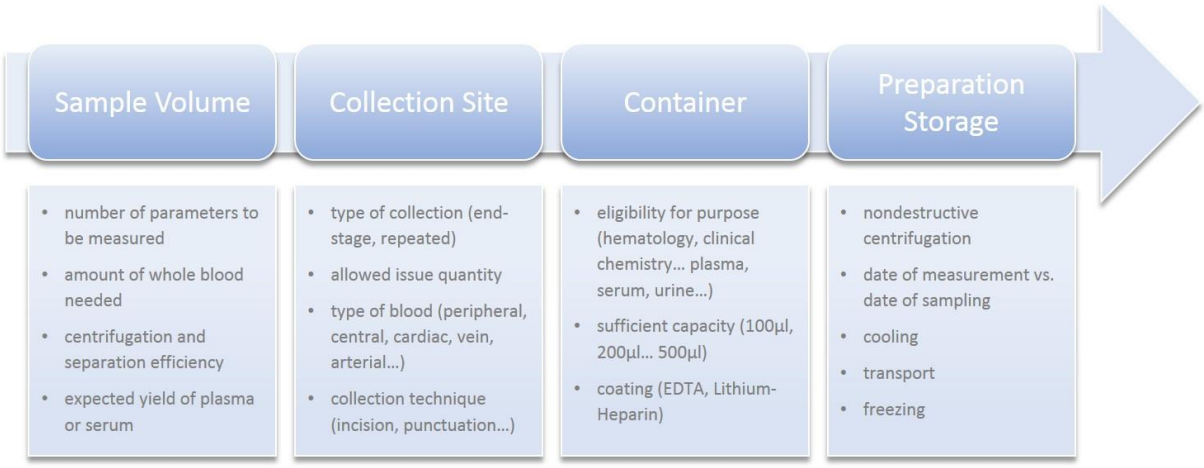
# Guidelines of Sample Preparation and Handling

In order to achieve the best and most reliable data of any measurement in fields of clinical chemistry and hematology it is absolutely necessary to follow some instructions. Here, you will find some basic information and references of correct sampling and sample handling. This document is not intended for replacing a comprehensive consultation of the pathophysiology staff!

## General considerations prior to blood sampling




## Animal Handling

It is always mentionable that animals, including laboratory animals, have to be reckoned among sensitive beings. Due to this, they considerably underlie exterior influences such as stress and excitement. You have to be aware of these effects especially in terms of animal handling and blood sampling.

The murine stress response is able to react within seconds, leading to altered values measurable in clinical chemistry and hematology. So, such negative influences should be minimized.

It might be useful to accustom your animals to procedures of sampling (if possible) prior to real blood sampling by repeated training over several days. Animal handling and blood collection itself should be conducted by trained personal only.

It is important to take all samples the same way every time, because the composition of blood may be different at different collection sites (peripheral sites, central veins, cardiac etc.). Don’t change the site and way of sampling during an experiment!

Since animals, such as mice, underlie a distinct circadian rhythm, larger deviations of the day-time of sampling should be avoided, too.

However, any effects of experimental treatment can be exclusively assessed in relation to suitable controls, i.e. untreated or non-affected animals of the same genetic strain, age, gender etc. that are handled the same way as the treated/affected ones.

## Blood Sampling

It is very important to make a point on the quality of your blood samples – before, during and after sampling. Please note that blood is a kind of fragile substance! Reliability of your laboratory values depends on sample integrity.

We strictly recommend to use the less invasive and traumatic method of sampling as possible depending on your experimental design. For example, due to blood-sampling in final experiments you might give consideration to the exsanguination by apical punctuation of the heart instead of decapitation to prevent blunt traumata and contamination of dripping blood.

A short overview of "Methods of Blood Collection in the Mouse" is given by Janet Hoff in *Lab Animal Volume 29, No. 10*.

The right choice of your sample container is also very important. Most of the biochemical parameters are measured in blood-plasma. Therefore, you might avoid any coagulation by using anticoagulant coated sample-tubes. For example, lithium-heparin coated tubes (orange EU-coded) are best suitable for sampling, preparation and measurement in clinical chemistry.

***NOTE:*** *The use of other anti-coagulant tubes, e.g. EDTA-treated vials, may risk the inactivation of enzymes like alkaline phosphatases in your sample due to complexing cofactors.*

For determining hematologic blood counts, EDTA-coated tubes (red EU-coded) are the mostly used. Each kind of tube is available for different amounts of blood (100µl, 200µl… 500µl). It is important to choose the right size of the pre-treated tubes for your targeted sample volume!

***ATTENTION:*** *You have to gently agitate tubes immediately after filling to ensure complete mixing of blood with anti-coagulant!*

## Sample Preparation

Subsequent to sampling, whole blood has to be handled very carefully until centrifugation. Strictly avoid vigorous shaking or dropping of your sample! Otherwise, containing erythrocytes might burst, leading to hemolysis and severe disturbances of the measurement process.

Of course, final centrifugation is a critical step in sample preparation by itself. You have to take care about the right speed of your centrifuge to avoid hemolysis again. In general, blood-samples should not be treated with more than 2000x g for 5 minutes.

***NOTE:*** *After successful sample preparation and centrifugation, your sample should exhibit two different phases. A nontransparent dark-red bottom phase, consisting of erythrocytes amongst others, and a transparent yellowish upper phase containing your sample-plasma/serum. There should be absolutely NO reddening of the upper Phase, which would indicate hemolysis!*

It is up to you to collect the supernatant in fresh tubes for storing and subsequent analyses. But it is recommended to conduct any measurements as prompt as possible without previous freezing of your sample. For this reason, you may hand in fresh samples of whole blood in appropriate tubes stored at 4°C (on ice, but not frozen). Final centrifugation and immediate measurement will then be done at the facilities site.

# Sample Volumes

For the correct and reliable measurement of laboratory values in clinical chemistry and hematology, sufficient amounts of sample material have to be collected. This sufficiency is of particular importance for all measurements in clinical chemistry!

Here, you can find basic instructions of calculating the correct sample volumes as well as typical reasons and pitfalls, leading to insufficient volumes and not feasible measurements.

Nevertheless, you should definitely confer with the pathophysiology staff regarding appropriate sample volumes in line with your experiment.

## Clinical Chemistry

The Fuji DRI-CHEM NX500 is a half-automated system for the assessment of blood parameters in clinical chemistry. The internal pipet-robot is not draining the pipet-tip completely. An initial dead volume of approximately 20µl will be kept for technical reasons during the entire measurement. Hereby, the subsequent release of the exact sample volume to the measurement-chip is guaranteed. Please note that it is not possible to measure any parameter* in samples (i.e. plasma, serum or urine, but NOT whole blood**) below 30µl volume.

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|---|---|
| For example: | **3 parameters*** to be measured in plasma |
| | 10µl plasma per parameter needed |
| | 20µl plasma dead volume |
| Calc.: | 3 x 10µl + 20µl = **50µl** total plasma volume |

50µl of plasma (or serum, or urine, but NOT whole blood**) are sufficient to measure three biochemical parameters*

***NOTE:*** *The electrolytes sodium (Na), potassium (K) and chloride (Cl) are measured by a different method, i.e. by potentiometry with an **i**on **s**elective **e**lectrode (ISE). A single ISE-chip is able to measure Na, K and Cl simultaneously. 50µl sample volume are needed for a single ISE-test. Again, please note that additional 20µl are needed to compensate the technical caused dead volume.*

* *applies to parameters, that are measured by colorimetric tests (NOT the Na-K-Cl ISE-Chip)*

** *plasma or serum have to be separated from whole blood by centrifugation before measurement (yields usually 30-50% $^{v}/_{v}$)*

Please keep in mind, that the volumes above are absolute minimum volumes needed to conduct any measurement. For your own guaranty of complete measurements, we strictly recommend to take enough blood for at least two or more additional measurements. Surplus sample material can be stored and returned to you after completed measurements.

Due to technical reasons, particular tubes for low sample volumes (100µl and 200µl) cannot be used with the Fuji DRI-CHEM NX500. That affects all tubes with conical shaped inner tubes (e.g. Microvette®, Sarstedt). In this case, the upper phase of plasma/serum has to be transferred after centrifugation into a fresh tube of appropriate size. Please note that the qualitative transfer of sample material is associated with loss of sample volume. Therefore, slight higher sample volumes have to be included!

### Clinical Hematology

Sample volumes for measurements in clinical hematology are essentially easier to handle. Complete blood cell counts can be assessed with 10µl whole blood, approximately. The practicability of measurement is limited to the fluid level within the tube, only. So, appropriate sampling tubes have to be chosen in order to gain at least 5mm of fluid level. Keep in mind that immediate mixing of the sample with precast anti-coagulant is absolutely necessary to prevent any blood clotting and that small sample volumes are significantly more difficult to mix than bigger volumes!

## Course of Action

To avoid the typical pitfalls of blood sampling and further handling, it is useful to follow at least these simplified instructions:

1. Read (all) the information given on the pathophysiology pages! **Ask** the pathophysiology staff!
2. Calculate the appropriate **sample volume**!
3. Choose the right **sample container** regarding volume and coating!
4. Choose the right **sampling technique** for your purpose!
5. Use pre-treated instruments for blood collection, e.g. heparinized syringes etc.!
6. Don’t forget to **gently agitate** the filled sample container immediately!
7. Keep blood samples cold, but not frozen, until centrifugation and measurement!
8. Choose the right **centrifugation speed** for plasma/serum separation!
9. Take off the supernatant **strictly qualitative**!