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SAMPLE QUALITY IN PREANALYTICS

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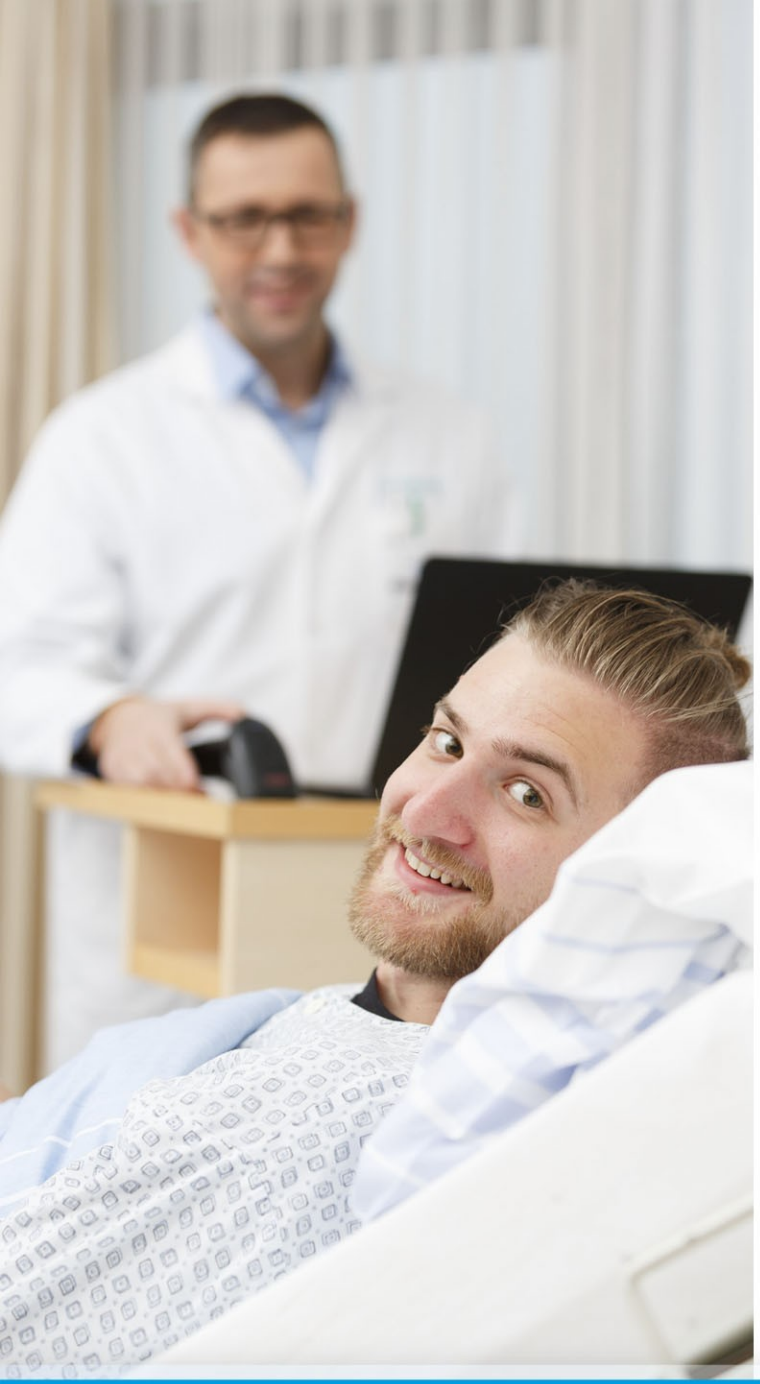
What is preanalytics?

Preanalytics includes all influencing factors and processes that have an effect on samples before they are analysed in the laboratory.

IT INVOLVES:

- Preparing the patient
- Patient and sample identification
- Sample collection
- Sample preparation
- Storage and transport of the sample
- Handling of the sample in the laboratory prior to analysis



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Sample quality in preanalytics

Patient-related influencing factors

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Influencing factors

PHYSIOLOGICAL FACTORS
INFLUENCE LABORATORY PARAMETERS
and must be taken into account

These include:

ENDOGEN FACTORS

- Gender
- Age
- Pregnancy

EXOGEN FACTORS

- Activity
- Food intake
- Effects of nicotine, coffee and alcohol
- Medication

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Patient-related influencing factors

Taking patient-related influencing factors into account:

ACCURATE PATIENT DETAILS
ON THE REQUISITION FORM
are essential for comparison of the patient's results with the reference ranges.

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Patient-related influencing factors

– long-term changes

Gender-specific differences in selected parameters:

	Male	Female	
Alanine aminotransferase	< 50	< 35	U/l
Iron	6.3 – 30.1	4.1 – 24	µmol/l
Ferritin	18 – 360	9 – 140	mg/l
Uric acid	3.6 – 7	2.3 – 6.1	mg/dl
Creatinine	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	< 15	< 20	mm/1hr.

Source: Thomas L.: *Labor und Diagnose*, 6th edition

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Patient-related influencing factors
– long-term changes

Effects of **age:**

DECREASE WITH AGE

- Albumin
- Calcium
- Creatinine clearance
- Inorganic phosphate
- pO_2
- Quick value (prothrombin time)

INCREASE WITH AGE

- Cholesterol
- Erythrocyte sedimentation rate (ESR)
- Ferritin
- Glucose

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Patient-related influencing factors
– changeable

Effects of **body weight:**

The following substances, among others, increase **WITH BODY WEIGHT**:

- Cholesterol
- Triglycerides
- Uric acid
- Insulin
- Cortisol

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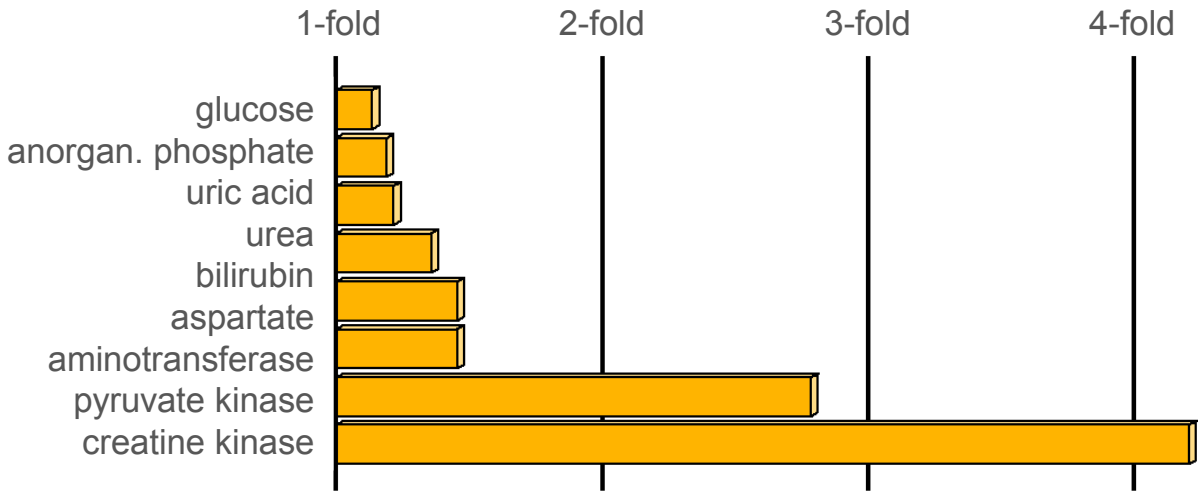
Patient-related influencing factors
– changeable

Effects of **various lifestyle habits:**



Changes in various serum concentrations following extreme physical activity – running a marathon

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Patient-related influencing factors
– changeable

Effects of **pregnancy:**

During pregnancy the PLASMA VOLUME increases by approx. 50%.

CHANGES IN CONCENTRATION can be observed in the case of a number of parameters:

- Key electrolytes are reduced
- Blood lipids are increased

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Patient-related influencing factors

– short-term changes

Fluctuations due to daily rhythms:

MAXIMUM IN THE **MORNING**

Adrenocorticotropic hormone (ACTH)	200%
Renin	140%
Noradrenaline (norepinephrine)	120%
Prolactin	100%
Aldosterone	80%
Cortisol	50%
Testosterone	50%

Adrenaline	20%
Haemoglobin	20%
Haematocrit	20%
Leukocytes	20%
Protein	20%
Thyroxine (T4)	20%
Bilirubin	20%

MAXIMUM AT **MIDDAY**

Iron	100%
Eosinophils	30%
Potassium	15%

MAXIMUM IN THE **EVENING**

Creatinine	100%
Urea	50%
Thyroid-stimulating hormone (TSH)	50%
Acid phosphatase	200%

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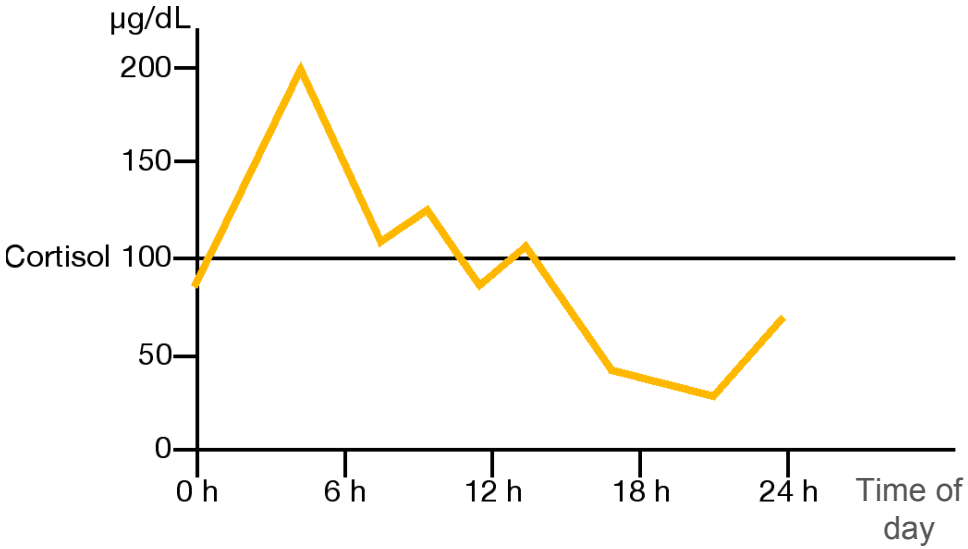
Patient-related influencing factors

– short-term changes

Fluctuations due to daily rhythms:



Fluctuations in cortisol levels due to daily rhythms

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Patient-related influencing factors
– short-term changes

Avoidance of the effects of daily rhythms:

The effects of daily rhythms are minimised by
COMPLIANCE WITH THE RECOMMENDATION
TO PERFORM BLOOD COLLECTION
between 7 a.m. and 9 a.m.

Patient-related influencing factors

– short-term changes

Physical strain:

In the case of physical strain, WATER and SMALL MOLECULES enter the extravascular space from the blood vessels.
That **INCREASES THE CONCENTRATION**, for example, of proteins and protein-bound substances.

That also occurs when SITTING UP AFTER LYING DOWN and during STASIS (use of a tourniquet).

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Patient-related influencing factors
– short-term changes

Avoidance of the effects of physical strain:

Before giving a blood sample as an outpatient, the patient should **SIT CALMLY** FOR AROUND 5 MINUTES.

Blood should NOT be collected FOLLOWING **PHYSICAL EXERCISE**, for example a morning jog.

Nor should ANY **EXHAUSTING PHYSICAL ACTIVITIES** be performed in the 3 days prior to the blood collection.

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Patient-related influencing factors
– short-term changes

Stress:

ANXIETY PRIOR TO BLOOD COLLECTION or the situation prior to an operation may result in extreme mental stress. That results in the **RELEASE OF VARIOUS HORMONES**,

such as aldosterone, catecholamines, cortisol, prolactin and renin.

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

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Patient-related influencing factors

– short-term changes

Avoidance of the effects of stress:

A **CALM ATMOSPHERE** and **REASSURANCE** before blood collection have a very beneficial effect in this respect.

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Patient-related influencing factors

– short-term changes

Food intake:

Following food intake, there are **MARKED DIFFERENCES** in various **PARAMETERS.**

Following a fatty meal, the effects are visible in serum in the form of turbidity (lipemia).

Lipemic samples are only of limited use.

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Patient-related influencing factors
– short-term changes

Avoidance of the effects of food intake:

The patient should **ABSTAIN FROM EATING FOR 12 HOURS** prior to blood collection, especially in the case of a lipometabolism diagnosis.

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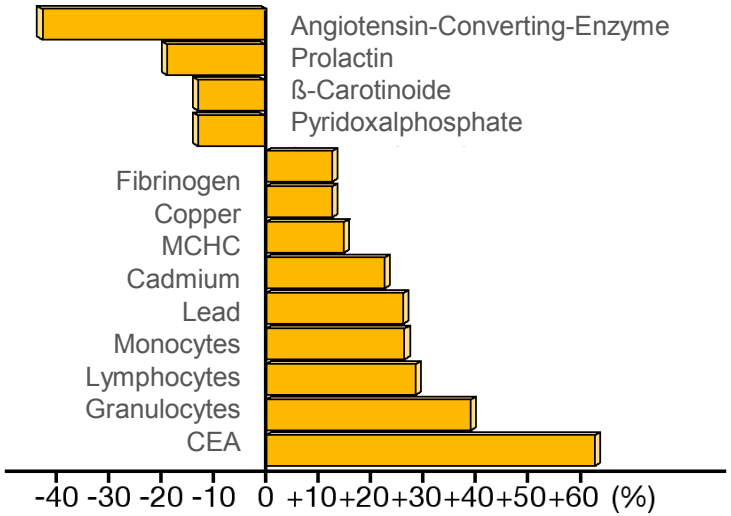
Patient-related influencing factors
– short-term changes

Nicotine:



Comparison between smokers and non-smokers

Smoking even one cigarette results in **HIGHLY SIGNIFICANT CHANGES** within an hour.

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Patient-related influencing factors
– short-term changes

Coffee:

Drinking coffee results in a **SHARP INCREASE IN CORTISOL** – of up to 40% in the case of 2 cups of coffee (200mg caffeine).

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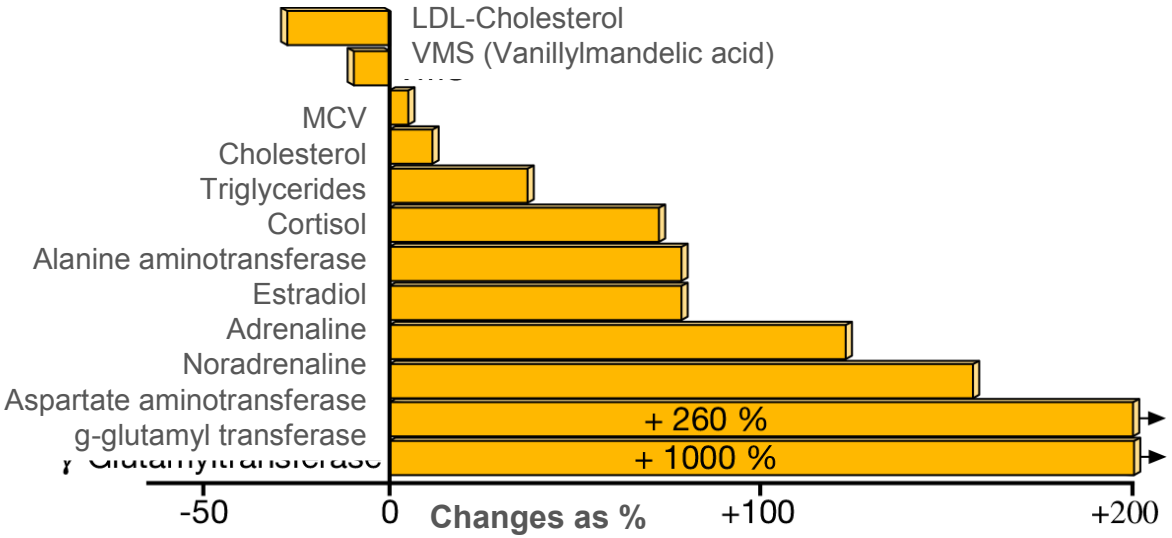
Patient-related influencing factors

– short-term changes

Alcohol:



Chronic changes
in the case of
alcohol consumption

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Patient-related influencing factors

– short-term changes

Avoidance of the effects of nicotine, coffee and alcohol:

It is recommended that the patient **DOES NOT SMOKE OR DRINK COFFEE** prior to the blood collection.
In addition, the patient should **ABSTAIN FROM DRINKING ALCOHOL FOR 24 HOURS** before the blood collection.

The patient should not have consumed alcohol in excess in the days leading up to the blood collection.

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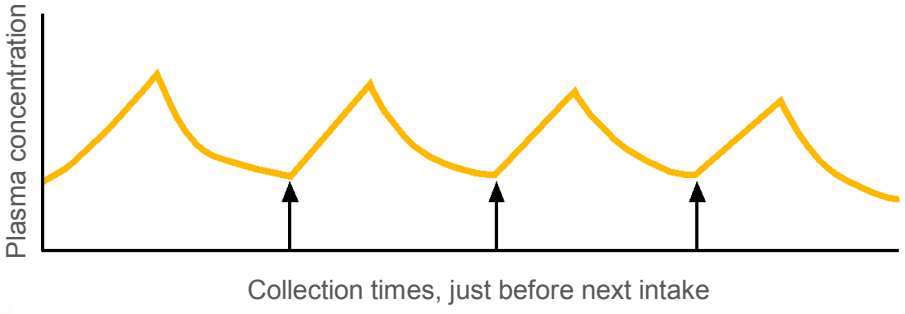
Patient-related influencing factors
– short-term changes

Taking the effects of drugs and medication into account:

For therapeutic drug monitoring, blood collection should be performed **IMMEDIATELY PRIOR TO INTAKE OF THE MEDICATION** if possible (measurement at trough level).
It should not be performed before the maximum serum concentration is reached. However, blood collection must be performed IMMEDIATELY if an overdose or intoxication is suspected.



Optimal time for the measurement of medication levels

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Patient-related influencing factors

Correct behaviour of the patient:

PATIENTS ARE UNAWARE of many of the INFLUENCING FACTORS DESCRIBED. Patients can only act correctly if they are aware of such factors and their effects.

- PROVIDING **PATIENTS WITH CORRECT GUIDANCE** helps to prevent errors.
- **MAKING ENQUIRIES** BEFORE BLOOD COLLECTION can identify incorrect behaviour.
- In the case of INORRECT BEHAVIOUR, **BLOOD COLLECTION** may have to be **POSTPONED**.

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Sample quality in preanalytics

Identification

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Identification

Identification errors:

Identification errors may result in misunderstandings, delayed test results or make it impossible to match the laboratory values and the patient.

This also includes the lack of samples or requisition forms and illegible labelling.

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Identification

Avoidance of errors in patient identification

Each patient must identify themselves immediately before the blood collection **BY STATING THEIR NAME**.

OBLIGATORY DETAILS on the requisition form

- Name and surname, date of birth
- Patient number, ward, room number, name or number of doctor's surgery
- Date and collection time
- Gender and age
- If applicable, week of pregnancy

Further details are required for various analytes or tests

- Collection times for daily profiles or functional tests
- Medication intake, including vitamins and hormones
- Height and weight
- Total quantity in the case of 24-hour urine collection

For small amounts of sample material, give the key parameters.

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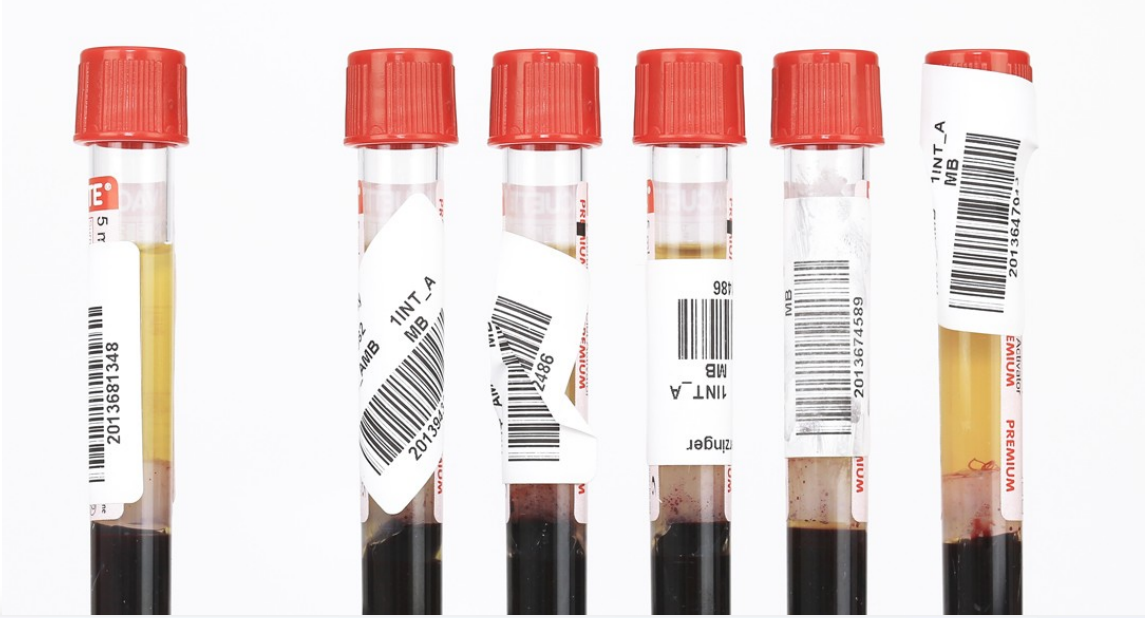
Identification

Errors in sample identification:

Incorrectly attached labels often result in identification errors.

Incorrectly attached labels

left: correctly attached label



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Identification

Avoidance of errors in sample identification

- Take care when filling in the label and ensure that the writing is **CLEARLY LEGIBLE**
- Only use **WATERPROOF PENS**
- Specifically indicate **STAT SAMPLES**
- Indicate **POTENTIALLY INFECTIOUS MATERIAL** on the sample tube and on the requisition form_
- Always **FILL IN THE LABEL PRIOR TO THE BLOOD COLLECTION** and attach it. Never do so after the blood collection
- **CORRECTLY POSITION** the label
- Always attach the label **TO THE COLLECTION TUBE**. Never attach it to the transport tube

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Sample quality in preanalytics

The significance of haemolysis and lipaemia

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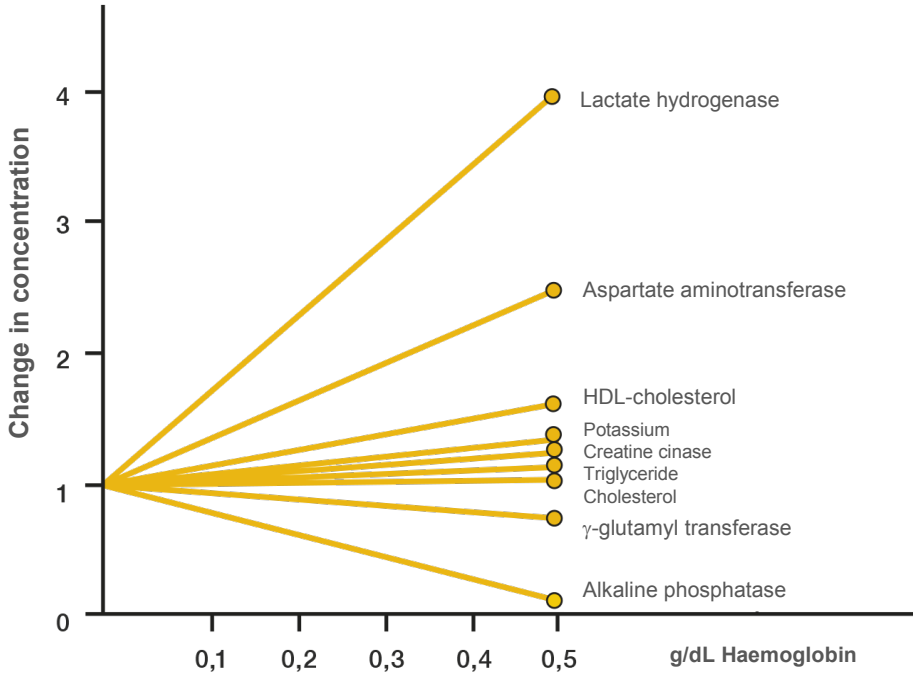
The significance of haemolysis

Errors due to haemolysis:



Change in various parameters in the case of haemolysis (haemoglobin 0.5g/dL)

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The significance of haemolysis

Errors due to haemolysis:

Samples with varying degrees of haemolysis

The significance of haemolysis

Errors due to haemolysis:

1. The release of components from the cells changes **CONCENTRATIONS** in the serum or plasma.

2. The red discolouration due to haemoglobin interferes with **PHOTOMETRIC MEASURING**.

3. **CHEMICAL REACTIONS** during analysis are influenced by cell substances.

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The significance of haemolysis

The following errors result in haemolysis and should be strictly avoided:

- Tourniquet applied too tightly
- Needles with too small a diameter
- Aspiration of the tissue fluid after piercing of the vein
- Transfer of blood from syringes into other containers
- Shaking of a sample, instead of gentle mixing
- Delayed separation of cells from serum or plasma >3 hours
- Centrifugation for too long or with excessive force
- Effects of temperature, heat and cold, e.g. during transport, or contact between the samples and the cooling elements
- Freezing of whole blood

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The significance of lipaemia

Errors due to lipaemia:

Samples with varying degrees of lipaemia

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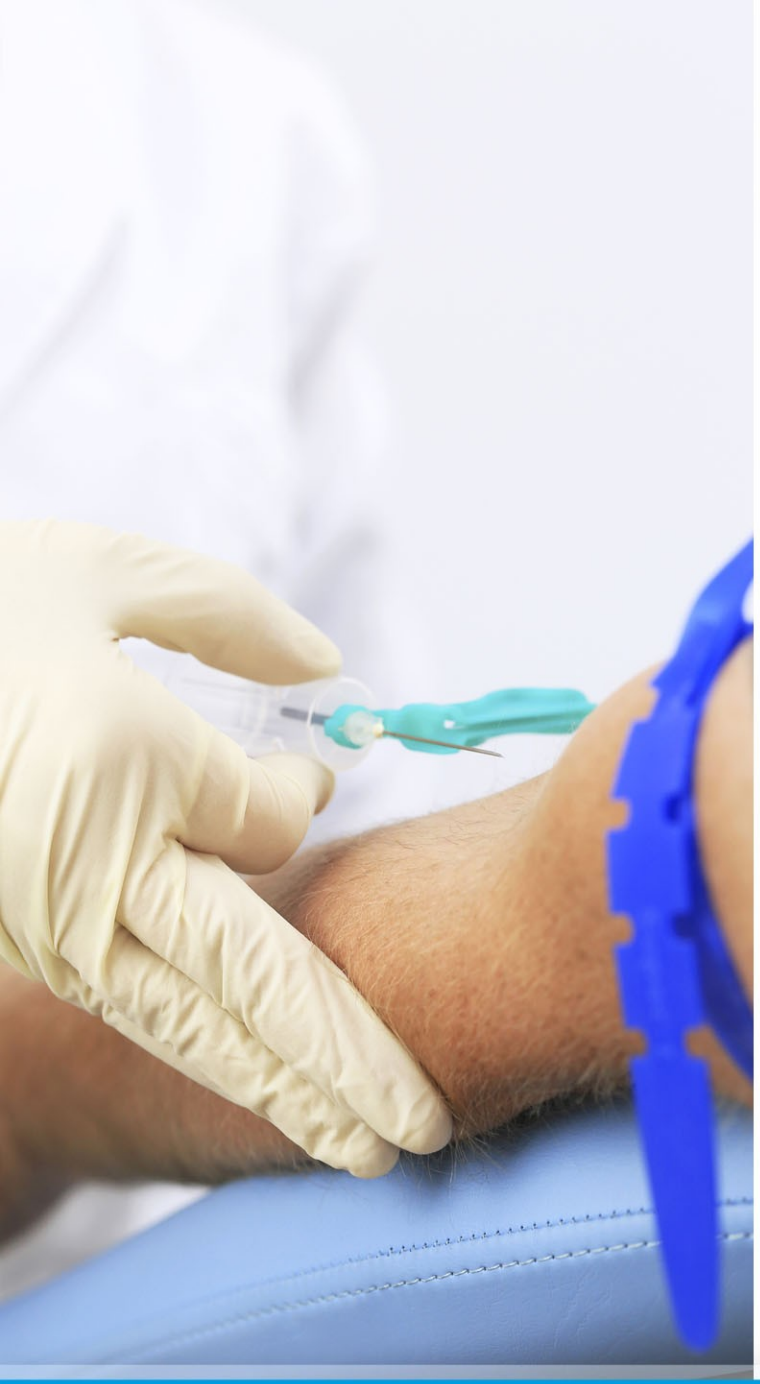
The significance of lipaemia

Errors due to lipaemia:

1. The lipoprotein particles can interfere with **PHOTOMETRIC MEASURING**.
   (methods that use lower wavelength are more affected by lipaemia)

2. **PHYSICAL REACTIONS** in electrophoretic methods
   (abnormal morphology of the alpha-2-globulin fraction)
   and in various immunoassays (antigen-antibody reaction)

3. **NON-HOMOGENEITY** of the sample
   (VLDL particles have low density and will be located at the top of the tube)



Sample quality in preanalytics

Blood collection

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Blood collection

Errors due to incorrect body position:

CHANGE OF **UP TO 10%**
Haemoglobin
Leukocytes
Total calcium
Aspartate aminotransferase
Alkaline phosphatase
Thyroxine
Immunoglobulins G and A
Albumins
Total protein
Triglycerides

CHANGE OF **BETWEEN 10 AND 20%**
Haematocrit
Cholesterol
HDL cholesterol
Apolipoprotein
Erythrocytes (red blood cells)

CHANGE OF **MORE THAN 50%**
Adrenaline
Renin
Noradrenaline (norepinephrine)

Effect of body position during the sample collection on various parameters:
INCREASE FROM LYING TO SITTING

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Blood collection

Avoidance of errors due to incorrect body position:

- Blood collection in a hospital should be performed with the patient **LYING IN BED**, rather than in a seated position.
- If that is not possible at the doctor's surgery, the sample should be taken with the patient in a seated position.
- It is important that blood collection is always performed in **THE SAME BODY POSITION** to ensure comparability of the results.

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Blood collection

Errors due to the tourniquet being applied for too long:

INCREASE OF **BETWEEN 6 AND 12%**
Alanine aminotransferase
Creatine kinase
Bilirubin
LDH
Albumin
Gamma-glutamyltransferase
Alkaline phosphatase
Total protein
Cholesterol
Triglycerides
Aspartate aminotransferase

DECREASE OF **UP TO 4%**
Glucose
Inorganic phosphatase
Leukocytes
Urea
Creatinine
Chloride

Percentage change of various parameters **AFTER A 6-MINUTE STASIS PERIOD**. Changes occur even after shorter stasis periods.

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Blood collection

Avoidance of errors due to incorrect stasis:

- Stasis lasting **UP TO 60 SECONDS** is acceptable and does not have a significant effect on the sample.
- The tourniquet should **NOT BE APPLIED TOO TIGHTLY** – it must still be possible to feel the pulse.
- In the case of good veins, the tourniquet should be **LOOSENED FOLLOWING SUCCESSFUL PUNCTURE OF THE VEIN** as soon as blood is flowing into the first tube.

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Blood collection

Errors due to **techniques used to locate the vein:**

IMPERMISSIBLE TECHNIQUES:

- Opening and closing of the fist
- Strong tapping of the vein

PERMISSIBLE TECHNIQUES:

- Only make a fist, **DO NOT PUMP**
- **APPLICATION OF WARMTH**

by using a warm arm bath, heating pad or local anaesthetic patch

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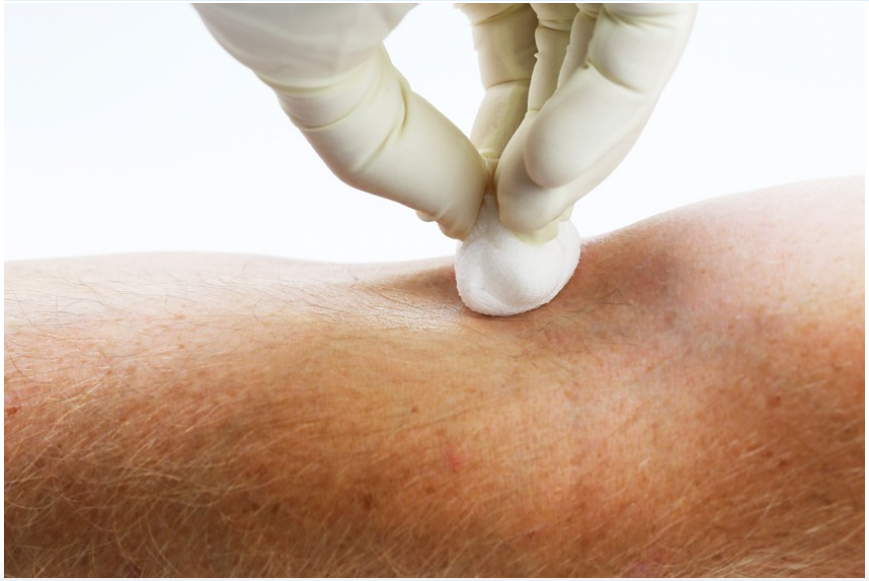
Blood collection

Errors due to **incorrect disinfection of the puncture site:**

In the case of incorrect disinfection, disinfectant may enter the sample and influence the analysis.

The disinfectant should be allowed to **DRY COMPLETELY** before the puncture is performed.



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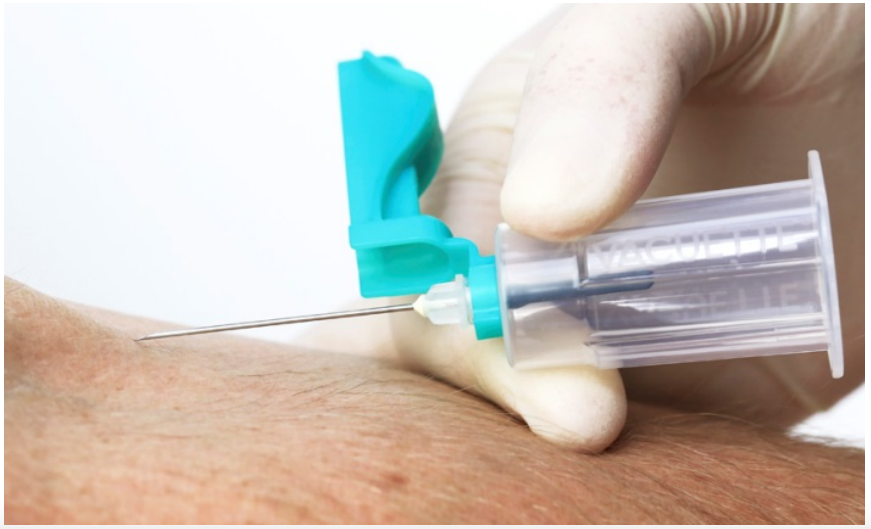
Blood collection

Errors **during puncture:**

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

- **DO NOT PROBE** tissue to locate the vein
- If necessary, **PUNCTURE** the other arm



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Blood collection

Order of draw:

Recommended **BLOOD COLLECTION ORDER**:

1. Blood cultures
2. Sodium citrate tubes
3. Serum tubes
4. Heparin tubes
5. EDTA tubes
6. Glycolytic inhibitor tubes
7. other additives

Winged needle devices cause underfilling of the first-drawn tube.
USE A DISCARD TUBE.

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Blood collection

Errors due to use of the **incorrect anticoagulant:**

A sample with the incorrect anticoagulant or in the incorrect tube is unusable.

Use the **CORRECT ANTICOAGULANT** or tube.

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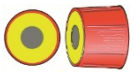
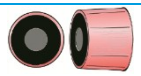
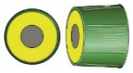
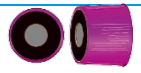
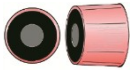
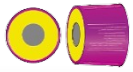
Blood collection

Avoidance of errors by selecting the correct anticoagulants:

VACUETTE® tubes	Colour code Cap	Additive	Intended purpose
Serum		Clot activator	Determinations in serum for clinical chemistry, microbiological serology, immunology, TDM
Serum Sep		Clot activator and separator gel	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 gel	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 gel	Determinations in EDTA plasma for molecular biological identification of viruses, parasites and bacteria

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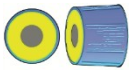
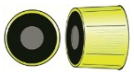
Blood collection

Avoidance of errors by selecting the correct anticoagulants:

VACUETTE® tubes	Colour code Cap	Additive	Intended purpose
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 with prevention of the artificial entry of platelet factors into the citrated plasma
Glucose		Anticoagulant Glycolysis inhibitor	Determinations in stabilised and anticoagulated whole blood for glucose and lactate testing
Trace elements		Sodium heparin	Determinations in heparinised plasma for trace elements testing
Blood groups		ACD-A ACD-B CPDA	Determinations in ACD / CPDA whole blood for blood grouping

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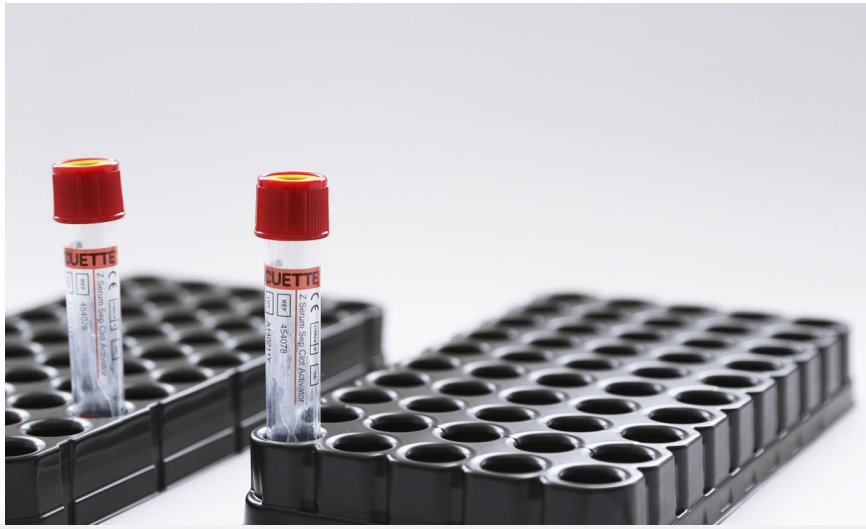
Blood collection

Errors due to tubes being **past their expiry date:**

Tubes that are past their expiry date are not suitable for use.

- Always **USE UP** tubes before opening a new box.
- Use products with the **EARLIEST EXPIRY DATE** first.



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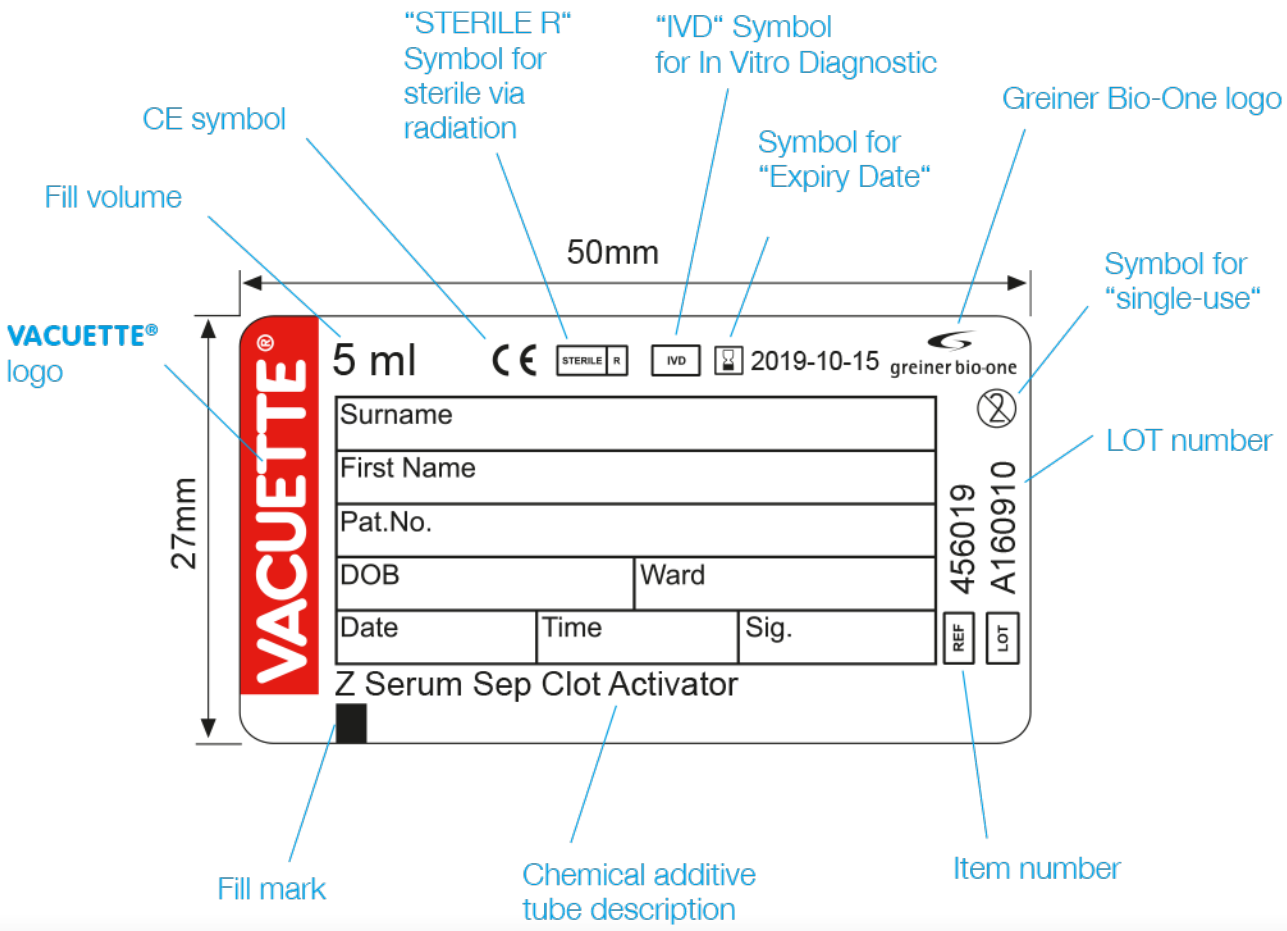
Colour-coded label with expiry date in compliance with ISO 6710:



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Blood collection

Errors due to **incomplete filling of tubes:**

- Tubes that have not been filled exactly result in an incorrect blood/anticoagulant mixing ratio.
- Particularly serious errors can occur if tubes for coagulation diagnostics are not filled exactly.

FILL THE TUBES EXACTLY and ensure that the mixing ratio is correct.

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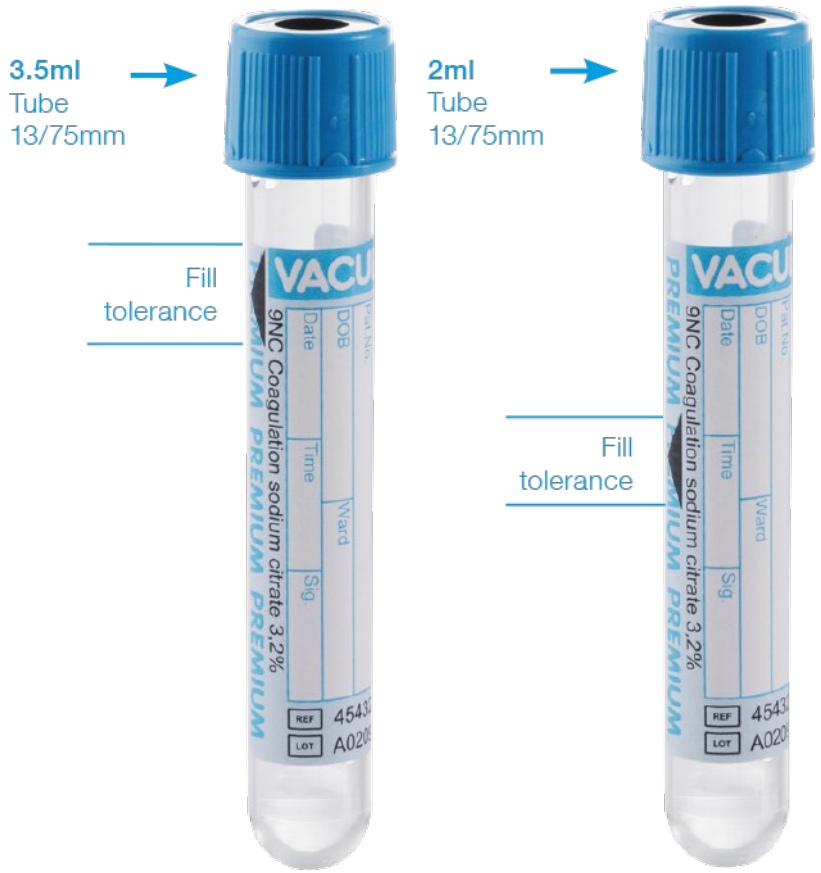
Blood collection

Fill tolerance of the VACUETTE® coagulation tubes:

The fill tolerance
CORRESPONDS TO THE
INTERNATIONAL STANDARDS
ISO 6710 and CLSI and is
indicated on the label.

Clinical and Laboratory Standards Institute (CLSI)



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Blood collection

Avoidance of errors when mixing blood with the tube additives:

- Insufficient or delayed mixing of the blood with the additives in the tubes may result in tiny clots or disrupt coagulation.
- Tiny clots can cause analyser blockages.

- **INVERT** tubes **5 – 10 times** immediately after filling (4 – 5 times in the case of coagulation tubes).
- Do not shake.
- **TAKE PARTICULAR CARE** when mixing tubes with a high fill level and little empty space.

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Blood collection

Avoidance of errors when mixing blood with the tube additives

An indicator of correct mixing is the **AIR BUBBLE**, which must **MOVE FULLY THROUGH THE TUBE** from top to bottom DURING EACH INVERSION.

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Sample quality in preanalytics

Sample storage

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Sample storage

Avoidance of errors by using the correct storage temperatures and storage times

- EDTA blood should be stored **AT ROOM TEMPERTURE**, not in a refrigerator.

- It can be kept for 24 hours at room temperature for blood cell counting, and for 2 – 3 hours for differential blood counts.

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Sample storage

Avoidance of errors by using the correct storage temperatures and storage times

- With a few exceptions, SERUM OR PLASMA SAMPLES should be stored in a refrigerator at a temperature of **4°C** following cell separation and analysis.

- Serum and plasma can be kept for up to 2 days above a separator gel at refrigerator temperature, and for longer in a secondary container.

- Since blood cells are destroyed during the freezing process, ONLY THE **FREEZING OF SERUM OR PLASMA** IS PERMISSIBLE.

- LONGER-TERM STORAGE of serum or plasma must take place at temperatures of **< -20°C**.
  - **FLASH FREEZING** to maintain the protein structures is important.
  - The **DEFROSTING PROCESS MUST BE SLOW**, either overnight in the refrigerator, or in a water bath with continual mixing.

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Sample storage

Avoidance of errors by using the correct storage temperatures and storage times

- CITRATED PLASMA for coagulation diagnostics can be kept for 3-6 hours **AT 20°C**.

YOUR LABORATORY will tell you which samples require **PARTICULAR STORAGE TEMPERATURES** or need to be deep frozen.

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Sample quality in preanalytics

Sample preparation

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Sample preparation

Centrifugation errors:

If the blood sample coagulates in a tube that is in a horizontal position, serum separation may be impaired.

coagulated in a horizontal position

coagulated in an upright position

COAGULATION of the sample in an **UPRIGHT** tube results in improved separation during centrifugation.

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Sample preparation

Centrifugation errors:

- If the waiting time before centrifugation is too short, fibrin fibres form in the serum due to post-clotting.
- Excessive cooling down or warming up in the centrifuge can result in haemolysis.
- Centrifugation for too long or at excessive speed can likewise result in haemolysis.
- Centrifugation in open containers results in evaporation of the sample, which is especially problematic in the case of small sample volumes.

- Samples should not be centrifuged earlier than **30 MINUTES** following blood collection.
- The temperature in the centrifuge should be between **20°C AND 22°C**.
- Only centrifuge **FIRMLY CLOSED TUBES**, for hygienic reasons, amongst others.

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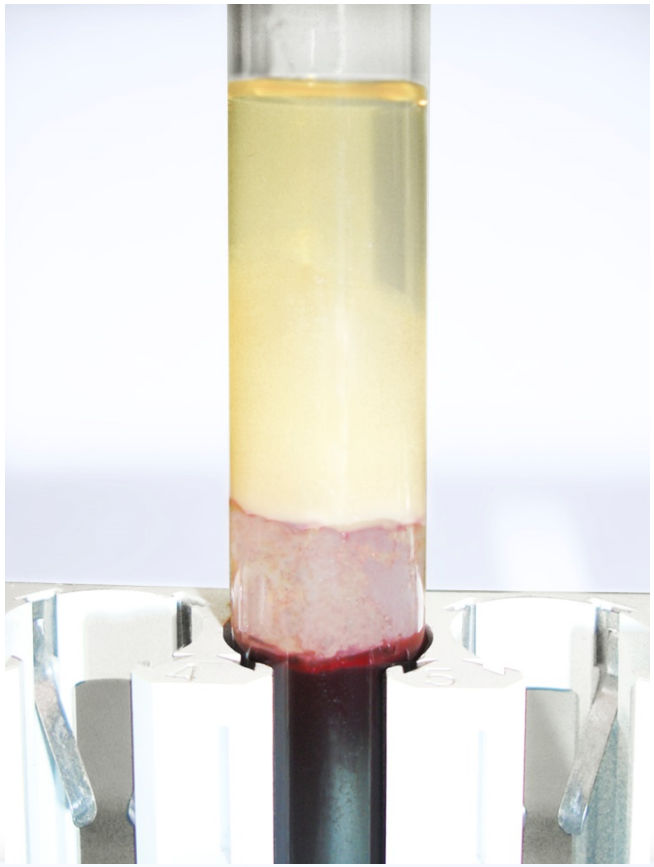
Sample preparation

Centrifugation errors:

In the case of patients undergoing anticoagulation therapy, coagulation is delayed.



Serum sample of a patient undergoing anticoagulation therapy, centrifuged too early

Centrifuge such samples only once RETRACTION OF THE BLOOD CLOT **HAS COMPLETELY FINISHED**.

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Sample preparation

Avoidance of centrifugation errors

	Centrifuge speed	Time
Platelet-rich citrated plasma (coagulation tube)	150g	5 minutes
Platelet-poor citrated plasma (coagulation tube)	1500 – 2000g	10 minutes
Platelet-free citrated plasma (coagulation tube)	2500 – 3000g	20 minutes
Serum tubes	1800 – 2200g	10 – 15 minutes
Serum sep tubes	1800 – 2200g	10 – 15 minutes
Heparin sep tubes	1800 – 2200g	10 – 15 minutes
ETDA sep tubes	1800 – 2200g	10 – 15 minutes
Glucose tubes	1800 – 2200g	10 – 15 minutes

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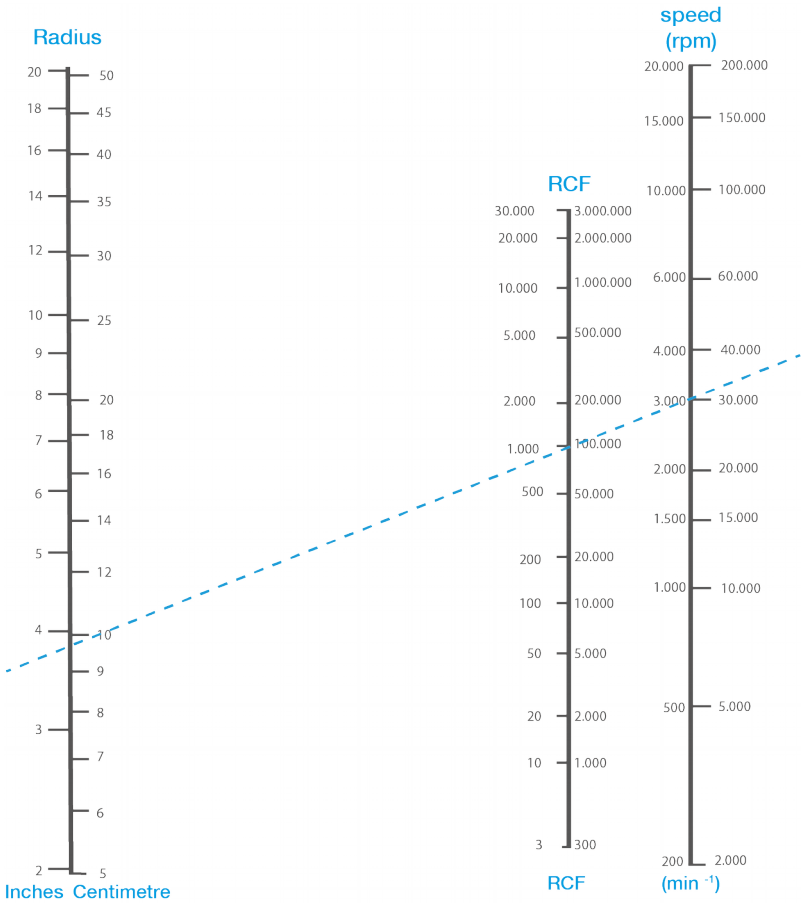
Sample preparation

Avoidance of centrifugation errors



$$g = RCF$$
$$= 1.118 \times 10^{-5} \times r \times (rpm)^2$$

g = RCF = relative centrifugal force
r = radius in cm
rpm = revolutions per minute

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Sample preparation

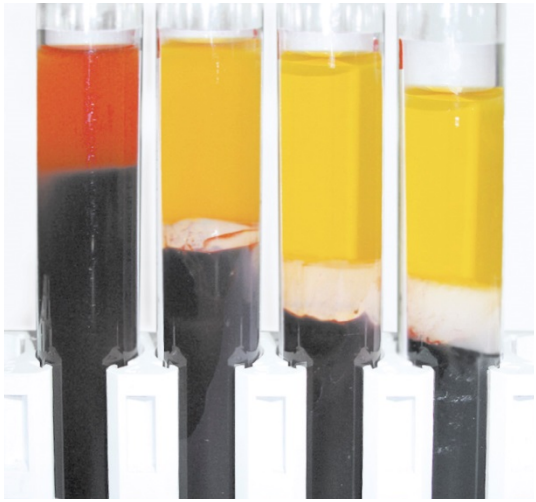
Centrifugation errors:

CENTRIFUGE **SPEED**
was incorrect.

from left to right:
INCREASING G-FORCE

right: correctly centrifuged sep tube

CENTRIFUGE **TIME**
was incorrect.



from left to right:
INCREASING CENTRIFUGATION TIME

right: correctly centrifuged sep tube

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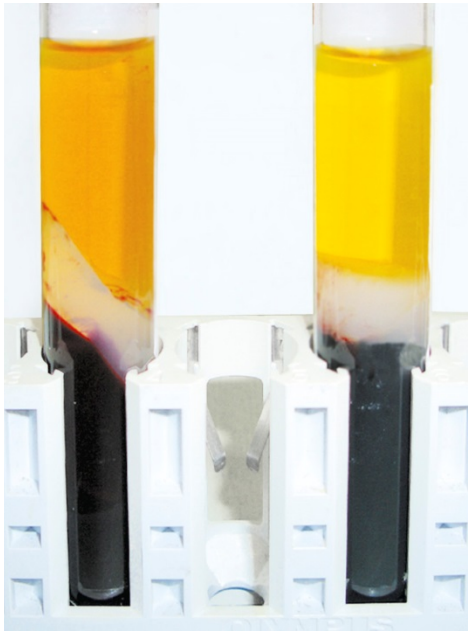
Sample preparation

Centrifugation errors:



Left:

Sep tube centrifuged in a **FIXED-ANGLE** CENTRIFUGE

Right:

Sep tube centrifuged in a **SWING-OUT** CENTRIFUGE

Sep tube centrifuged in a **FIXED-ANGLE** CENTRIFUGE and transported horizontally

Jolts may break the labile sloping gel barrier.

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Sample preparation

Avoidance of centrifugation errors

- Allow the sample to **COAGULATE** in an **UPRIGHT** position
- Centrifugation should be performed **AS SOON AS POSSIBLE**, but in compliance with the required waiting times
- Select the correct centrifuge **TEMPERATURE**
- Only centrifuge **FIRMLY CLOSED TUBES**
- Comply with the indicated CENTRIFUGATION **TIME** and CENTRIFUGATION **SPEED**

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