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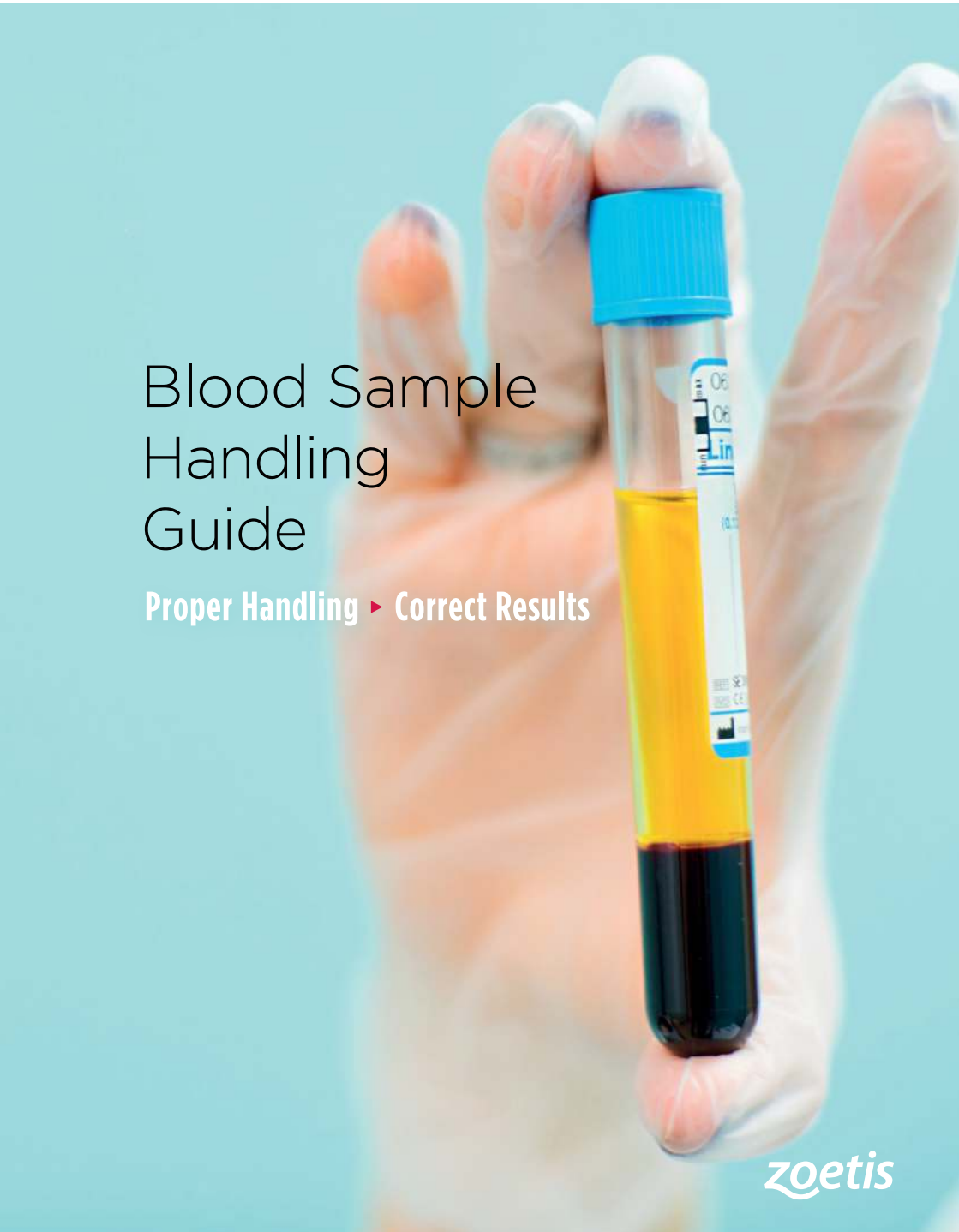

Blood Sample Handling Guide

Proper Handling ▸ Correct Results

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Samples for evaluation will provide useful information ***only*** *when obtained and processed correctly.*
These steps will help you to take advantage of laboratory technology and its medical diagnostic capability – and to ***properly collect samples*** *for the respective procedures.*

Dr. Anna Stemann
VETSCAN Product Management Team

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CONTENT

Syringe Needle Size for Blood Collection

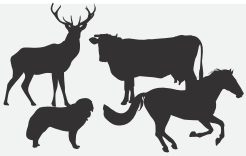
Before collecting a blood sample choose the right syringe needle size. The needle gauge will vary based on patient size. Use the needle with the largest bore governed by the size of the vein, to minimise the possibility of haemolysis. **Be especially careful** with ill animals, because they may have fragile erythrocytes.

SMALL Species
(adult weight up to 30 kg)

22G

MEDIUM Species
(adult weight 30-60 kg)

20G



LARGE Species
(adult weight over 60 kg)

18G

Collection Tubes

After you have collected blood with a syringe, choose the appropriate tubes for each type of diagnostic testing that will be performed.

There are more than **9 types of tubes** used for blood collection.
They contain the appropriate anticoagulant indicated by their **stopper colour.**

Did you know that there are two different colour-systems?
Be aware of which you are using – either the **US-code** or the **EU-code** to avoid mistakes.

Anticoagulant	Colour Code*	Analysis Type
Sodium Citrate	Blue	Coagulation, Fibrinogen
Serum (clot activator)	Red	Harvesting of serum for common biochemical determinations [1]
Lithium Heparin	Green	Plasma for Biochemistry [1]
EDTA (K2 or K3)	Lavender	Haematology [2] *preserves cell volume and morphology*

*Colour coding in blood collection based on ISO 6710. Colour coding may vary due to local rules and regulations.

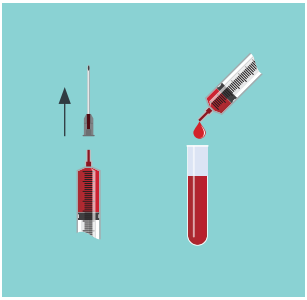
[1] Use for your VETSCAN VS2

[2] Use for your VETSCAN HM5

Transfer the Sample

After you have collected blood with a syringe, transfer it into the appropriate tube or draw the sample directly into the appropriate tube.

a) Make sure you remove the stopper from the tube and the needle from the syringe before dispensing the sample into the blood collection tube to avoid haemolysis.



b) The ratio of blood to anticoagulant is very important:
   - Fill sodium-citrate tube always to the fill-line exactly
   - Fill EDTA and Lithium heparin tube at least half full

Fill the tube properly!
Neither too little nor too much.

*If **not enough blood** is added into the collection tube, you may see:*

- a false decrease in haematocrit value due to dilution of the blood
- a false decrease of analyte values due to dilution of the blood
- inaccurate MCV, MCH, MCHC and HGB
- altered red cell shape: erythrocytes will shrink due to the high osmolality of the anticoagulant fluid
- artifactually prolonged clotting time

*If **too much blood** is drawn into the collection tube (over-filled tube), clots will form.*

- The clots in this case may be too small to visualize, but can affect results.

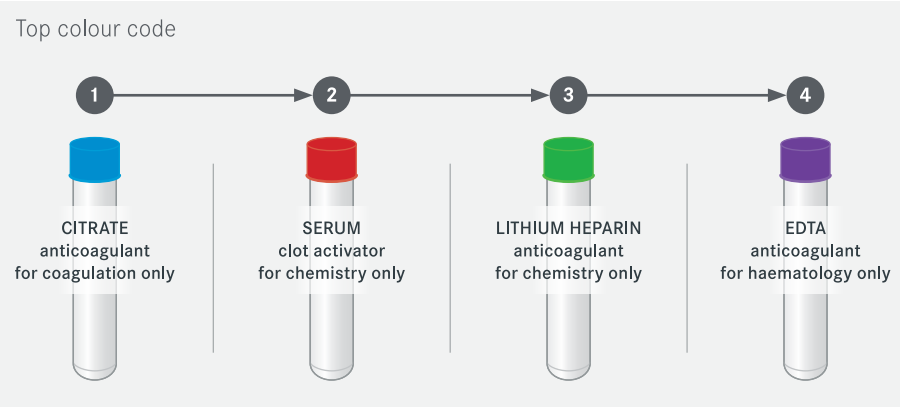
Note: If using a vacutainer, fill the tube until the vacuum is exhausted.

Order of Filling

It is recommended to follow the correct order of filling tubes to avoid cross-contamination of additives.

- The sodium citrate tube is to be filled first, followed by the serum tube, the lithium heparin tube and any EDTA tubes last of all.
- Filling out of order may lead to contaminating the blood samples with addititves and lead to invalid results in major biochemical parameters.

Tube Filling Order:

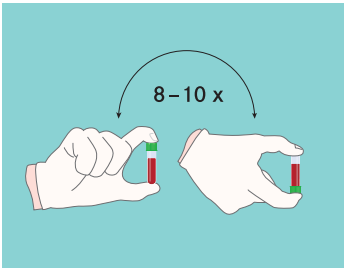


Sample Mixing

When: **Immediately** following collection.

Why: To **properly mix** the blood and anticoagulant.
To **prevent formation** of clots that will interfere with cell counting.

How: Holding tube upright, **gently** invert 180 degrees and back.
Ensure that it is thoroughly mixed by inverting the tubes 8–10 times.



Be careful: **Avoid shaking** samples. This can lead to haemolysis. **Do not transfer** blood from one tube to another e.g. EDTA to Lithium Heparin.

If not mixed properly:
The specimen may need to be re-drawn if blood clots form or, if mixed too vigorously, haemolysis occurs.

Centrifugation

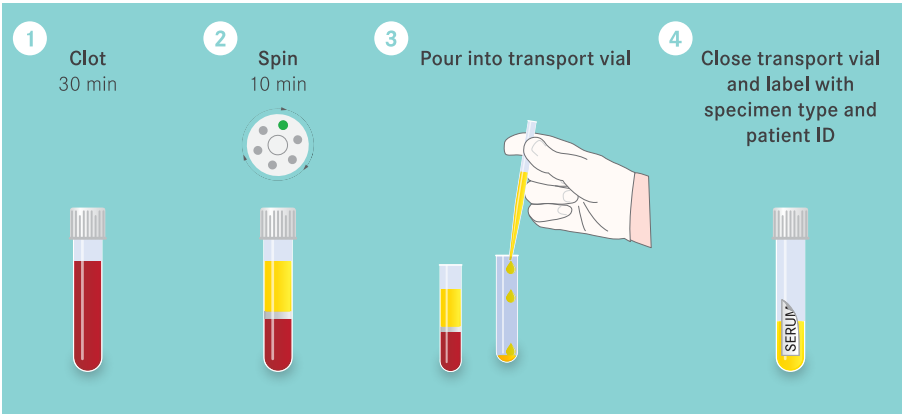
To obtain serum sample:

- After filling the tube without additives or with clot activator, allow the sample to clot for 30 minutes at room temperature.
- Centrifuge according to your protocol. Remove the serum and place in the storage tube.

For Plasma:

- Centrifuge the EDTA or lithium heparin directly after collection according to your protocol. Transfer plasma to a storage tube.

Standard Recommendation for centrifugation conditions:



Preparation	Additives	Time (min)	Spinning	Temp.
Serum	No	10	2,000 x g	18 - 25 °C
Plasma	Lithium-Heparin	10	2,000 x g	18 - 25 °C
	EDTA	10	2,500 x g	18 - 25 °C

Storage After Collection

Storage at room temperature:
Analyse Lithium Heparin whole blood within 60 minutes, EDTA whole blood – within 3–4 hours, Plasma/Serum – within 5 hours

Please Note: Storing whole blood or centrifuged but unseparated serum or plasma (allowing prolonged contact with cells) can result in hypoglycemia, hyperkalemia, and haemolysis.

Storage at 2–8 °C: EDTA whole blood: 8 hours, plasma or serum: 24–48 hours

Storage at min. -10 °C: Not appropriate for whole blood, for plasma or serum: 5 weeks

Sample	Room temp	2 – 8 °C	min. - 10 °C
 Lithium Heparin	1 hrs	no	no
 EDTA	3–4 hrs	8 hrs	no
 Plasma Serum	5 hrs	24 – 48 hrs	5 weeks

Sample Handling Errors

1. HAEMOLYSIS

What is it?

- Blood cells breaking and releasing cellular components: enzymes, electrolytes and hemoglobin.
- May interfere with results because of its red colour.
- The concentration of K+, LDH, AST and total protein may be falsely increased.

Why it happens:

Reasons for haemolysis are usually improper handling:

- Transferring blood through the needle of a syringe into collection tube with force.
- Mixing the tube too strongly after collecting the sample.
- Puncturing the vein before the alcohol swab dries, allowing alcohol to mix with sample.
- Frothing of the blood when the needle bore is only partially inserted in the lumen of the vein.

Techniques to prevent haemolysis:

- Mix all tubes with anticoagulant additives gently 8-10 times.
- Avoid drawing blood from a haematoma; select another draw site.
- If using a needle and syringe, avoid drawing the plunger back too forcefully.
- Make sure the venepuncture site is dry before proceeding with draw.
- Avoid a probing, traumatic venepuncture.
- Avoid prolonged tourniquet application (no more than 2 minutes; less than 1 minute is optimal).

2. LIPAEMIA

What is it?

- High concentration of fat in blood, which results in turbid or cloudy serum.
- May interfere with all chemical reactions as milky plasma distorts light absorption.
- May falsely decrease concentrations of certain analytes, e.g. electrolytes.

How to prevent:

Remind your client NOT to feed their pets for at least 10 hours prior to their appointment to avoid lipaemia.

Evaluating a sample to provide reliable result:

Haemolysis and Lipaemia can interfere with measured analytes through physical and chemical interactions. The interference can cause falsely elevated or falsely decreased results.

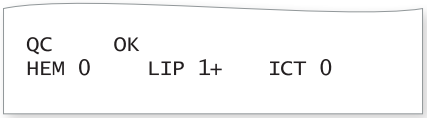
This leads to incorrect diagnosis and treatments with potentially unfavourable outcomes for the patient.

A standard procedure for checking the quality of the sample is a visual sample evaluation. Evaluate plasma or serum for signs of haemolysis or lipaemia. Based on deviation from normal colour (clear, yellow), you can decide whether to proceed with this sample or draw a new one.

Did you know that?
The VETSCAN VS2 Analyser performs a series of over 150 internal quality checks and provides specific information regarding the grade of haemolysis, lipaemia, hyperbilirubinaemia and its effect on the test result. You can always be assured that you are getting only reliable, high-quality results when using the VETSCAN VS2 chemistry analyser.

In every run, the Intelligent Quality Control system of the VS2 checks the quality of each blood sample and reports the measured values for each physical interferent. The sample indices are printed on the bottom of each result to inform the operator about the levels of interference present in each sample. These indicate the degree of lipaemia, haemolysis and icterus in the sample, measured on a scale of 0 (clear), 1+ (slight), 2+ (moderate), and 3+ (gross). The degree 3+ not necessarily causing a VS2 result to be supressed.

- HEM/LIP/ICT 0 means no interference at all
- HEM/LIP/ICT 1+ means slight interference etc.



Icterus (ICT): Although Icterus is not caused by sample handling errors, icterus in blood samples can cause interferences that can affect the quality of results.
What is it? An increase of bilirubin in the blood (hyperbilirubinaemia). May interfere with results because of yellow/orange/brown colour. The concentration of alkaline phosphatase, total protein and chloride may be falsely increased. The concentration of triglyceride and creatinine may falsely be decreased.

Good news for VETSCAN HM5 haematology analyser users:
The advantages of the impedance method, implemented in the HM5 performs correct counting of cells independent of lipaemia and icterus level.

FAQs

Can I use any anticoagulant to perform a Biochemistry Analysis?

To get a proper result, please use only Lithium Heparin (whole blood or plasma) for your biochemistry or no anticoagulant at all (serum).

Why?

Sodium Citrate and Sodium Heparin contain Na^+. This will falsely elevate Na^+ concentration in your sample.

EDTA is usually a salt with Potassium. Using EDTA will falsely elevate K^+ concentration. Also EDTA binds $Ca2^+$ and $Mg2^+$. This leads to false decrease in $Ca2^+$ and $Mg2^+$ concentration.

And what about Haematology?

For haematology it is important to preserve the size and morphology of the cells. This you can achieve only with EDTA. All other anticoagulants will prevent the coagulation but will also change the size of the cells.

I used EDTA for Haematology, but the red blood cells are still shrinking in size.

Erythrocytes can decrease their cell-volume when the proportion of blood to EDTA is wrong: too much EDTA, with not enough blood. Pay attention on properly filling tubes or try vacuum-tubes.

The instructions for use from my tube supplier has instructions that are different than what is suggested in this guide. What should I do?

This guide is designed to give guidance on how to handle blood samples in order to ensure the best quality results. Specific manufacturers may have instructions that differ from this guide; please follow the instructions for use from the supplier of your blood collection tubes.

If you have any questions, please consult our ABAXIS Product Support Team at:
1800 270 727

Notes

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