Tips & Techniques in Preanalytics





Preanalytics & S-Monovette®

Optimal preanalytical work is a basic prerequisite for precise and conclusive laboratory diagnostics.

Laboratory values can be correct only if and when all conditions prevailing at the time of blood collection are standardised.

"Tips & Techniques in Preanalytics" is intended to support you in becoming familiar with, assessing and enhancing preanalytical influences.

Please be aware that the topics addressed in "Tips & Techniques in Preanalytics" covering the fields of

Venous Blood Collection,
Capillary Blood Collection, and
Urine Collection

are recommendations only and do not, under any circumstances, replace medical, scientific or technological expertise.

With the compliments of

SARSTEDT AG & Co.







Preanalytical Influence Factors

"Preanalytics encompasses any and all procedures prior to laboratory work".

Note:

Preanalytical issues can never be settled by individuals alone but require the cooperation of physicians, nurses and laboratory personnel involved in the overall procedure.

Patient-related influence

Permanent

- Population (race)
- Sex

Long-term

- Age
- Pregnancy
- Nicotine / illegal Drugs / alcohol

Short-term

- Circadian fluctuations
- Posture
- Physical strain

Permanent influence

Population (race)

There are significant differences between the black compared to the white population..

- Significantly lower leucocyte count
- Vitamin B12 concentration is 1.35 times higher
- Higher CK and α -amylases

Sex

Apart from individual sex-related components (hormones), a person's muscular mass, for example, is one of the factors that determine pertinent parameters.

- The proportion of CK and creatinine depends on the muscular mass.

Long-term influence

Age

As a person grows older, the cholesterol level frequently increases in both men and women (although this depends primarily on nutrition).

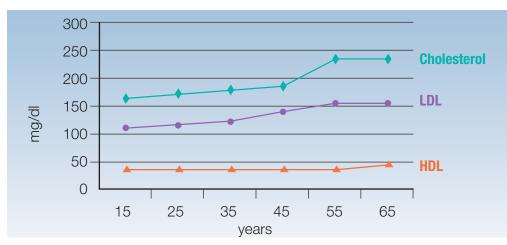


Fig.: Guder, Narayanan, Wisser, Zawta "Samples: From the Patient to the Laboratory" GIT-VERLAG GmbH & Co. KG, Darmstadt





Preanalytical Influence Factors

Smoker or non-smoker?

Chronic nicotine abuse is known to increase leucocyte count, a number of enzyme values and tumour markers (particularly the CEA value).

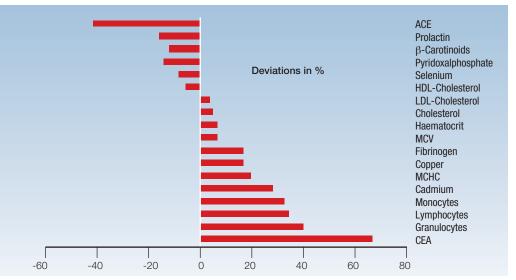


Fig.: Guder, Narayanan, Wisser, Zawta "Samples: From the Patient to the Laboratory" GIT-VERLAG GmbH & Co. KG, Darmstadt

Alcohol

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

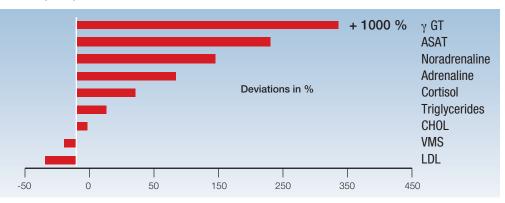


Fig.: Guder, Narayanan, Wisser, Zawta "Samples: From the Patient to the Laboratory" GIT-VERLAG GmbH & Co. KG, Darmstadt

Short-term influence

Posture

Increase in concentration when moving from the horizontal to an upright position.

Parameters	increase in %
Haematocrit	13 %
Erythrocytes	15 %
HDL Cholesterol	10 %
Aldosterone	15 %
Epinephrines	48 %
Renin	60 %

Physical strain

Increase in various analytes after extreme physical strain, e.g. a marathon.

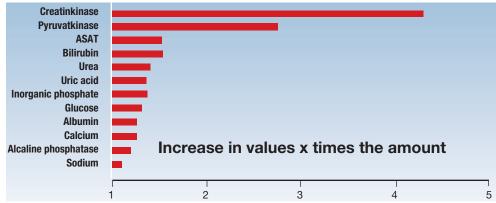


Fig.: Guder, Narayanan, Wisser, Zawta "Samples: From the Patient to the Laboratory" GIT-VERLAG GmbH & Co. KG, Darmstadt

Effects of constriction time

Comparison: 1 min. vs. 3 min.

Parameter	deviation in%
Bilirubin	+ 8
Cholesterol	+ 5
Creatinine	- 9
Creatine kinase	- 4
Iron	+ 7
Glucose	- 9
y-Glutamyltransferase	-10
Potassium	- 5





Preparation and Identification

Patient preparation

Informing the patient

 Inform the patient of the forthcoming procedure in order to alleviate possible anxiety and stress.

Explaining particular preconditions

that must be observed is an essential part of this information, e.g.

- Consumption of pharmaceutical drugs
- Restriction to a special diet
- Sample collection on an empty stomach (except for emergency diagnostics)

Precise instructions for use

should be given to explain the use of urine and faeces collection containers.

Carefully explain the forthcoming procedures to children using terms which are easy for them to understand in order to prevent unnecessary distress.

Patient identification

Correct patient identification

is a fundamental necessity (surname, first name/s, date of birth, admission number, ward, room number).

Errors do not only occur with popular names.

Patients should always identify themselves when directly addressed.

Partially/completely deaf or cognitively impaired patients might answer questions like "You are Mr. Miller, aren't you? with an affirmative nod.

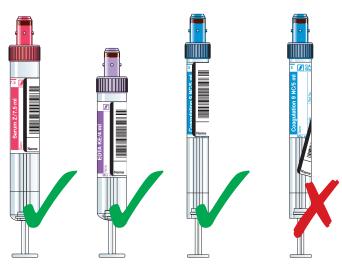
A person sitting on the edge of the bed might just as well be a visitor.

If the patient's identity is not entirely clear, the phlebotomist should either refuse to perform blood collection or carry out the procedure subject to further clarification.

Sample identification

- Never analyse sample tubes which are not clearly identified.
- Barcode labels enable reliable sample identification.
- The identification should always be provided on the primary tube.
- Only use water-proof felt tip pens on glass or plastic tubes.
- Additives (anticoagulants, clotting activators, gel) are identified by a colour code on the sample tubes. Due to the absence of international standardisation, additional identification may be required from case to case.

Never provide sample identification on the cap, outer packaging or transport container.



- Sample tubes are correctly labelled, provided:
 - ▶ they enable unrestricted visibility of the tube content
 - ► they enable control of the filling volume
 - ► the screw cap can be easily removed
 - ▶ the tube and label do not get jammed or stick together in the centrifuge

Clearly mark the tube and request form to indicate that the material is known to be infectious.





Blood Collection

Phlebotomist identification

The identity

of the phlebotomist should be ascertainable for each sample taken and,

• if possible, also noted on the request form

Questions concerning the type and time of blood collection as well as problems, if any, during sampling, the patient's condition and other important issues might be helpful in the event of unclear analysis results.

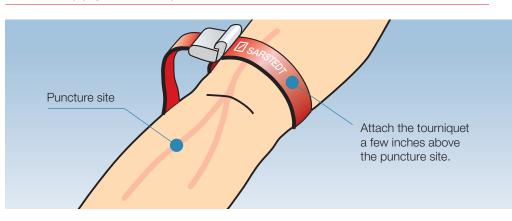
Ordering doctor's identification

The identity

of the ordering doctor enables further enquiries in case of

- illegible requests (e.g. certificates of referral)
- erroneous requests (e.g. prostata phosphatase for a female patient)
- restriction to essential parameters in the event of small sample volumes

How to apply a tourniquet



Procedure:

- Disinfect the selected puncture site.
- After 30 to 60 seconds, wipe off the disinfectant using a dry swab.
- Attach the tourniquet a few inches above the puncture site.
- The pulse must be perceptible (tourniquet pressure: 50 100 mm Hg)
- Maximum constriction time: 1 min.

Recovery of diagnostic samples

- Wear gloves
- Have you checked the vein conditions?
- Disinfect the puncture site
- Do not touch the puncture site again
- Release extended constriction and attach tourniquet again
- Remove protective sleeve from the needle
- Hold the bevelled side of the needle away from the skin
- Puncturing angle: less than 30°
- Keep the vein in place by tightening the skin
- "Warn" the patient
- Release the tourniquet as blood starts to flow
- Collect blood with due regard to the order of draw





S-Monovette® Safety-Needle

Problems prior to / during blood collection:

Poor vein conditions:

- Select new puncture site
- Apply thermo-pad or pre-heated cloth
- Use Safety-Multifly®-Needle (set for difficult veins)

Penetrating the vein:

• Slightly withdraw the needle

Interruption of blood flow during collection:

- Needle position has been changed
- Vein has collapsed

Wrong handling during blood collection:

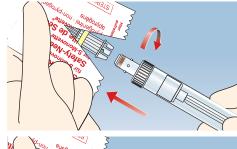
- "Pumping" the fist to enhance blood flow leads to a rise in K⁺ and Mg²⁺ due to increased muscle activity
- Extended constriction changes parameters, e.g. K+, γ-GT
- "Bending" the needle is not necessary when using the S-Monovette® system because of a generally very flat penetration angle. Lumen changes caused by bent needles may damage the cells (haemolysis).
- Haemolysis can also be the result of a needle that is too thin.

Incorrect handling after blood collection:

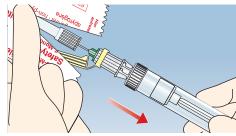
- Insufficient mixing of the sample (micro clots)
- · Excessive mixing (shaking) causes haemolysis
- Ensure compliance with coagulation times prior to centrifuging serum samples (approximately 30 minutes after sampling) to prevent post-coagulation (gelatinisation).
- Ensure observation of the centrifugation recommendations for improved sample quality



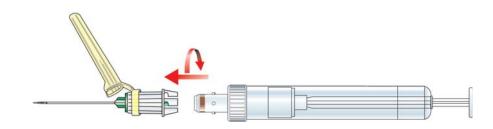
 Open the packaging at the tear-off line



Attach the needle



Remove the protective sleeve



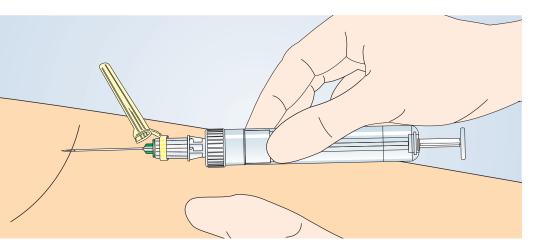
IMPORTANT:

• Do not lock the Safety-Needle of the S-Monovette® into place by slightly turning clockwise until immediately prior to venipuncture.

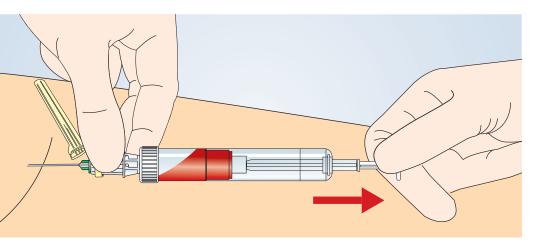


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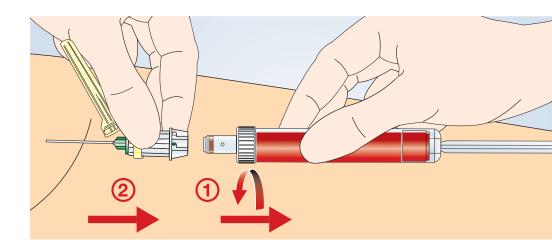
S-Monovette® – Aspiration Principle



• Use the thumb of your free hand to tighten the skin and hold the vein in place. "Warn" the patient and puncture the vein.

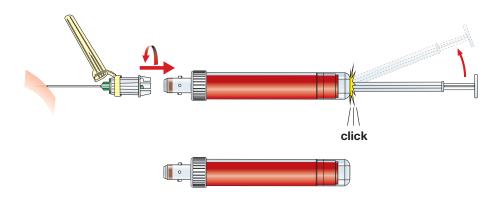


• Loosen the tourniquet and slowly withdraw the plunger. Wait until the blood flow stops.



• For multiple sampling, remove S-Monovettes from the needle by slightly twisting anti-clockwise. The needle remains in the vein.

After blood collection



• Remove the S-Monovette® from the needle and then withdraw the needle from the vein.

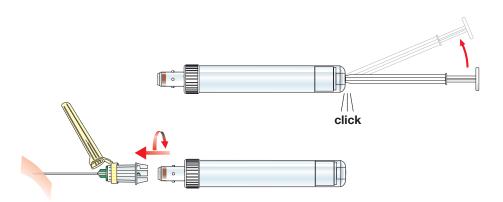
IMPORTANT:

When blood collection is complete, withdraw the plunger into the "click" position and break off.

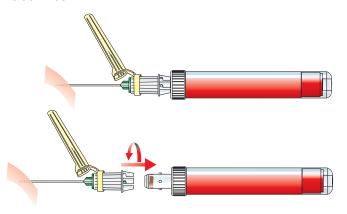




S-Monovette® - Safety Products

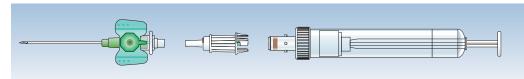


- Prior to blood collection, the Safety-Needle must already be in the vein.
 To begin blood collection gently, we generally recommend using the first S-Monovette® with the aspiration method. Then continue with the vacuum method.
- Immediately before blood collection withdraw the plunger into the "click" position and break off.
- Push the evacuated S-Monovette® onto the needle and secure by twisting clockwise.



• Wait until the blood flow stops. Remove the S-Monovette® from the needle by twisting anti-clockwise. Then withdraw the Safety-Needle from the vein.

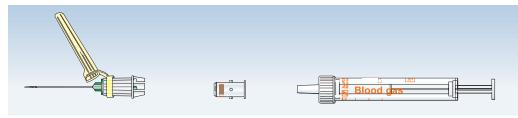
Multi-Adapter



• For Luer connections already in situ, e.g. indwelling cannulas.

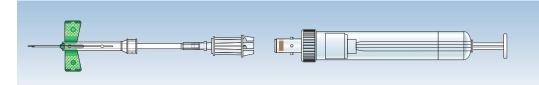
Discard the first S-Monovette® in case the patient has received an infusion.

Membrane-Adapter



• Blood collection with Luer systems (e.g. Blood Gas Monovette®) from an S-Monovette® needle already in situ

Safety-Multifly®-Needle



- Application:
 - Difficult vein conditions

Source of error:

- The air contained in the tubing is drawn into the first S-Monovette[®]. Incorrect mixing ratio for ESR and coagulation analysis. Discard the first tube, if necessary.





S-Monovette® – User Guide

Safety Needle

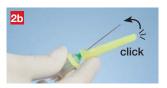


After blood collection:

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



Hold the Safety-Needle at the adapter, place the needle protector on a stable, flat surface and slightly press the needle downwards until it noticeably and audibly locks into the needle protector.



Alternatively, you can also activate the needle protector using your index finger.

For safe operation, make sure to press against the lower end of the protection device.



After activating the protective mechanism:

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

Safety-Multifly®-Needle



Hold the needle protector at its back end with your thumb on top and your forefinger below and withdraw the Safety-Multifly®-Needle from the vein. Affix the tubing by pressing it slightly against the palm of your hand and push the needle protector over the needle ...



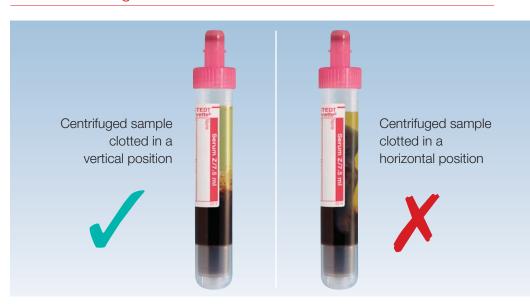
...until the needle is noticeably and audibly locked into the protective casing.



After activating the protective mechanism:

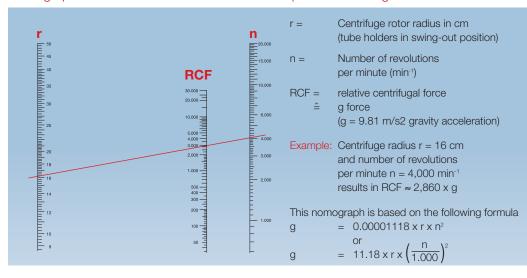
Discard the safely locked Safety-Multifly®-Needle into a disposal box.

Before Centrifugation



Centrifugation

Nomograph to convert the number of revolutions per minute into g-force



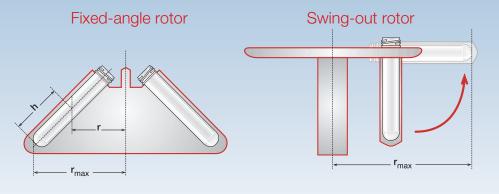


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S-Monovette® - General Information

Difference between fixed-angle and swing-out rotors

Please refer to the information provided by the centrifuge manufacturer for the centrifuge rotor radius ($r_{\text{\tiny max}}$) required for calculation or determine the radius by means of the following illustrations.



S-Monovette® – Centrifugation conditions

S-Monovette® Serum		10 min.	2,000 x g	20°C
S-Monovette® Serum-Gel*		10 min.	2,500 x g	20°C
S-Monovette® Li-Heparin		10 min.	2,000 x g	20°C
S-Monovette® Li-Heparin-Gel*	or	10 min. 15 min.	3,000 x g 2,500 x g	20°C 20°C
S-Monovette® EDTA-Gel*		10 min.	2,500 x g	20°C
S-Monovette® Citrate		10 min.	1,800 x g	22°C

*We recommend processing of S-Monovettes with gel preparation in swing-out rotors only.

Refer to the nomograph on the previous page or the centrifugation calculator at www.sarstedt.com/ User information/Centrifugation to convert the g-force into the number of revolutions per minute.

Sample storage and transport



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

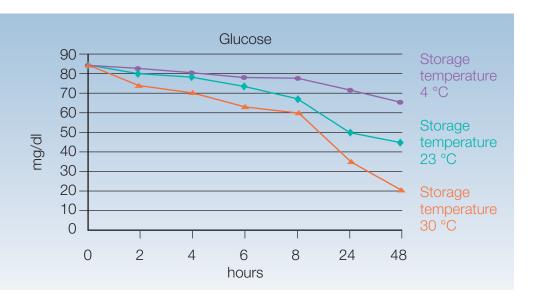
Whole blood without serum/plasma separation must not be frozen under any circumstances as this process would result in a total haemolysis.

- For long-term storage, the serum should be stored in enclosed containers at 2 to 4°C.
- Serum or plasma samples can be stored at -20°C for extended periods of time.
- Special cool transport containers should be used to protect samples during prolonged transit.
- Some analytes must be transported to the laboratory without delay (e.g. Ammonia: within 15 min.).





Influence of temperature and time



Communication of results

- As a rule, analysis results should be communicated to the requesting institution in writing only.
- Exception: Emergency diagnostics
- The communication of results by phone should remain an exception. Results should be disclosed to the doctor in charge only.
- Conveying the analysis results to the patient as well as their interpretation is restricted to the doctor in charge.
- The patient decides if results are to be disclosed to third parties.
 If the patient is unable to do so, the decision should be left to the doctor in charge.

A patient's laboratory data is private and it is a doctor's duty to maintain confidentiality.

Fields of application

Preparation **Application** Serum Clinical Chemistry, Serology, special analyses Serum-Gel Clinical Chemistry, Serology (only in routine diagnostics) Lithium-Heparin Plasma recovery for Clinical Chemistry, Serology EDTA K Haematology (e.g. Hb, HK, erythrocytes, leucocytes) Citrate 1 in 10 Coagulation analyses (e.g. Quick, PTT, TZ, Fibrinogen) ESR determination to the Westergren method Citrate 1 in 5 or using the S-Sedivette® Fluoride Glucose determination (24 h stability) and enzymatic Lactate GlucoEXACT Glucose determination (48 h stability at RT)

Order of draw*

Blood culture



Citrate blood



Serum-/Serum-Gel blood



Heparin-/Heparin-Gel blood



EDTA blood



Fluoride-/Citrate-Fluoride blood





^{*}It is recommended to draw a discard tube first when a citrate tube is the first tube needed.

^{*}Recommendation in accordance with CLSI standard H3-A6:

[&]quot;Procedures for the Collection of Diagnostic Blood Specimens by Venipuncture; Approved Standard - Sixth Edition"

Capillary Blood Collection & Microvette®







Capillary Blood Collection

What is capillary blood?

A mixture of blood from arterioles, venules and capillaries as well as intestinal and intra-cellular fluids.

Note:

Capillary blood collection does not necessarily involve the use of an end-to-end capillary.

Fields of application

- Paediatrics
- Geriatrics
- Adults: Blood Gas analysis, Glucose and Lactate determination
- Point-of-Care tests

Criteria that exclude capillary blood collection

- Volumes > 1 ml (e.g. blood culture)
- Coagulation analysis
- Inflammations
- Patient in shock

Capillary blood collection

- Preparation
 - Material
 - Patient
 - Puncture site
- 2 Puncture
- Sampling





Preparation

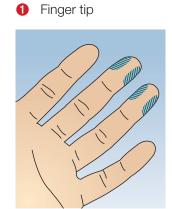
Material required:

- Gloves
- Swab
- Disinfectant
- Semi-automatic disposable lancet (Safety-Lancet)
- Sample tube (Blood Gas capillary, Microvettes, Bilirubin capillaries etc.)
- Multi-Safe Disposal Box
- Plaster, if required (not advisable with small children risk of swallowing)

Patient preparation

- Patient identification
- Inform the patient about the reason for blood collection and explain the procedure.
- Select the puncture site.
- Warm up the puncture site for enhanced blood circulation, if required.

Puncture sites











Advantages of warming the puncture site

- Enhanced blood flow by up to seven times the normal volume
- Precondition for capillary Blood Gas analyses

Enhancing the blood circulation leads to an arterialisation of the capillary blood and, as a result, to an acceptable comparability with the analyses obtained from arterial blood.

How to warm up the puncture site

- Wrap the patient's foot or hand with a cloth heated to 39 to 40°C.
 For optimal result, pull on a rubber glove.
 Duration: 3 to 5 min.
- For capillary Blood Gas analyses from adults, rub a hyperaemisating ointment into the earlobe.

Puncturing and sampling

- Wear gloves
- Skin disinfection
 - Disinfectant
 - Leave to dry (until the disinfectant has completely dried up)
- How to hold the finger or foot
- Puncturing with a Safety-Lancet





Product features





- Primed system ready for use one application step less.
- cannot be reused.
- Easy handling secure firing button avoids the risk of unintentional activation and deactivation of the system
- Ridged lancet body ensures safe grip
- Small contact face for precise puncturing
- Variety of options

• The sterile, disposable product

1. Twist the cap until it separates from the Safety-Lancet.

User Guide



2. Press the Safety-Lancet firmly against the chosen and cleaned puncture site and press the firing button.



3. Discard the Safety-Lancet into a suitable sharps container.



4. Collect blood

Product range

- 5 Different options
- Version for heel puncturing

	7	7	7	7	
Description	Mini	Normal	Extra	Super	Neonatal
Penetration Depth	1.6 mm	1.8 mm	1.8 mm	1.6 mm	1.2 mm
Needle size	28 G	21 G	18 G	Blade 1.5 mm	Blade 1.5 mm
Blood volume	Low	Medium	Medium to high	High	Medium to high

Important information

- Discard the first drop of blood.
- Hold the punctured limb downwards.
- Avoid blood smears.
- Ensure that the sample tube is held in the correct position.
- Avoid repeated strong pressure (milking).

This causes haemolysis and contamination of samples with tissue fluid.



Product features:



- For the collection of even the smallest blood volumes from 100 μl to 500 μl.

Different inner tube options – conical tube for a high supernatant after centrifugation or cylindrical tube for enhanced mixing results.

Range of collection techniques

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

Microvette® - Order of draw*



EDTA



Lithium-Heparin / Lithium-Heparin-Gel



Fluoride





Serum / Serum-Gel

SARSTEDT

Microvette® - Collection methods

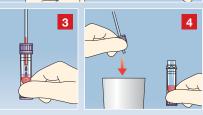
- 1 Capillary method using the end-to-end capillary
- 2 Sampling with the collection rim

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

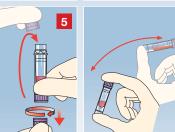
1. Capillary method



- 1. Hold the Microvette® in a horizontal or slightly inclined position and collect the blood sample with the end-to-end capillary.
- 2
- 2. Collection is complete when the capillary is entirely filled with blood.



- 3. Hold the Microvette® upright to allow blood to flow into the collection tube.
- 4. Twist to remove the cap including the capillary and discard as a complete unit.



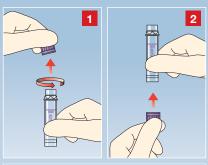
- 5. Remove the cap from the tube base and close the tube ("click" position).
- 6. Mix samples thoroughly and gently.



^{*}Recommendation in accordance with CLSI standard H4-A6: "Procedures and Devices for the Collection of Diagnostic Capillary Blood Specimens; Approved Standard – Sixth Edition"

Microvette®

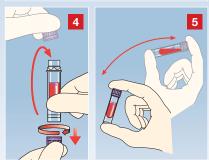
2. Sampling with the collection rim



- 1. Slightly twist the cap to detach.
- 2. Attach the cap to the tube base.



3. Use the special rim to collect the blood drops.



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

Microvette® – Centrifugation conditions

Microvette® Serum	5 min.	10,000 x g	20°C
Microvette® Serum-Gel	5 min	10,000 x g	20°C
Microvette® Heparin	5 min.	2,000 x g	20°C
Microvette® Heparin-Gel	5 min.	10,000 x g	20°C
Microvette® Fluoride	5 min.	2,000 x g	20°C

Information on the centrifugation conditions is also printed on the inner box label.



Preanalytics & Urine-Monovette®







Types of urine samples

Preanalytics:

Reliable results in urine analysis are subject to duly observed collection, transport and storage conditions.

Depending on the time and type of urine collection, we differentiate between:

- Mid-stream urine
 - First morning urine
 - Second morning urine
 - Spontaneous urine
- Bladder puncture urine
- Catheter urine
 Urine collected in cases of one-time catheterisation
 and a permanent catheter
- 24 h urine

Mid-stream urine

Correct sampling:

- 1 Thorough cleaning of external genital area
- 2 Once urine has been flowing for approximately 3 seconds, 10 to 20 ml urine are passed into a sterile collection container without interrupting the urinary stream.

Make sure to avoid contamination.

Note:

- Especially important for microbiological analyses
- Precondition: Patient must be able to cooperate

First morning urine

The constituents contained in the first morning urine are of particularly high concentration.

Application:

Suited for bacterial analyses, test strips, sediment, clinical-chemical analyses, protein diagnostics

• Advantage:

Due to the extended retention time in the bladder, morning urine is ideally suited for nitrite and protein determination.





Types of urine samples

Second morning urine

The constituents in the second urine passed after the first morning urine are of a medium concentration.

- Application: Test strips, glucose, protein
- Disadvantage:
 Not suited for nitrite testing

Spontaneous urine

Urine collected at any given time.

- Application:
 Entirely sufficient for many chemical and microscopic parameters
- Advantage: Easy to collect
- Disadvantage:
 Dilution error always take into account the specific weight (density) for correct determination

Bladder puncture urine

Urine collected through bladder puncture is suited for bacterial analysis, primarily in case of infants and small children.

Note:

Reduced risk of infection compared to catheterisation.

Catheter urine

One-time catheterisation:

Collecting urine by means of one-time catheterisation is very rarely done as it is painful for the patient and involves a high risk of infection.

Permanent catheter:

If collecting urine from a permanent catheter is an absolute requirement, this must be done through the sterile catheter puncture.

Note:

For diagnostic purposes, urine should not be collected from the urine drainage bags.





24h Urine Collection

The term "Urine Collection" generally describes any urine volume collected over a particular period of time, whilst the 24 h period is the interval most frequently applied.

- Application:
 - e.g. catecholamines, creatinine clearance
- Advantage:

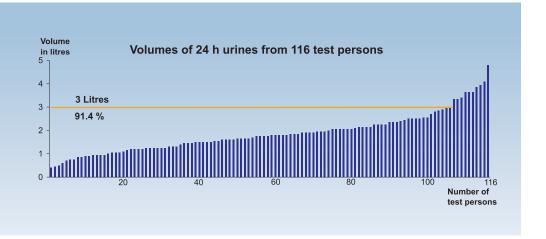
Eliminates any fluctuation in the parameters as may be caused by a difference in concentration.

• Disadvantage:

Extended collection periods, sufficiently dimensioned collection containers, precise patient instructions, correct stabiliser

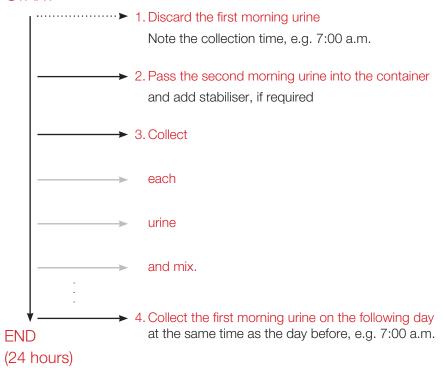
Urine container volumes

- Studies have revealed that a 3,000 ml container accommodated 91.4 % of all 24 h urines collected.
- A 2,000 ml container can hold only 60 %.



How to collect 24 h urine

START



UriSet 24 - "The complete set"







1 Dry chemical urine analysis by means of a test strip to determine early symptoms (Screening-Test)

Note:

The screening result alone is not sufficient for a direct diagnosis as it only renders an indication of the existence and approximate amount of a particular substance. The results obtained serve as a basis for more detailed microscopic, bacterial or clinical-chemical analyses.

2 Sediment analysis in case of unusual results obtained in dry chemistry.

Urinalysis and preanalytics

• Use fresh, non-stabilised and non-centrifuged mid-stream urine (not stored for longer than 2 hours).

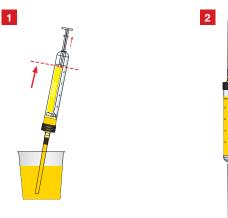
Extended storage may cause the following changes, e.g.

- Disintegration of leucocytes and erythrocytes
- Bacterial growth
- Glycolysis caused by bacteria
- Mix urine thoroughly immediately before using the test strips
- Overall wetting of all test fields
- Ensure observation of the incubation time
- Correct centrifugation to obtain the urine sediment (5 min. 400 x g)

The Urine-Monovette® is suitable for urine sampling, transport, analysis and centrifugation.



Urine-Monovette® User Guide



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



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



Remove the tip, break off the plunger and attach cap.





Microbiological urine diagnostics

Note:

- Native urine is the ideal material to determine the bacteria causing urinary infections provided the sample is analysed within 2 hours when stored at ambient temperature, and within 4 hours when kept in a cool environment.
- We suggest the use of morning urine (mid-stream urine).
 During the day, advise patients not to pass urine at least 4 hours before collection.
- No antibiotic treatment 2 to 3 days prior to collection.

Urine-Monovette® with boric acid



In a filling volume of 10 ml, the boric acid concentration is 1.5 %.

Micro organisms are stabilised for up to 48 hours when stored at room temperature.

Important:

- Observe the nominal volume
- Mix thoroughly after urine has been aspirated into the Urine-Monovette®
- Not suitable for clinical-chemical analyses, test strips, etc.

Recommendations for urine collection

- Analyse urine within two hours
- If possible, use mid-stream urine for analysis
- Proficient sampling
- Use clean, disposable containers
- · Correct identification of containers prior to sampling



Errors in preanalytical work are the figures in front of the comma.

Errors in laboratory analytics are the figures behind the comma.



Notes







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