLECTION

ERRORS ARE OFTEN
MADE DURING BLOOD
COLLECTION. THEY
CAN AFFECT BOTH
PATIENT WELL-BEING
AND THE SUBSEQUENT
LABORATORY ANALYSIS.

In addition to comprehensive patient preparation and correct blood collection, the collection time also plays an important role and circadian fluctuations in parameters should be taken into account. Filling different blood collection tubes in the wrong order or choosing the wrong anticoagulant may lead to the sample being falsified. Such samples are then unusable for the laboratory.

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 „Short-term changeable influencing factors“ on page 18). 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.

TIME OF BLOOD COLLECTION

The influence of fluctuations due to daily rhythm (see chapter „Daily rhythms and biorhythms“ on page 18) 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.

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.

Increase when changing from lying down to sitting up	Parameters
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

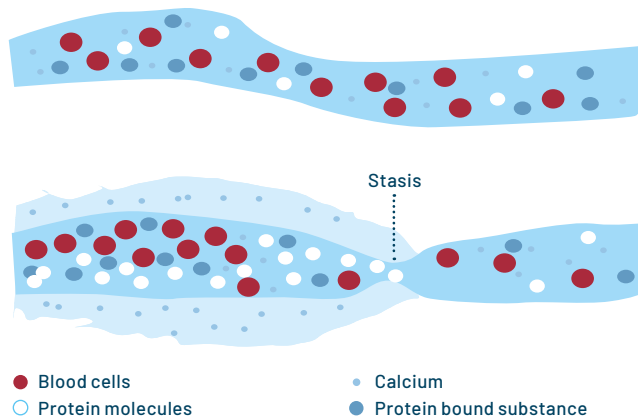
Influence of body position during sample collection

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.

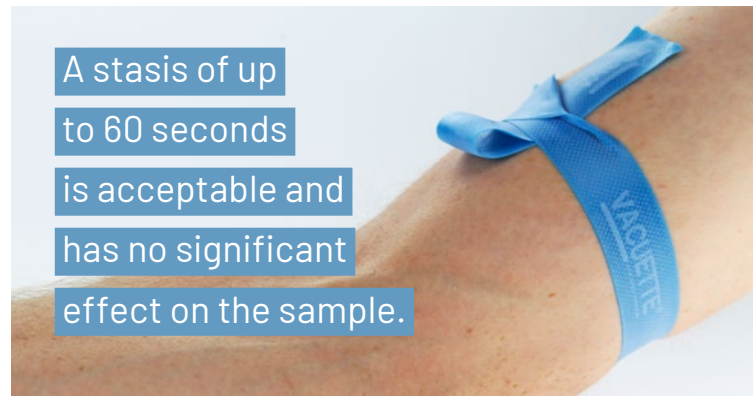
INTENSITY AND DURATION OF THE STASIS

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 „Body position“. The change in concentration depends on length and intensity of the stasis.

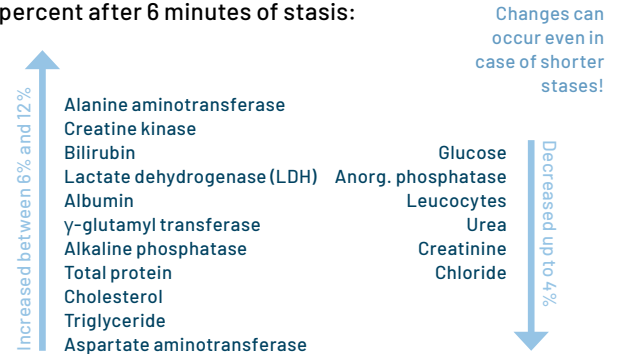


Haemoconcentration due to transfer of plasma and small molecules from intravascular to interstitial space

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.



Change in different parameters as percent after 6 minutes of stasis:



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.

- / The tourniquet should not be applied too tightly – it should still be possible to feel the pulse.
- / In case of good veins, the tourniquet should be loosened immediately on successful puncture of the vein, before the blood collection is started.

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.

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.

IMPROPER TECHNIQUES ARE

opening and closing the fist as well as tapping of the puncture site!



In the booklet "VACUETTE® Blood Collection Techniques", the procedure for successful venipuncture is described.

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.

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.

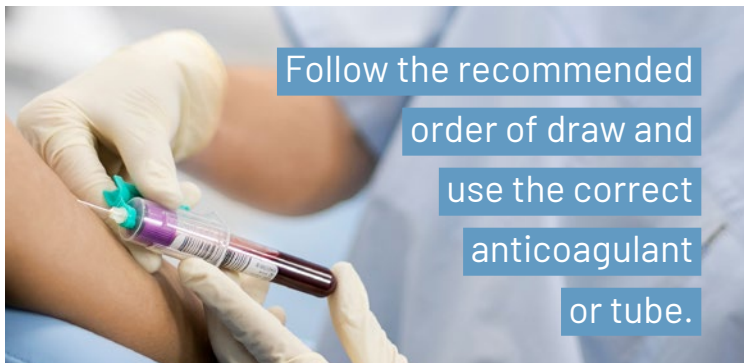
DO NOT PROBE,
when locating the vein. If necessary, puncture the other arm.

COLLECTION FROM CATHETER

Blood collection directly from an IV catheter is an option as long as the intended use of the catheter allows this. Accessories with Luer lock or Luer slip connectors are recommended for this. Internal guidelines are to be followed at all times.



Luer-Lock on the example of the VACUETTE® SAFELINK (left) and Luer-Slip on the example of the HOLDEX® Single-Use Holder (right).



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.

* If a winged blood collection set is used, the first tube in the series will be under-filled. Therefore, if a Sodium Citrate specimen is drawn first, a tube without additive is recommended to be drawn prior to this tube to ensure the proper additive-to-blood ratio.

In addition, even though studies have shown that PT and aPTT tests are not affected if drawn first in a tube series, it is advisable to draw a second tube for other coagulation assays, since it is not known whether or not these tests will be affected.

(CLSI GP41-A7 Order of Draw p. 26)

- 1 **Blood Culture**
 - 2 **Sodium Citrate/CTAD***
 - 3 **Serum**
with and without Separator
 - 4 **Heparin**
with and without Separator
 - 5 **EDTA**
with and without Separator
 - 6 **Glycolytic Inhibitor**
 - 7 **Other Additives**
-

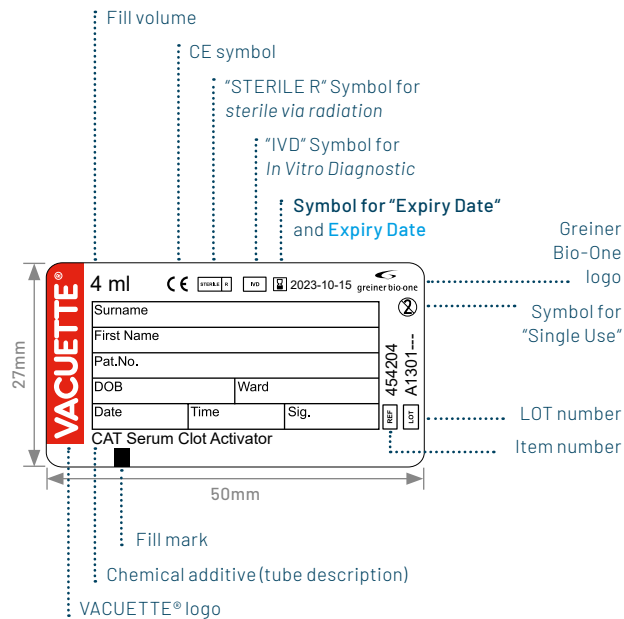
WRONG ANTICOAGULANT

Thanks to the coding system for blood collection tubes, confusion is largely 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.

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.

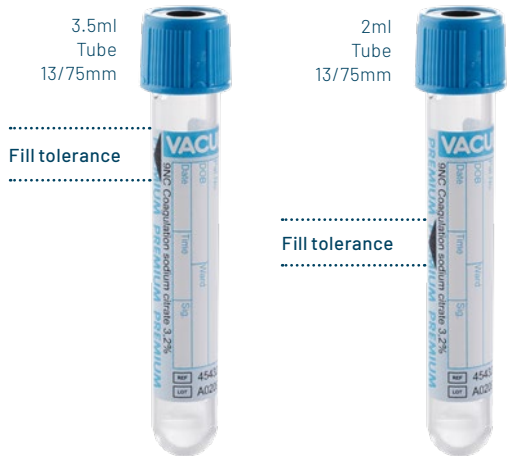


Colour-coded label with expiry date according to ISO 6710

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

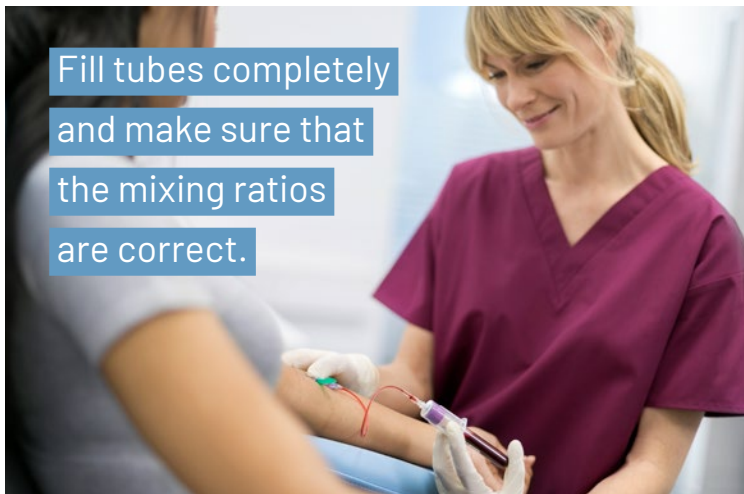
MIXING RATIOS AND SPECIMEN VOLUMES

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.



The fill tolerances correspond to the international standard ISO 6710.

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



Fill tubes completely
and make sure that
the mixing ratios
are correct.

If using a winged blood collection set, the first tube taken may be underfilled. For this reason, in case of taking a sodium citrate sample first, it is recommended to begin with a discard tube (no additive) to ensure the correct additive-to-blood ratio.

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 removal from the holder, so that the additive can mix with the blood. Coagulation tubes are inverted 4-5 times, all other tubes 5-10 times (FC Mix tubes 10 times).

- / All tubes should be fully inverted 5 times immediately after sample collection – **do not shake!**
- / Even serum tubes contain additives 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:



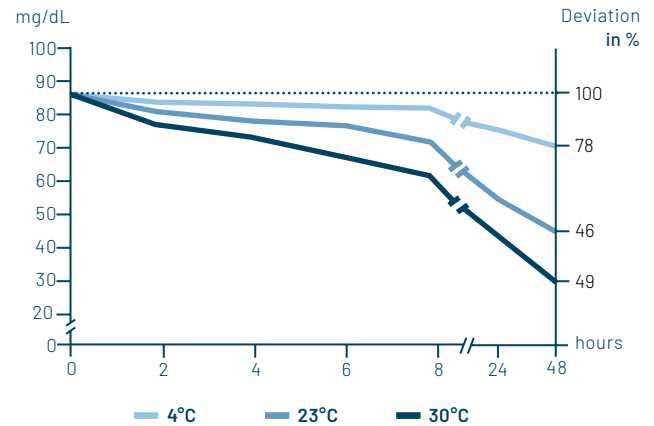
Air bubble as indicator for mixing

FREQUENT ERRORS WHEN STORING AND TRANSPORTING SAMPLES

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.


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



Influence of time and temperature on, e.g. glucose without stabiliser

**FOR INFORMATION ON THE
CORRECT SAMPLE MATERIAL,
STORAGE AND STABILITY,**

please refer to the instructions
for use of the assay used!



Only store samples
in closed containers
to avoid evaporation.

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 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 hours.

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

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

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.

SPECIMEN MAILING

The ADR (*Accord Européen Relatif au Transport International des Marchandises Dangereuses par Route*) regulations are applicable for sample transport.

This is a European Agreement concerning the International Carriage of Dangerous Goods by Road. 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: Infectious substance

Category B: Biological substances

The shipment of blood samples for diagnostic purposes generally falls into Category B. If a diagnostic sample is suspected of containing a Category A pathogen, the regulations for the shipment for Category A substances must be observed.

When shipping Category A samples, the samples must be packed in accordance with instruction P620 for infectious substances.

When shipping Category B samples, the samples must be packed in accordance with instruction P650 for biological substances. The shipment of Category B materials must be assigned to UN3373.



The packaging of patient samples must consist of three components:

1. Leakproof primary container with sample (certified for 95 kPa)
2. Leakproof secondary packaging with absorbent insert
3. Sufficiently strong outer packaging

The label "Biological Substance, Category B" and the UN symbol "UN3373" must be visibly printed on the outer packaging.

The shipper is always responsible for the classification, identification, packaging, marking, labelling and required documentation of a hazardous substance under the Dangerous Goods Regulations. Employees who pack, ship and transport samples must be appropriately trained.

**OBSERVE SPECIMEN
SHIPPING REGULATIONS!**

WIDESPREAD ERRORS IN SAMPLE PREPARATION

ERRORS WHEN CENTRIFUGING

Waiting too long before centrifugation can cause changes in serum / plasma above the cells. (see chapter „Storage conditions“ on page 53)

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



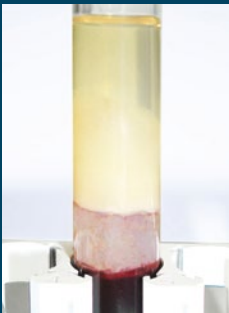
Samples that have coagulated in horizontal and upright positions.

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. Standard serum tubes should not be centrifuged within 30 minutes after blood collection.

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. Serum samples should only be centrifuged when clotting is complete.

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 recommendation¹⁰). According to the WHO, 18-25°C are also tolerable.

Centrifuging for too long or at too high a speed can also lead to haemolysis.

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 recommendations of VACUETTE® tubes:

Tube type	Inversions (mixing)	Recommended g-force / relative centrifugal force(RCF)	Time [min]
Serum Fast with Separator	5-10 times	1800g	10
		3000g	5
Serum Tube with/without Separator		1800-2200g	10-15
EDTA Tube with/without Separator			
Heparin Plasma Tube with/without Separator			
Standard Glucose Tube	10 times	2000-2200g	10
Homocystein Detection Tube		1800g	10
VACUETTE® FC Mix Tube	10 times	1800g	10
Coagulation Tubes			
- Platelet tests (PRP)	4-5 times	150g	5
- Routine tests (PPP)		1500-2000g	10
- Preparation for deep freeze plasma (PFP)		2500-3000g	20

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

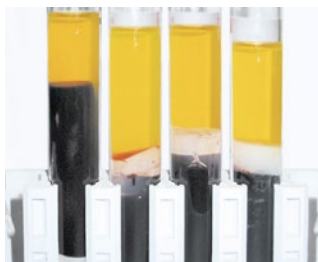
FOR AN OPTIMAL GEL BARRIER

in serum sep tubes a swing-out centrifuge should be used and the recommended duration and speed of centrifugation should be applied.

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.



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.



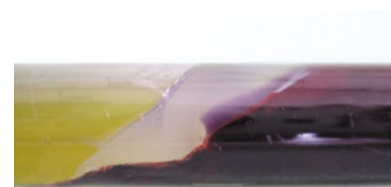
In a fixed-angle centrifuge, a sloping gel barrier will be formed. In this position, the gel barrier is less stable, and even the slightest of shakes during road transportation could cause the barrier to break down, especially if horizontal.

Left: Separator gel tubes centrifuged in a fixed-angle centrifuge

Right: Separator gel tubes centrifuged in a swing-out centrifuge



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



- / 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.



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.

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 suitable.



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 „Mixing blood and tube additives“ on page 48)

**ALWAYS MIX CAREFULLY
PRIOR TO ANALYSIS,**
not just thawed samples but
newly arrived samples.

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.

IDEAL TRANSPORT CONDITIONS

must be ensured.

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 according to manufacturer's instructions.
- / Always collect blood sample prior to beginning with antibiotic treatment.
- / 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.

- / Fresh puncture should always be preferred to collection from catheters. However, there are exceptions, for example if contamination of the catheter is suspected.
- / Follow the manufacturer's instructions for use when filling aerobic and anaerobic blood culture bottles.
- / All information that is relevant for a quick and correct performance of the requested analyses should be provided on the consignment note.
- / Immediate transport to the laboratory.
- / Never store in the refrigerator.

If the in vitro multiplication of a fastidious pathogen, such as a virus, 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.

The recommendations are largely taken from the international standard CLSI M47 Ed2. Always follow the recommendations of the manufacturer of the blood culture bottles!



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.

When collecting samples, there
is a difference between random
urine, morning urine and
collection urine.

WHEN SHOULD A URINE SAMPLE BE TAKEN

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.

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.



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:

- / If the urine must be stabilised, add appropriate preservatives.
- / Discard first morning urine and collect all the following urine throughout the 24-hour period .
- / Pay attention to 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.

TECHNIQUES FOR COLLECTING AND PREPARING URINE

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 native urine sample should be brought to the laboratory within 2 hours.

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 microscoped. 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.

BLADDER CATHETERS AND BLADDER PUNCTURE

are reserved for special cases.

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 „Mid-stream urine“ on page 74)
- / 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.

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.

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.

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 endogenic biomarkers, for example saliva amylase or cortisol.

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

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.
- / Stick the label onto the collection tube, never on the transport 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.
- / 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.
- / Only freeze serum or plasma - never freeze whole blood.
- / 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 according to manufacturer's instructions.
- / Always collect blood sample prior to beginning with antibiotic treatment.
- / Thoroughly disinfect the skin prior to sample collection.
- / 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.
- / Fresh puncture should always be preferred to collection from catheters.
- / Follow the manufacturer's instructions for use when filling aerobic and anaerobic blood culture bottles.
- / All information that is relevant for a quick and correct performance of the requested analyses should be provided on the consignment note.
- / Immediate transport to the 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 URINE COLLECTION

- / If the urine must be stabilised, add appropriate preservatives.
- / Discard first morning urine and collect all the following urine throughout the 24-hour period .
- / Pay attention to 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.

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.



FURTHER INFORMATION ON OUR PRODUCTS

can be found on our
corporate website
www.gbo.com.

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NOTES

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