Tips & Techniques in Pre-analytics

ANIMAL HEALTH CARE

Volume 1: Blood analysis in companion animals

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Introduction

Clinical laboratory diagnostics is an integral part of the veterinary practice. After the case history and clinical examination, tests are ordered to confirm or rule out the suspected diagnosis. Hematology and clinical chemistry frequently provide essential information for identifying disease. Especially since molecular biology has found its way into laboratories, the etiology of some diseases can be better clarified. Laboratory results continue to be interpreted in monitoring, therapy follow-up and prognosis of diseases.

Prof. Dr. med. vet. Andreas Moritz

The quote from the information brochure Tips & Techniques in Pre-analytics, prepared by LABOKLIN and SARSTEDT "… with the highest precision of modern analytical equipment, the measured result can only be as good as specimen quality allows" illustrates the particular importance of this subject. Pre-analytics refers to the part of the laboratory diagnostics process which takes place before the actual sample analysis. On completing the case history and patient preparation, this comprises correct sample collection, storage and transport to ensure that the results are reliable and diagnostically conclusive. While implausible values in the analysis process in the laboratory can be checked by repeat measurements, pre-analytical errors cannot usually be remedied. Therefore it is important to perform all steps carefully to minimize potential errors.

In the following eight sections, the basic facts on the topic are conveyed and illustrated ian informative and easily understood manner: What is Pre-analytics, – Patient preparation, – Overview of sample materials and sample tubes, – Common errors in pre-analytics, – Sample collection, – Safety throughout the sample collection, – Preparation of the laboratory test, – Labeling, storage and transport. Easily remembered texts in grey highlighted boxes support understanding, learning and remembering. The production and analysis of blood smears is – rightly – given plenty of space. The cell images from dog, cat and small mammal blood smears are positively conspicuous and go somewhat beyond pre-analytics.

This booklet is highly recommended not only for veterinarians, veterinary assistants and medical technicians as a handy little reference for the practice, but also for students of veterinary medicine as interesting learning material.

Prof. Dr. med. vet. Andreas Moritz studied veterinary medicine at Justus-Liebig University (JLU) in Giessen. Following his doctorate and habilitation in the field of internal medicine and clinical laboratory diagnostics, he was appointed university lecturer at the Department of Veterinary Medicine of JLU. After spending time abroad in St. Paul, Minnesota, USA, and Gent, Belgium, he returned to JLU and was appointed professor for clinical pathophysiology and clinical laboratory diagnostics in 2006. In addition to directing the department central laboratory, from 2017 onwards he took over the directorship of the Clinic for Small Animals, Internal Medicine. He is a specialist in veterinary internal medicine and specialist in veterinary clinical laboratory diagnostics as well as EBVS® European Veterinary Specialist in Small Animal Internal Medicine (dipl. ECVIM-CA) and associate member of the European College of Veterinary Clinical Pathology (ECVCP). Alongside training student of veterinary medicine, Prof. Moritz is involved in the national and international professional qualification of veterinarians in the field of internal medicine of small animals and clinical laboratory diagnostics. Currently he is president of the German Small Animal Veterinary Association (DGK-DVG).

ANIMAL HEALTH CARE

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Link to the PDF version: www.sarstedt.com/laboklin-blood-test-vet-us

animalhealthcare.
sarstedt.com

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1. What is pre-analytics?

“Hello mouse, you're a lab expert, right?”

“Yes, I'm ready to assist you with help and advice!”

“That’s super! What is actually meant by pre-analytics, and why is this important to know?”

“Pre-analytics includes all processes that take place before the laboratory analysis and may influence the measured result. Ultimately, even with the highest precision of modern analyzers, the measured result can only be as good as specimen quality allows.”

Pre-analytics covers all areas prior to actual analysis of the sample material. These include the case history and the clinical examination, which provide the indication for testing. Also included are patient preparation, selection of the correct sample tubes, sample collection, sample preparation, sample transport as well as sample storage, sample processing and the path of the sample until testing begins.

As is evident from the list, most pre-analytical tasks take place before the sample reaches the laboratory. The attending veterinarian can therefore in many cases provide a crucial contribution to good sample quality and thus a representative and evaluable result. Due to the large number of potential sources of error, it makes sense to familiarize oneself with various aspects of pre-analytics ahead of an analysis.

2. Patient preparation

A pleasant and stress-free visit to your practice for patients and owners/keepers is not only good for your image, but also has a significant influence on the sample quality.

The animal owner should be informed in advance about the influence of physical activity and stress on the results of blood analysis. Increased levels in particular of muscle-associated enzymes, such as CK, LDH and AST, can be detected in the serum after physical exertion. In addition, elevated serum levels of glucose and lactate are also to be expected. Additional aspects which should be clarified beforehand will be considered below.

Note

"Carnivores should best be presented for blood collection strictly fasting (around 12 hours abstinence), as postprandial lipemia can influence many measured variables, leading in the worst case to non-evaluable samples."*

** (Moritz et al., 2014)*

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2.1. Influencing factors

An overview is presented below of influencing factors which may represent potential sources of error in diagnostics. Signalment, therapy, suspected diagnosis and test procedures should therefore be known before each examination.

2.1.1. Age, breed and living conditions

- Young animals: For some parameters (e.g. AP, phosphate …), they may exhibit higher or lower values than adult animals (Humann-Ziehank and Ganter, 2012).

- Breed-specific peculiarities: With some parameters, there are breed-specific deviations (e.g. greyhounds: lower thyroxine concentration, leukocytes and platelet concentration, higher hematocrit; Cavalier King Charles Spaniel: macrothrombocytopenia possible (Zaldívar-López *et al.*, 2011).

2.1.2. Test selection

Before blood collection, a few considerations should be made about the desired test and the requirements:

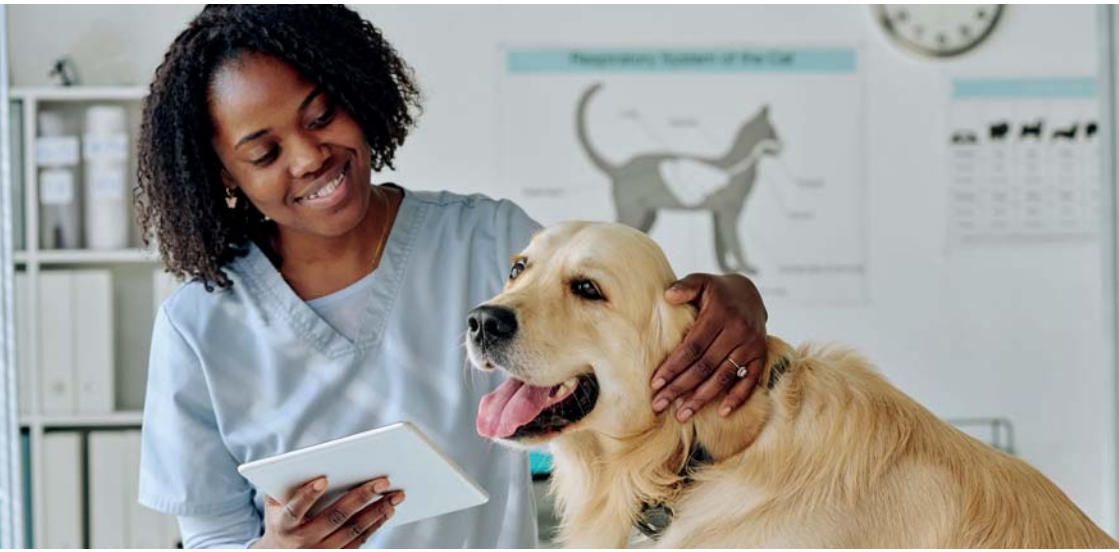
- Is the requested test available for the species of animal?
- Does the patient have to be in a specific condition for the intended test (e.g. fasting) or remain in the practice for a certain time?
- Does it involve a therapy follow-up or a check of correct medication adjustment?
- Are suppression or stimulation tests required?
- What material is needed? Is the desired analysis using the available sample material possible and useful? Example: Coagulation tests are possible only with citrate plasma!
- How much sample volume is necessary for the test?
- Can the sample withstand transport to the laboratory so that measuring the desired parameter is still feasible? It is advisable to check the parameter stability beforehand and to clarify the required pre-analytical conditions.

Stress, agitation and extreme physical exertion are always to be avoided before blood collection (Moritz *et al.*, 2014). This applies in particular to wild animals, but also to animals that are handled infrequently. (Braun *et al.*, 2015).

2.1.3. Medication and biorhythm

- Is the patient on a particular medication that influences the test results and may potentially lead to a false interpretation? (Examples of problematic combinations are phenobarbital treatment in an epilepsy patient when tested at the same time for hypothyroidism (Gaskill *et al.*, 2000), glucocorticoid treatment in a dog that is to undergo an ACTH stimulation test, or antibiotic pretreatment prior to microbiological testing.)
- Is there a biological rhythm for the requested parameter, which needs to be observed?

It is evident that not all pre-analytical influencing variables can be monitored. Nevertheless, as many factors as possible should be documented (Braun *et al.*, 2015) to facilitate the interpretation of the results later.



2.1.4. Order of collection

Using a fixed, established routine is recommended for the order of collection, for example the order of collection according to Gurr. The meaning of the different color codes is explained in chapter 3.2.1.

ISO color code according to NCCLS	
	Citrate – blood
	Serum/Serum Gel – blood
	Heparin/Heparin Gel – blood
	EDTA – blood
	Fluoride/Citrate Fluoride – blood

Gurr *et al.*, (2011)

"Oh, no! We collected the blood sample into the EDTA tube instead of the serum tube. I'll just quickly pour it in the other tube!"

"Please don't! The blood was already mixed with EDTA during the collection process, which then distorts the measured results! Samples must never be poured off from one treated tube to another."

(Moritz et al., 2014)

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3. Overview of sample materials and sample tubes

Several components are available for blood testing. They are introduced briefly here.

There is whole blood, serum and plasma. These differ in composition. Further processing of the sample depends on the sample material.

Whole blood is utilized with an added anticoagulant (e.g. heparin or EDTA). This prevents coagulation of the blood and facilitates separation of the liquid blood components from the solid components.

Plasma is obtained from whole blood with an added anticoagulant (e.g. heparin or EDTA) and immediately afterwards is separated by centrifugation into blood cells (solid components) and plasma (liquid components). In contrast to serum, plasma contains all coagulation factors of blood, as these were not used for coagulation.

Serum is also obtained from whole blood, however without addition of an anticoagulant, but generally with a coagulation inducer. Hence the sample will and should coagulate and may be centrifuged 30 minutes standing upright. Serum contains no coagulation factors, as these are used during the coagulation process (Moritz *et al.*, 2014).

Note

"If the time the serum tube is left standing is too short, the serum may form a gel-like consistency making testing difficult or impossible!"

(Moritz et al., 2014)

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3.1. Serum vs. plasma – what is the difference?

Serum	Plasma
… cell-free supernatant from a fully coagulated sample	… cell-free supernatant from a sample with anticoagulants added
… contains no fibrin	… contains fibrin
… lower yield	… higher yield
… the sample first has to coagulate before it can be centrifuged.	The sample can be centrifuged immediately.

Coagulum forms in the shape the blood cells take in the tube.

This means if the S-Monovette® is lying flat after blood collection, the blood cells sediment along the horizontal tube and form an elongated shape.

This formation can be compressed during centrifugation. After centrifugation, however, it expands again.

Note

"The serum from such a sample cannot be readily pipetted. Therefore it is important to store serum samples upright after blood collection."

Sample coagulated upright after centrifugation

Sample coagulated horizontally after centrifugation

3.2. Overview of sample materials

Commonly used sample tubes and their primary uses are listed in the table below:

Sample materials	Applications
Citrate	Blood coagulation
Sodium fluoride	Glucose/lactate
Serum gel	Clinical chemistry
Lithium heparin	Blood count / Clinical chemistry
EDTA	Blood count

3.2.1. Test tube color codes

Don't be bothered by the different tube colors!

The quick-release caps of the sample tubes indicate the preparations (anticoagulant / coagulation inducer) in the vessels. Note that there are two different color codes.

- “EU color code”
  (is based on the British Standard BS 4851)
- “ISO color code”
  (is based on the international Standard ISO 6710)

It's best to agree, both the practice and the laboratory, on one of the two color codes. Nevertheless, the exact preparation is also printed on the tube in addition to the color coding.

3.2.2. Lithium heparin tubes

These are the heavy hitters among tubes, as lithium heparin blood can be used for determining blood count, and lithium heparin plasma can be used for analysis of numerous clinical-chemistry parameters (to avoid hemolysis, ideally centrifuged in advance if a blood count is not required from the same sample). The yield from plasma is also somewhat higher than from serum because, with serum, a larger proportion of fluid always remains in the coagulum (Moritz *et al.*, 2014). Lithium heparin tubes are particularly popular among all those who care for small mammals or exotic pets, as usually only a small sample volume can be collected from these animals.

Caution: Samples in lithium heparin tubes are not suitable for PCR analyses because of the inhibitory effect (Schrader *et al.*, 2012).

Note

"Important: Wherever possible, the same material (serum or plasma) should be used for the clinical chemistry analysis. This applies in particular to repeat analyses, since for some analytes, such as potassium, differing concentrations may be present in serum and plasma."

(Humann-Ziehank and Ganter, 2012; Braun et al., 2015)

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3.2.3. EDTA tubes

EDTA (ethylenediaminetetraacetic acid) is a chelating agent. It functions as an anticoagulant by binding metal ions, in particular calcium ions necessary for blood coagulation. There are different forms of EDTA, including dipotassium and tripotassium EDTA. (Moritz *et al.*, 2014). Blood in EDTA tubes is predominantly used for determining blood counts.

It is important to gently invert the sample immediately after blood collection to prevent clots, as otherwise the sample can no longer be reliably tested (Vap *et al.*, 2012)! EDTA tubes should never be filled first, as there is a risk of contamination of the needle with EDTA. This can then result in incorrect measurements for numerous parameters (Sharratt *et al.*, 2009).

EDTA samples are not only needed for determining blood counts, but also for determining serological blood type. They can also be used for genetic tests and pathogen detection by PCR (polymerase chain reaction) if the pathogen sought is present in the blood. However, for many investigations, such as ACTH or pro-BNP, centrifuged and pipetted EDTA plasma is needed (see p. 48 ff. for more details on sample centrifugation and labeling).

Note

"Important! With reptiles and some bird species, use of EDTA is contraindicated, as EDTA may cause hemolysis that makes later evaluation impossible."

(Nardini et al., 2013)

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3.2.4. Serum tubes

Serum tubes generally contain coagulation inducers, which lead to rapid coagulation. Serum can be used for the analysis of many clinical-chemical parameters and for numerous serological test methods. Serum electrophoresis, as the name suggests, should also be performed with serum.

Serum gel tubes:
These have a similar use to serum tubes. Their advantage is that, after centrifugation, the gel sits between the layers, making it unnecessary to pipette off the supernatant.

"Here I have a tube with the abbreviation CAT. Is that only for cats?"

"No, serum tubes are also labelled with the abbreviation CAT, which stands for 'Clot Activator'."

(Moritz et al., 2014)

Neutral tubes:
Neutral tubes are marked with “Neutral or Neutral Z”. They are non-coated and are used either as serum tubes or for transferring plasma samples (e.g. citrate plasma).

3.2.5. Citrate tubes

The mixing ratio for citrate blood is generally 9:1. Citrate blood and/or citrate plasma is necessary for coagulation analyses. Several important points should be considered for correct handling:

1. Citrate tubes should never be filled first (Moritz *et al.*, 2014) as coagulation is initiated as a result of the stasis process.

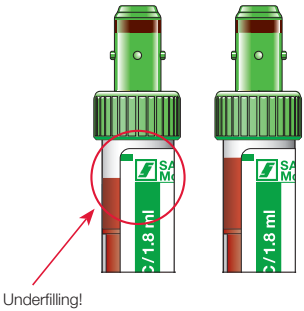
2. Even though it should be ensured for all tubes that the expiration date is not exceeded (Braun *et al.*, 2015), it is particularly important for these tubes! If in doubt, new tubes should be ordered.

3. It is essential that the tubes are filled exactly to the mark during sample collection. Over or underfilling leads to unreliable coagulation analysis results. In some cases the sample can longer be tested

4. For most coagulation parameters, it is recommended to centrifuge and pipette the sample promptly and use cooled citrate plasma (see 3.2.4. neutral serum tubes) for the analysis. However citrate whole blood is required for thrombelastography!

Optimal filling volume varies among tubes. See arrow for filling level mark to be observed for the two right tubes.

It is essential to avoid under and overfilling! Clots not only lead to erroneous hematalogical test results, but can also block the capillaries of the hematology equipment (Moritz *et al.*, 2014).



3.2.6. Sodium tubes

These are suitable only for lactate and glucose determination. They are particularly important when glucose cannot be determined directly in the practice, but is sent to an external laboratory. Sodium fluoride should stop glucose degradation in the sample, therefore sodium fluoride tubes can be used for the determination of glucose concentration (Braun *et al.*, 2015).

3.3. Which sample type is suitable for which test?

Not every sample material can be used for every test. The following table provides an overview of which tests are reasonably possible from which blood component.

Possible uses of different sample types

Anticoagulant	Component	Blood count	Blood smear	Clinical chemistry parameters	Serology	Coagulation
EDTA	Whole blood	Yes	Yes	No	No	No
EDTA	Plasma	No	No	Limited	Limited	No
Lithium heparin	Whole blood	Yes	Limited	No	No	No
Lithium heparin	Plasma	No	No	Yes	Yes	No
Citrate	Whole blood	No	Yes	No	No	Limited
Citrate	Plasma	No	No	No	No	Yes
NaF (sodium fluoride)	Plasma	No	No	Glucose, lactate	No	No
Serum tubes without anticoagulant	Serum	No	No	Yes	Yes	No

4. Common errors in pre-analytics

4.1. Interference factors

Hemolysis, underfilled sample tubes and blood clots are among the most common interference factors in pre-analytics. The following table provides an overview of the causes and informs about the consequences.

Common interference factors in analytics and their potential causes

Interference factor	Cause	Affected parameters
Clots	Overfilling of the EDTA, lithium heparin or citrate tube	Blood count including platelet count, coagulation parameters
Hemolysis	Sample not centrifuged, improper centrifugation, pipetting error, long storage time, very high temperatures, frost	Various clinical chemistry parameters, including false high potassium concentrations and false low calcium concentrations
Lipemia	Patient not fasted, medications, endocrinopathies as well as various other pathological changes	Various clinical chemistry parameters as well as occasionally blood count parameters; for example, it may be that the hemaglobin content measurement yields false high results in lipemic samples.
Medication	Therapy (e.g. infusions, glucocorticoids, antibiotics, sedatives)	Varies depending on medication and parameter (false high or false low), for example, with glucocorticoid administration elevated liver enzymes in dogs (in particular alkaline phosphatase) are typical.
Over or underfilling	Specified fill level not observed	Coagulation parameters

Note

"EDTA contamination, for instance due to incorrect order of sample collection, can lead to typical changes, for example very high potassium values and low calcium values."

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4.1.1. Hemolysis

Hemolysis can have various causes. Hemolysis frequently occurs *in vitro*, for example from prolonged tourniquet application, physical shear forces (needle too thin, needle bent), traumatic vein puncture (“poking”), too vigorous inversion or shaking the blood sample, too high or low temperatures, too high rotation speed during centrifugation, contamination with water or disinfectants as well as samples that are too old. Even though hemolysis occurs more frequently *in vitro*, hemolysis *in vivo* should always also be taken into consideration.

Consequences of hemolysis

Release of cell contents – concentration differences

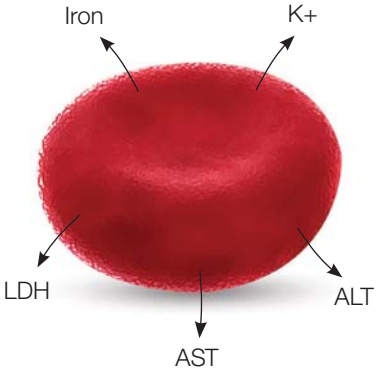
Substances present at higher concentrations in red blood cells (intracellular concentration) escape into the serum/plasma (extracellular concentration) as a result of the destruction of the erythrocyte cell membrane during hemolysis. The consequences are false high measured results.

Release of cell contents – optical interference

During hemolysis, hemaglobin, the red blood pigment, is also released into the serum/plasma. This can lead to false measurement signals in photometric analyses due to the endogenous extinction of hemaglobin.

False measurement signal = False result

(Humann-Ziehank and Ganter, 2012)



Note

"Hemolysis refers to the escape of intraerythrocytic substances following damage to the cell membrane. As a result of the red coloration of the serum/plasma, problems arise during the measurement, in particular during photometric tests."

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4.1.2. Hyperlipidemia (lipemia)

Lipemia may influence numerous parameters (Braun *et al.*, 2015). Depending on the degree of lipemia, the result may be that some parameters cannot be determined at all. Carnivores are best presented for blood collection strictly fasting (around 12 hours abstinence). The causes of lipemia can vary – while in the simplest case it is only a matter of postprandial hyperlipidemia, dietary and medicinal influences as well as endocrinological, inflammatory, neoplastic or genetic causes can also play a role (Xenoulis and Steiner, 2015). Repeatedly occurring hyperlipidemia should therefore be clarified further.

Lipemic sample

Note

"Correct laboratory results are fundamental for further treatment decisions – but require good pre-analytical sample quality."

4.1.3. Jaundice

Diverse interferences in measurement can occur with jaundiced samples (Martínez-Subiela *et al.*, 2002; Berlanda *et al.*, 2020). It is therefore important that the samples are inspected visually for yellowing as well as by means of a specific measurement. *Caution*: It is important to know that plasma/serum from horses has a physiological yellow coloration!

Icteric sample

Note

"Good that, using the indices for hemolysis, lipemia and jaundice, I immediately have an impression of the quality of my sample! That way, I can better assess how the results of the respective tests are to be interpreted"

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5. Sample collection

Depending on animal species, different veins are suitable for venous blood collection. The following graph provides an overview of which veins are commonly used for which animal species.

Note

"Always make sure to also protect yourself during collection and wear gloves!"

Possible puncture sites for blood collection

Species	*Jugular vein*	*Lateral saphenous vein*	*Medial saphenous vein*	*Cephalic vein of forearm*	*Auricular vein*	*Facial vein (buccal plexus)*
Dog	×	×		×		
Cat	×		×	×		
Rabbit/hare		×		×	(×)	
Ferret		×		×		
Guinea pig		×		×		
Rat, mouse, gerbil		(×)				×
Horse	×		(×)	(×)		

(Moritz et al., 2014)

In small animals, the blood is collected preferably with a 20-G needle from the *cephalic vein of forearm* (Moritz *et al.*, 2014).

The right solution for every animal – together for improved animal welfare.

Veterinary practices/clinics encounter many different animal species, all with their own highly individual requirements. SARSTEDT offers you the right solutions for pre-analytics in veterinary medicine.

The S-Monovette® – safe, gentle and hygienic blood collection from small animals

The Multivette® – collect blood from smaller pets gently and easily using only venous pressure

The Micro needle – even from especially delicate animals, you can reliably collect each drop, e.g. in a micro sample tube or a Microvette®

Venous blood is generally required for most analyses. In rare cases, capillary or arterial blood may be necessary/advantageous, for example for blood gas analysis (arterial blood) or in cases of suspected babesiosis (capillary blood).

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The following steps are recommended:

1. Prepare sufficient quantities of required utensils!
2. Bring in assistants!
3. Hand disinfection! Gloves!
4. Examine veins and make selection!
5. Shear and disinfect!
6. Stop patting the puncture site!
7. Have assistant place the tourniquet!
8. Remove protective sheath of safety needle!
9. Bevelled side of needle facing upwards!
10. Puncture angle less than 30°!
11. Tension skin; fixate vein!
12. If applicable, “pre-warn” owner!
13. Loosen tourniquet when blood is flowing!
14. Collect samples; note order of collection!

“Do you have any other tips for blood collection?”

“Definitely! Avoid prolonged use of the tourniquet, it can affect several parameters, such as potassium. Pumping should also be avoided.”

(Moritz et al., 2014)

Watch video!

Blood collection in a large dog with an S-Monovette® and a safety needle

The **S-Monovette® blood collection system** enables closed blood collection using two techniques. The aspiration technique facilitates blood collection that adjusts to the blood flow, making it gentler. For a high blood flow, the dual system of the S-Monovette® also allows for the conventional vacuum technique for blood collection.

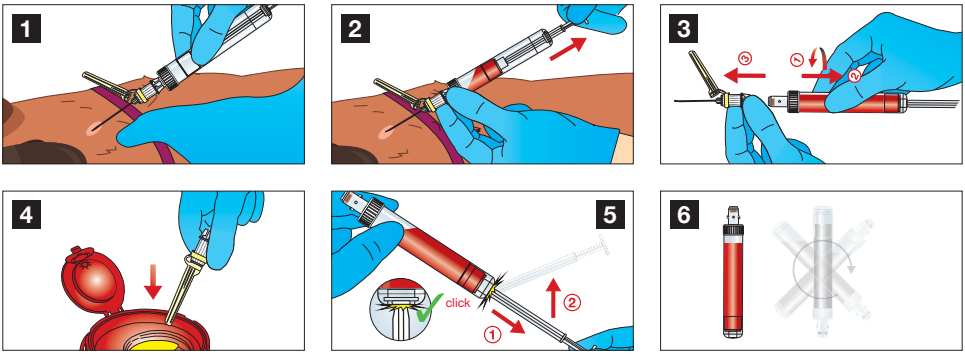
Collection using the S-Monovette® and safety needle markedly reduces the risk of EDTA transfer.

(Sulaiman, 2011)

Note

"If you are ever unsure whether the sample volume is sufficient for all desired tests, you can simply specify the desired processing order. Important: If, for example, lithium heparin blood is sent only once and a clinical chemistry parameter is noted as the first point in the sequence, it will no longer be possible to determine the blood count after centrifugation."

Blood collection with the S-Monovette® blood collection system



Once the patient and supplies have been prepared for the blood collection, the following steps are performed.

1. The selected vein is punctured with the safety needle connected to the S-Monovette®.

2. The piston of the S-Monovette® is pulled back slowly according to the blood flow, to the end of the tube until the blood flow stops.

3. The S-Monovette® can easily be detached from the safety needle with a twisting motion. This enables multiple sampling with one needle.

4. After blood collection, the safety needle can simply be locked into the needle guard and disposed of.

5. Once all S-Monovettes are collected and the patient cared for, all S-Monovette pistons are retracted until an audible click is heard. Then the pistons are broken off.

6. Invert several times and the sample is ready for the laboratory.

To use the vacuum technique with the S-Monovette®, the piston is pulled back into the locking position at the base and snapped off. This produces a “fresh” vacuum in the sample tube. When this S-Monovette® is then connected to the needle, the sample tube fills as a result of the vacuum.

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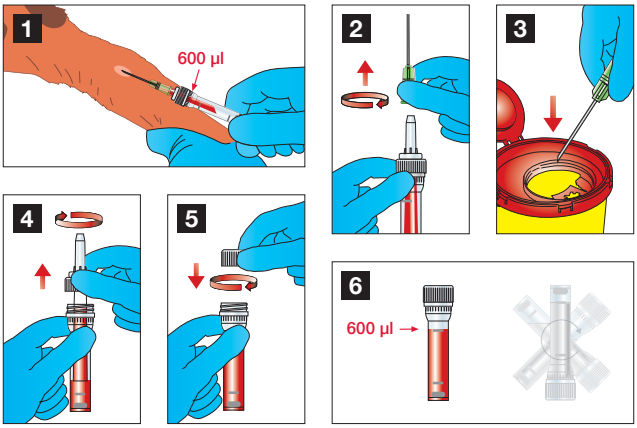
Blood collection from a cat using a Multivette® 600 and a Luer needle

Watch video!

The **Multivette® 600** is designed for small blood volumes of 600 µl. Blood is collected in a practically closed state, via natural venous pressure. This makes collection especially easy and gentle.

The blood sample can be centrifuged directly in the Multivette® 600. This eliminates sample transfer and saves time. The small inner diameter makes pipetting after centrifugation easy. In addition, the sample can be closed securely and sent to the laboratory.

Blood collection with the Multivette® 600



Once the patient and and supplies have been is prepared for the blood collection, the following steps are performed:

1. The selected vein can be punctured easily with the Multivette® and a commercially available Luer needle. Thanks to the internal capillary, the Multivette® 600 is self-filling due to venous pressure, making blood collection especially gentle and easy. The fill line indicates when the Multivette® 600 is completely full.

2. After collection, the Luer needle is removed.

3. Luer needles are disposed of in an appropriate disposal box.

4. The special design of the Multivette® 600 helps blood flow from the capillary when the Multivette® is held upright and twisted open.

5. The Multivette® is sealed with the enclosed screw cap.

6. Now invert several times and the sample is ready for the laboratory.

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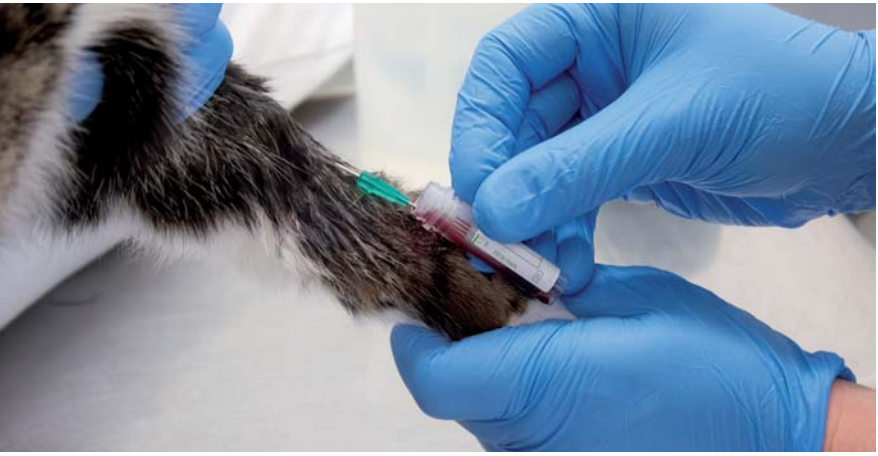
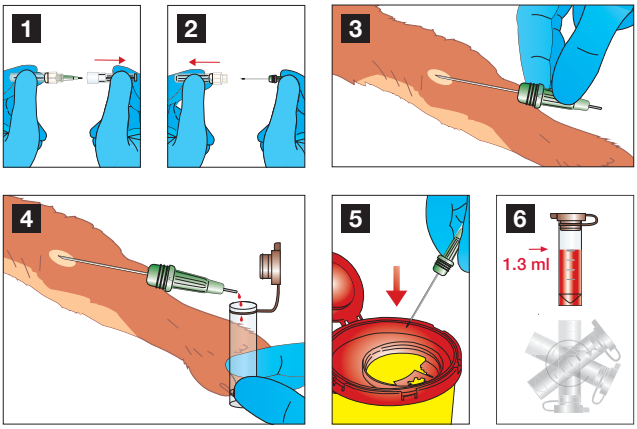


Watch video!

Blood collection from a cat using a Micro needle and a Micro sample tube

Especially for very small animals and difficult vein conditions, every drop of blood counts. The special **Micro Needle** ensures that every drop of blood flows into the sample tube and does not clot beforehand. The correct sample tube can be selected according to the expected sample volume. Micro sample tubes are ideal for volumes of 1.3 ml. For smaller patients, the Microvette® is the right choice for volumes from 100–500 µl.

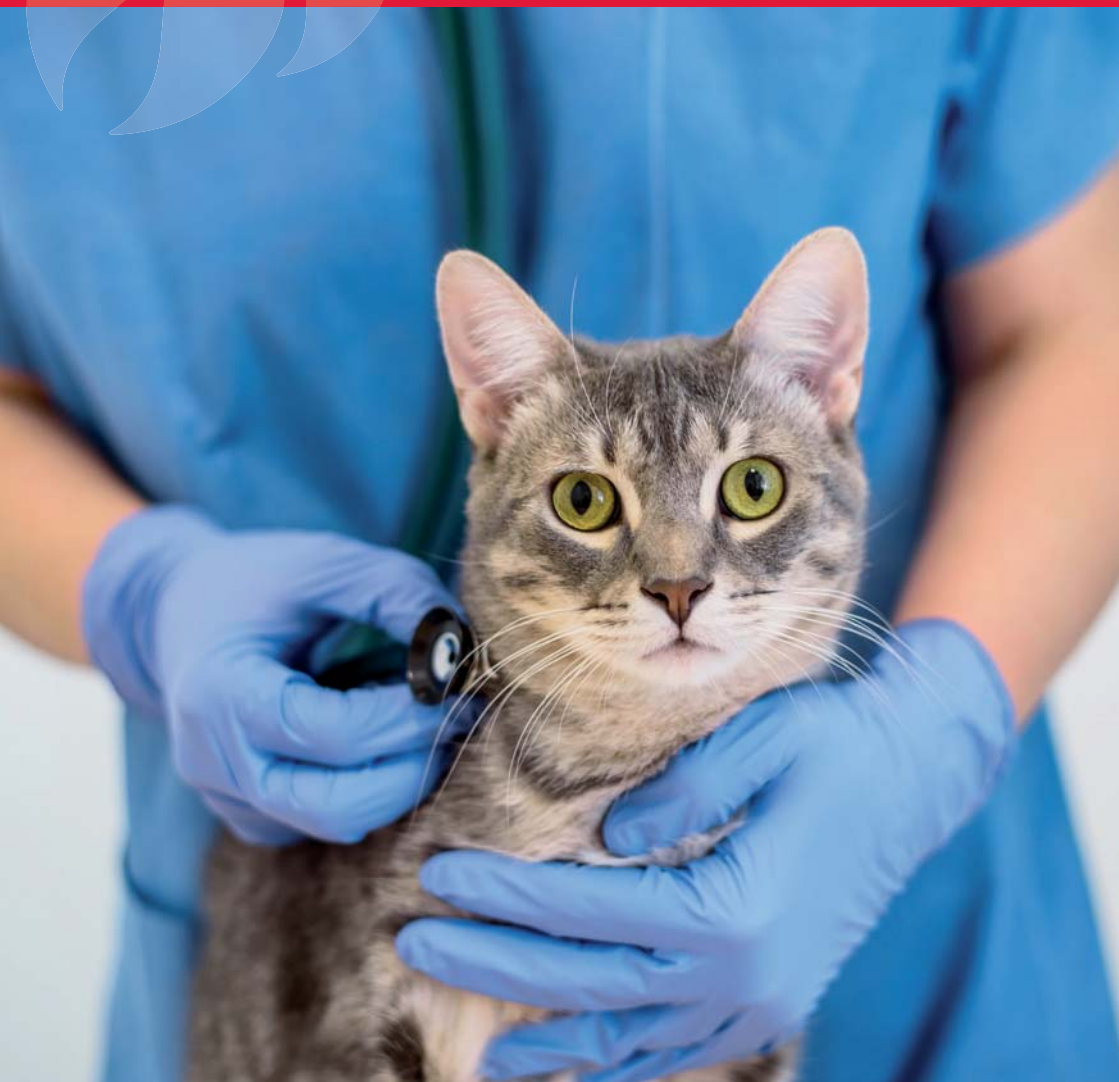
Blood collection with Micro Needle and micro sample tube



Once the patient and everything else is prepared accordingly for the blood collection, the following steps are performed:

1. The Micro Needle is opened at the transparent side.
2. Then the Micro needle is gripped at the ribbed holder to remove the protective cap.
3. The selected vein is then punctured.
4. The blood is collected in a suitable sample tube.
5. After collection, the Micro needle is securely disposed of in an appropriate disposal box.
6. Now invert several times and the sample is ready for the laboratory.

6. Safety throughout sample collection



Diverse zoonotic pathogens and their hazards for humans are known, for example Bartonella, Brucella, Campylobacter, Chlamydia, Coxiella, Dermatophytes, Giardia, Cryptosporidia, Leptospira, Listeria, Pasteurella, rabies virus, Salmonella, Toxoplasma and numerous others (Jackson and Villarroel, 2012). Therefore it is advisable to implement and also regularly rehearse specific safety rules in the practice. This includes observing general hygiene and complying with appropriate occupational safety measures (gloves, face mask if necessary, covering open wounds, etc.). (Re-)use of potentially contaminated equipment must be avoided! It should also be ensured that vaccinations are always up to date. The Standing Committee on Vaccination also recommends rabies vaccination for veterinarians (Epidemiologisches Bulletin [Epidemiology Bulletin], 2023).

Suitable waste containers must be provided and used for collecting pointed or sharp objects. They should not be overfilled.

Safety information

- Use only boxes of a size suitable for holding the items to be disposed of
- Before filling is started, the lid must be mounted and locked into place
- Secure the box with the recommended adhesive adapter by unscrewing it or by attaching it to the wall bracket to prevent it from falling over
- Take special care disposing of scalpels in the box (risk of canting and damage to the walls and base of the box)
- Drop items to be discarded only vertically into the box
- Do not force objects into the box
- Do not place liquids in the box
- Do not reach into the box with your hand or other means (risk of injury!)
- Do not throw the box down, do not shake or drop it
- Before closing the box, make sure that no objects are protruding from the opening
- Before disposing of the box, check carefully that the lid is tightly closed

Recommendation:

Fill Multi-Safe to only approx. 2/3 of the capacity!

Do not overfill Multi-Safe:
Risk of injury!
Observe fill line!

Fill line observed
see marking

overfilled

Sample processing

Important: Before any further processing, it must be ensured that the samples can be clearly traced to the patient and are correctly labeled! Further information on this is found in section 8 “Labeling, storage and transport”.

Sample tubes are correctly labeled if:

- contents are clearly visible.
- it is possible to check the fill level.
- the screw cap can be removed unhindered.
- tubes and labels do not become jammed or stuck together in the centrifuge.

“So then I'll take the samples straight away for further processing ...”

“Stop! Samples can only leave the collection room and the patient if they are labeled with the barcode assigned to the respective patient.”

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7. Preparation for the laboratory test

Before samples are analyzed on site or shipped to an external laboratory, the blood samples must be prepared appropriately. **Use of serum or plasma instead of whole blood has several advantages and is in many cases the preferred method for the following reasons:**

- Stability and shelf life: Serum samples generally have a longer shelf life than whole blood samples. Storage-related hemolysis can be prevented in this way. This is particularly important if the sample is transported over long distances or tests are carried out at a later date.
- Standardization: The use of serum or plasma samples is standardized in laboratories and analytical equipment.
- Better precision and reproducibility: Removing the blood cells can help improve the precision and reproducibility of laboratory tests (especially applicable for in-house equipment).

However, there are also situations in which whole blood samples are required, especially when specific tests based on the blood cells themselves have to be carried out. This is the case with all hematological examinations. Since the blood cells react very sensitively to storage, temperature, transport and time-related changes, the examination should ideally take place within a few hours to a maximum of 2 days after blood collection (whole blood stored refrigerated). To prevent these changes (cell degeneration begins immediately after blood collection), blood smears should also be prepared immediately.

7.1. Centrifugation

Centrifugation of blood samples is used to separate solid components (cells, blood clots) from liquid components.

Different centrifuges can be used for this – however it is crucial that the samples are centrifuged directly in the practice. It is important that a distinction is made between speed and g-force (gravitational force). The g-force is the value that is relevant for a good centrifugation result. It is very important when programming the centrifuge.

G-force can be calculated using the radius (cm) and the revolutions/minute (rpm):

Note

"Centrifugation is a physical separation process based on different density ratios of substances, such as blood cells and plasma."

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

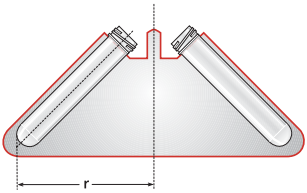
r = radius in cm

n = revolutions per min (rpm)

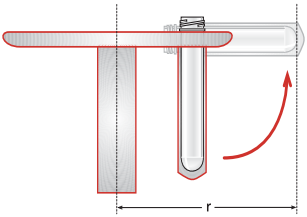
To convert g-force into revolutions/minute [rpm] or the other way around, you can use the centrifugation calculator at **www.sarstedt.com/en/service/centrifugation/**.

Please refer to the centrifuge manufacturer's information for the centrifuge radius r or determine it using the following illustration:

Fixed-angle rotor



Swing-bucket rotor



Difference between fixed-angle rotor and swing-bucket rotor

For gel Monovettes, we recommend using only swing-bucket rotors. The sample tubes in a fixed-angle centrifuge is rigidly mounted at an oblique angle. The sample tubes of a swing-bucket rotor moves from a vertical to a horizontal position during centrifugation. Hence the force can act evenly from the lid towards the base during centrifugation. The result is a well-formed, horizontal gel layer.

Serum and plasma samples are commonly centrifuged at 2000 × g for around 10–15 minutes.

After centrifugation, the serum or plasma is separated from the rest of the blood sample in order to avoid subsequent hemolysis.

Minimum centrifugation time

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

n.v. = not validated Centrifugation at 20 °C

* Applies to all S-Monovettes except Ø 8 mm (S-Monovettes for pediatrics).

Re-centrifugation

Re-centrifugation of sample tubes is not recommended (CLSI, 2010).

In such cases, lysed blood components can diffuse back from the centrifuged blood cells into the serum or plasma. As a result, changes occur, for example in cell-sensitive parameters such as potassium, phosphate, glucose or LDH (Hue *et al.*, 1991).

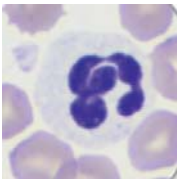
7.2. How do I make a blood smear?

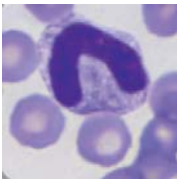
"Can I use any blood for the blood smear?"

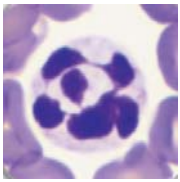
"No. The blood must be anticoagulated. Whole blood for serum or the last blood remaining in the collection needle must not be used. The anticoagulant of choice is EDTA. However, if necessary, lithium heparin or citrate may be used."

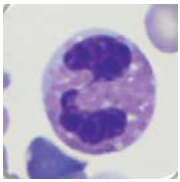
As noted above, one or more blood smears should be sent for each blood test. This applies especially if a cytomorphology test is also desired in addition, for example to verify the platelet count of the equipment or if nucleated red blood cell precursors, changes in red blood cell morphology, atypical white blood cells, left shift or agglutinates are suspected.

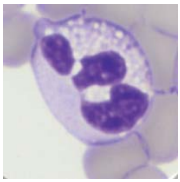
Hematology – Dog

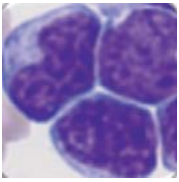

Segmented neutrophil granulocyte

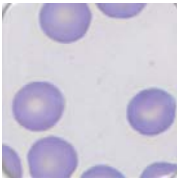

Band neutrophil granulocyte

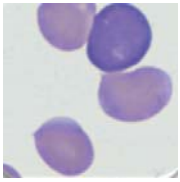

Hypersegmented neutrophil granulocyte

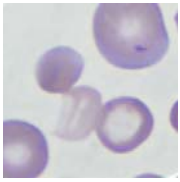

Segmented eosinophil granulocyte

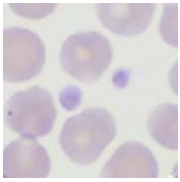

Segmented eosinophil granulocyte in greyhound


Large atypical neoplastic lymphocytes

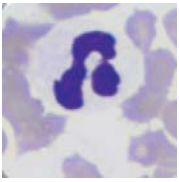

Physiological red blood cells

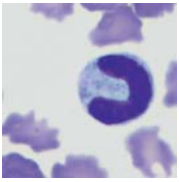

Polychromasia

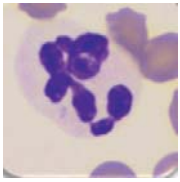

Anisocytosis

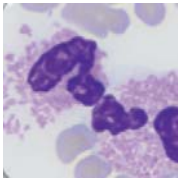

Physiological platelets

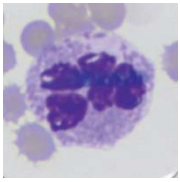
Hematology – Cat

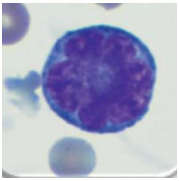

Segmented neutrophil granulocyte


Band neutrophil granulocyte

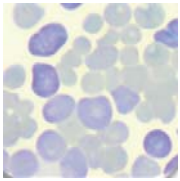

Hypersegmented neutrophil granulocyte

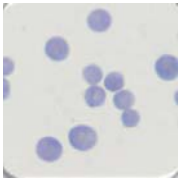

Segmented eosinophil granulocytes

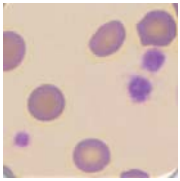

Segmented basophil granulocyte

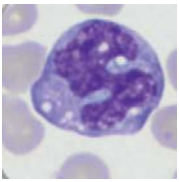

Medium-sized dark basophilic, atypical-reactive lymphocyte


Physiological red blood cells

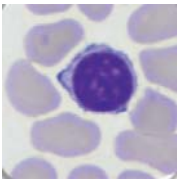

Polychromasia


Anisocytosis

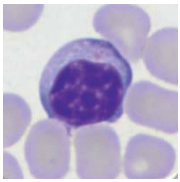

Physiological platelets



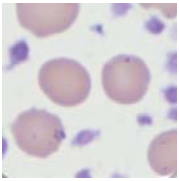
Activated monocyte



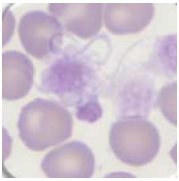
Small mature standard lymphocyte



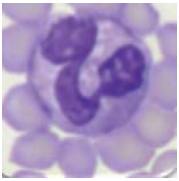
Small mature reactive lymphocyte



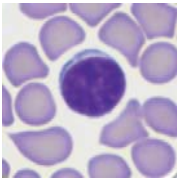
Thrombocytosis



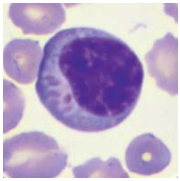
Macrothrombocytes



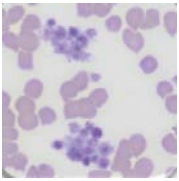
Monocyte



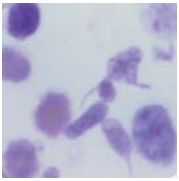
Small mature standard lymphocyte



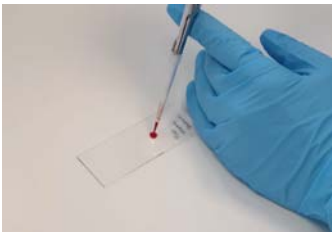
“Large granular lymphocyte” (LGL)

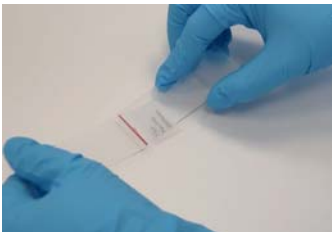


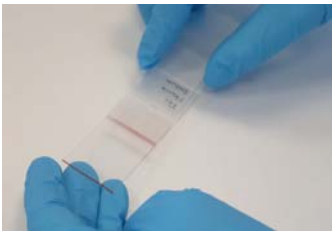
Platelet aggregates

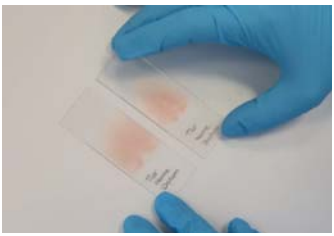


Atypical platelets



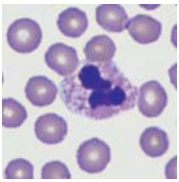




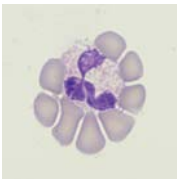


SARSTEDT

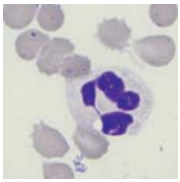
Hematology – Small mammals



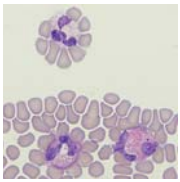
Segmented heterophil granulocyte, rabbit



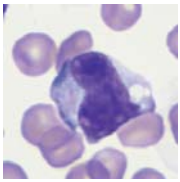
Segmented heterophil granulocyte, guinea pig



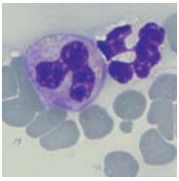
Segmented neutorphil granulocyte, ferret



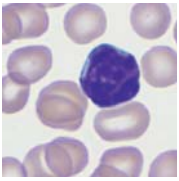
Heterophil (top), eosinophil (bottom) Guinea pig



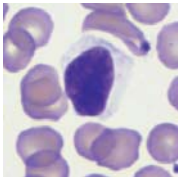
Activated monocyte, rabbit



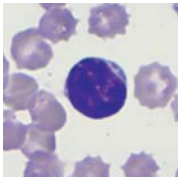
Eosinophil granulocyte, ferret



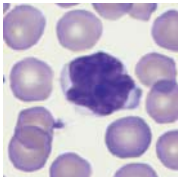
Small mature standard lymphocyte, guinea pig



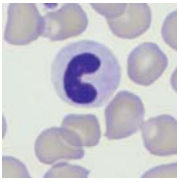
Small mature reactive lymphocyte, rabbit



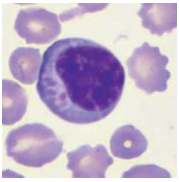
Standard lymphocyte, ferret



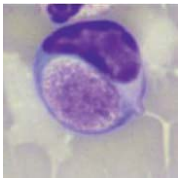
Indented atypical-reactive lymphocyte, rabbit



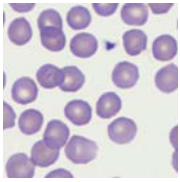
Band neutophil granulocyte, ferret



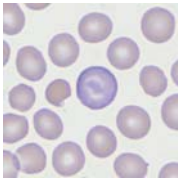
"Large granular lymphocyte" (LGL), guinea pig



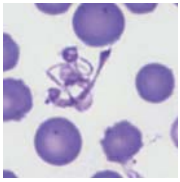
Foa-Kurloff cell, guinea pig



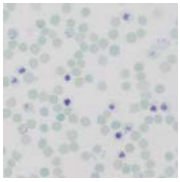
Physiological red blood cells, rabbit



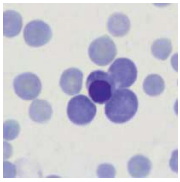
Anisocytosis, polychromasia, rabbit



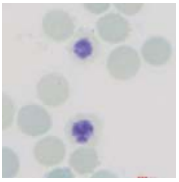
Atypical platelets, guinea pig



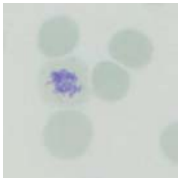
Reticulocytes overview, rabbit



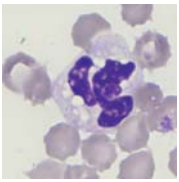
Nomoblast, rabbit



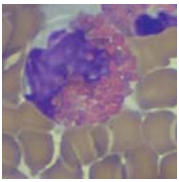
Reticulocytes group I, rabbit



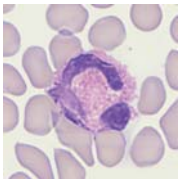
Reticulocytes group II, rabbit



Monocyte, ferret

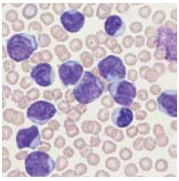


Eosinophil granulocyte, rabbit

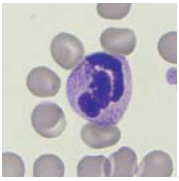


Eosinophil granulocyte, guinea pig

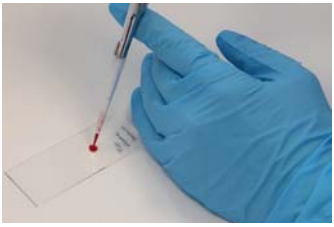
Puncture sites in guinea pig
Left: Saphenous vein: Puncture lateral of the Achilles tendon (middle third of lower leg) at an angle < 45° Right: cephalic vein of forearm

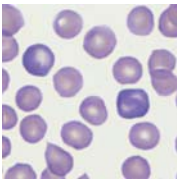


Lymphoma stage V
Large atypical neoplastic lymphocytes, guinea pig

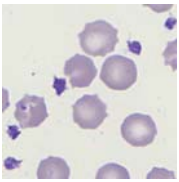


Basophil granulocyte, guinea pig

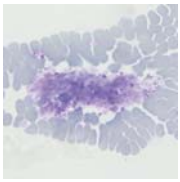




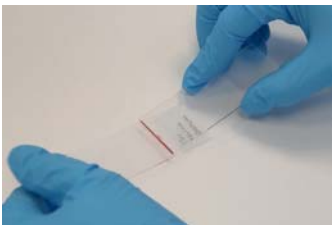
Polychromasia, rabbit

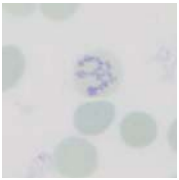


Platelets, ferret

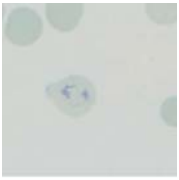


Platelet aggregate, ferret

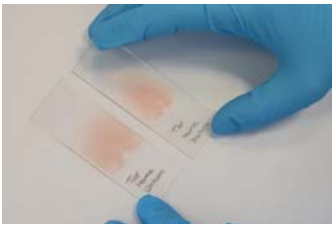




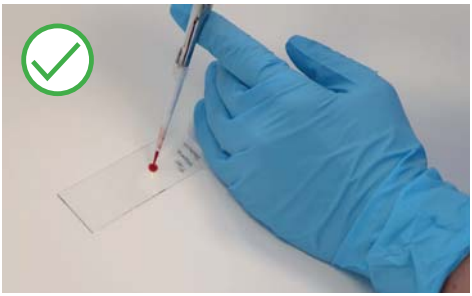
Reticulocytes group III, rabbit



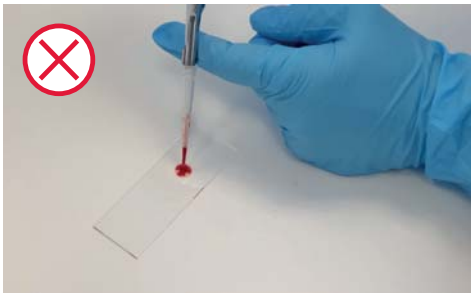
Reticulocytes group IV, rabbit



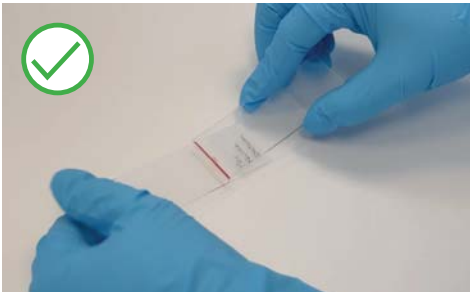
Dos and Don’ts of blood smears



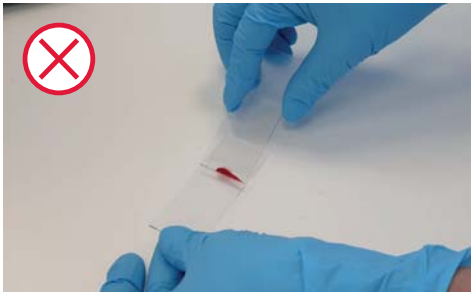
The microscope slide is labeled in pencil (or alcohol and water-resistant pen. One drop (approx. 10 µl) of blood is applied to the microscope slide.



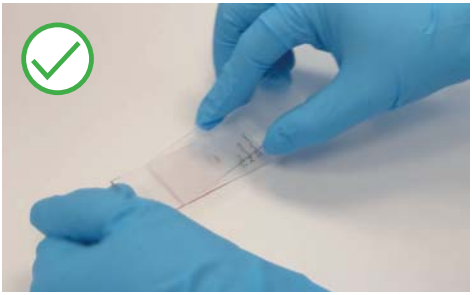
If the label is missing, the smear cannot be assigned to a customer. Also, the blood drop is much too large.



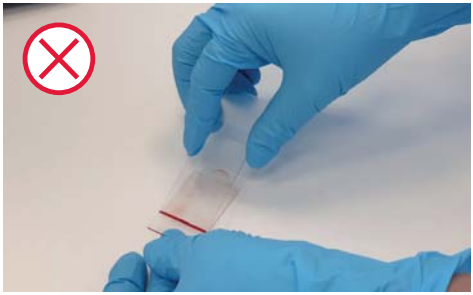
A second microscope slide is placed in front of the drop of blood. The slide used for streaking is drawn back toward the blood drop until all of the blood is distributed along the edge.



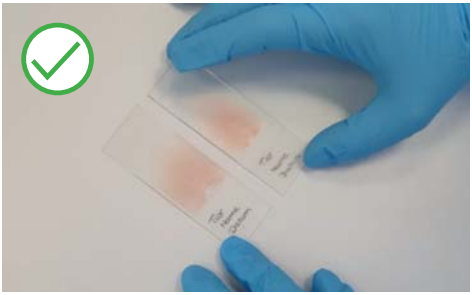
The blood drop does is not distributed evenly along the edge of the slide. Cause: The microscope slide is not sitting flat or the pressure distribution is uneven.



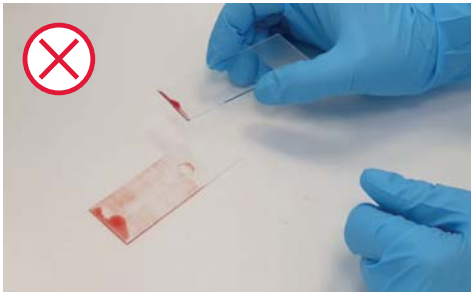
With a quick, even movement, it is pushed to the other end of the horizontal slide (approx. 45° angle).



Streaking too slowly leads to “stops”. Too much pressure or too large a sample volume cause all the blood to be pushed to the end of the smear.



A “tongue-shaped” finished smear is seen, from left to right: body, monolayer (evaluation zone) and feathered edge. Important: Allow air drying before staining and packing in shipping boxes!



No separation into body, monolayer and feathered edge is seen.
The blood smear is very inhomogeneous with no evaluation zone and is thus not assessable!

Tips and Tricks
Blood smear from dog (F) and mouse (A):

"Mouse, I have problems making nice blood smears – can you help me?"
"Of course, what exactly would you like to know?"

The blood smear doesn't look like a tongue, it is the same thickness from start to end and extends beyond the edge. What mistake was made?

Presumably the drop of blood / blood volume was too large. It should work better with less blood. If the smear is then too short, just slowly increase the blood volume. With anemic animals with very thin blood, it makes sense to use a smaller drop of blood.

It may also be that the smear angle is too flat (< 45°). The angle determines the length of the smear. A steep angle (> 45°) produces a short, a flat angle (< 45°) a long, smear.

My blood smear is not homogeneous but contains "stops". How do I change that?

These "stops" can have various causes. They frequently arise through very slow smearing out of large sample. It is also important not to slow down when smearing. Once the smearing has started, it should be finished swiftly.

My blood smear has different thicknesses on the two sides and contains streaks. Why?

Very probably the microscope slide / cover slips used for streaking were not placed flat ("even") on the other slide or the pressure conditions during streaking were not evenly distributed. Ideally the microscope slide / cover slips used for streaking should be placed far in front of the drop of blood and moved back and forth. That way, you get a feeling for the pressure and also notice if there is any grating.

Sometimes the cut is not ideal or there is dirt on the microscope slide. You can try to wipe this off or use a new one.

The cells in my blood smears are damaged, why?

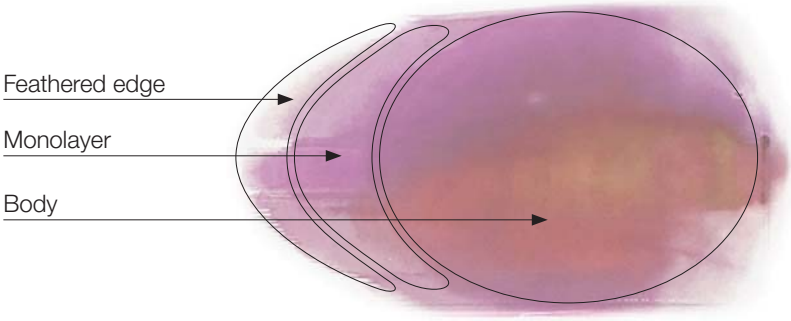
Possibly too much pressure was used for the smear. That can be practised by smearing with a cover slip. It breaks very quickly under too much pressure. Now and again however, the blood cells are also very fragile and are quickly damaged (for example in the case of leukemia, high-grade inflammation or anemia).

My blood smear is much too thick and too short, what's the reason?

It may be that the microscope slide is being held at too steep an angle. A flatter angle leads to a longer smear.

How many blood smears do I have to do before I get the “perfect one”?

A good question which I unfortunately do not have an all-inclusive answer for. But I can promise that it will work at some point and then you can always repeat it. Producing a good, evaluable blood smear is purely a matter of practice. Whether (or not) there is an art to it, is in the eye of the beholder.



Different zones of a blood smear

8. Labeling, storage and transport

Depending on the test parameters, there are a few things that also need to be taken into consideration after blood collection. While many analyses are possible from older and non-refrigerated material (mainly analyses on genetic material, for instance pathogen diagnostics by PCR), constant cooling must be ensured for others.

In any case, major temperature fluctuations should be minimized, even during preparation for shipping to an external laboratory, for example by avoiding overheating of the sample in the warm transport vehicle (Humann-Ziehank and Ganter, 2012). For some analyses, light protection is additionally recommended, for example bilirubin testing (Braun *et al.*, 2015). The exact requirements in each case can be found in the information on the test request next to the respective test. Specific lists of particularly sensitive parameters are available on request. It should be noted that not only the deep-freeze/cooling packs, but also the samples, will have to be brought to the appropriate temperature prior to transport, as the cooling/freezing capacity of the pack and box alone is insufficient to adequately cool down or freeze samples. Special boxes can be obtained, which contain a polystyrene box and a special sample cooling/freezing pack, enabling all-round cooling of 2 sample tubes. These may be personalized upon purchase and returned after receipt of the samples, just as with cooling packs, provided these are adequately labeled. Centrifuged material should strictly be stored in the refrigerator, for longer storage a storage temperature of -20 °C is recommended, better still -70 °C (Moritz *et al.*, 2014).

“The quality of many centrifuged and pipetted serum samples generally benefits from freezing the sample – however repeated freezing and thawing should be avoided.”

“Precisely! But beware: Whole blood must on no account be frozen! Complete hemolysis would result!”

“And blood smears should neither be frozen nor kept in the refrigerator.”

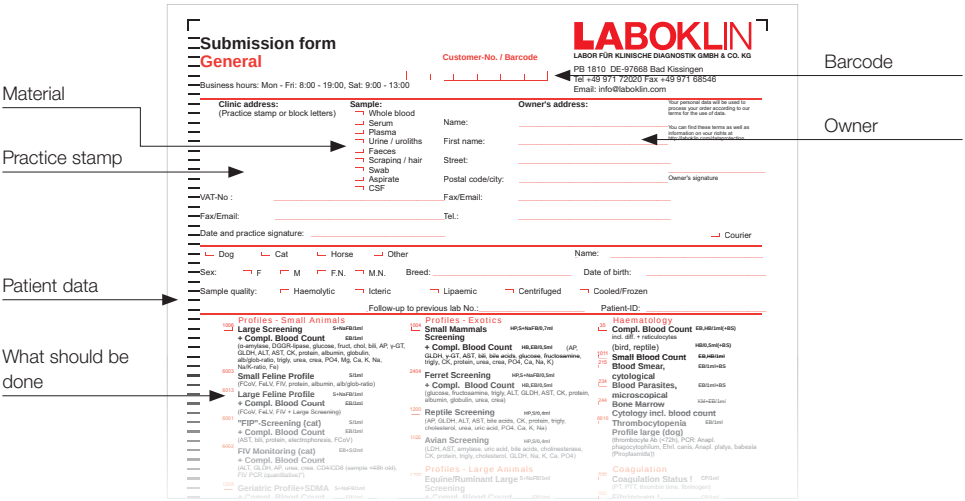
(Vap et al., 2012)

Checklist for transport

- Seal samples (avoid evaporation)
- Store serum/plasma at 4–8 °C
- Store upright
- Store EDTA for blood counts at room temperature
- Avoid repeated freezing and thawing
- Protect light-sensitive parameters (“sun parameters”) from sunlight (e.g. bilirubin)
- Use a special preparation for stabilization (e.g. S-Monovette® HCY-Z-Gel for homocysteine)

Careful packaging and labelling are also important. This ensures correct allocation of the sample in the laboratory, prevents sample loss through leakage and serves the purpose of protecting all persons coming into contact with the sample. For example, examination gloves are not adequate packaging for fecal samples, there is a risk of breakage as well as the possibility of falsely elevated glucose values if urine samples are in jam jars, and needles still on the syringe are an unnecessary (and unfortunately often underestimated) source of risk. Where samples with particularly high zoonotic potential are involved (for example from monkeys or dogs suspected of having leptospirosis) the laboratory should be informed in advance and the corresponding information affixed to the outer packaging so that it is already clearly visible.

Example of a sample order:



Ideal labeling on the analysis request: Select desired test without marking any other fields! Important: Do not forget a preliminary report and any pretreatments!

Note

"The correct sample material should always be marked on the order next to the desired analysis. If there are any uncertainties, a brief phone call to the laboratory can help!"

SARSTEDT

Labels contain not only patient data including the practice-specific barcode, but also details on sample type (Gunn-Christie *et al.*, 2012) – is the sample urine, serum or CSF? Centrifuged EDTA plasma, citrate plasma or lithium heparin plasma? This is not always immediately evident but can determine whether a specific analysis is possible from the material sent in. Time and resources can be saved if the material is already correctly marked.

To ensure a sample is assigned to a patient, the respective patient data should not only be noted on the test order, but also directly on the sample. Of particular importance here is: The barcode on the sample must match the barcode on the test order!

Caution in the case of tubes with flip-top caps – there is a risk of opening and leaking during transport.

Correct outer packaging (secondary tube) for each individual sample, including an absorbent liner, ensures maximum protection. All samples including the secondary tube must then be packed again in an outer packaging!

Note

“The sender is always responsible for correct sample transport!”

All samples must be labeled on the outer packaging: either with “exempt veterinary sample” for non-infectious samples, or with the adhesive label UN3373 in accordance with the Hazardous Substances Ordinance for potentially infectious veterinary samples. The adhesive labels necessary for this can be requested in the laboratory.

The most important packaging requirements are:

- Sufficiently resistant, so that impacts/stresses (vibrations / temperature/ humidity/pressure changes) during normal transport cannot lead to damage / leakage of contents.
- A sample tube / primary tube and in addition shipping tube / secondary packaging as well as outer packaging are required, with either the secondary or the outer packaging (e.g. protective sleeve / shipping bag) having to be rigid. For air transport, rigid outer packaging is always required, which must withstand an internal pressure of 95 kPa (0.95 bar) and temperatures of -40 to 55 °C (see respective company information for IATA/Post/DHL requirements).
- One surface of the outer packaging must measure at least 100 × 100 mm.
- The shipping item must pass a drop test from a height of at least 1.2 m.

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