MDC MAX DELBRÜCK CENTER FOR MOLECULAR MEDICINE IN THE HELMHOLTZ ASSOCIATION

Max-Delbrück Centrum for Molecular Medicine

Animal Phenotyping Facility

Guidelines of Sample Preparation and Handling

for Clinical Chemistry and Hematology

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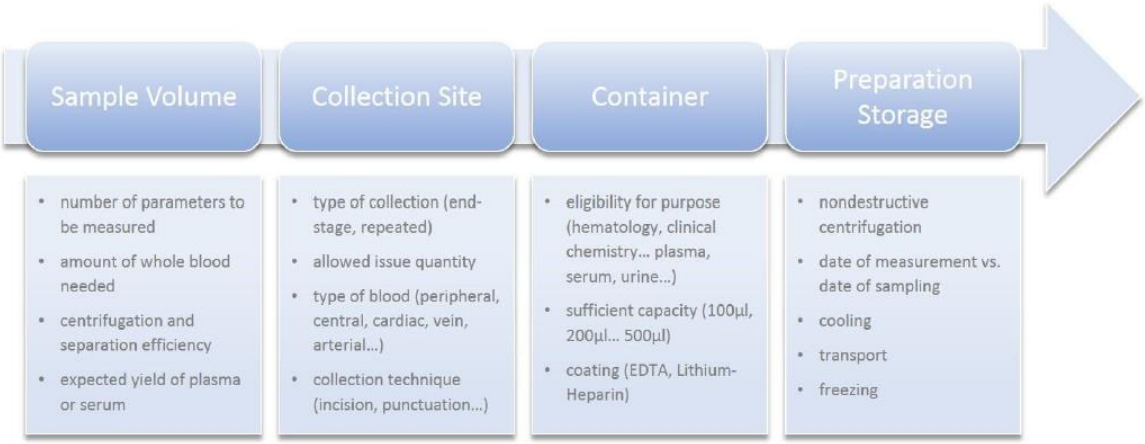
Guidelines of Sample Preparation and Handling

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 to replace a comprehensive consultation of the animal phenotyping 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. 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 a trained person only.

It is important to take all samples the same way every time. 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 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-serum while others are best measured in plasma.

When plasma is to be measured, you might avoid any coagulation of your samples by using anticoagulant coated sample-tubes. Lithium-heparin coated tubes (orange EU-coded) are best suitable for sampling, preparation and measurement of most parameters in clinical chemistry in plasma but not for all.

Vice versa, you might optimize coagulation of your serum samples by using tubes coated with clotting activators.

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!

Dedicated tubes for blood collection and separation are available at your animal phenotyping platform.

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

ATTENTION: *You have to* ***gently slew*** *tubes immediately after filling to ensure complete mixing of blood with anti-coagulant or clotting activator! But* ***do not vigorously shake*** *your samples!*

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. In general, blood-samples should not be treated with more than 2000x g for 5 minutes if any anticoagulant is used.

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 no reddening of the upper Phase, which would indicate hemolysis!*

Most of the analytes are stable for several days up to weeks when stored at 2-8°C. Longer storage at -20°C is possible. But you should be aware of that enzyme activity may suffer from freezing. Therefore, it is very important to handle all samples the same way within one experiment.

Dedicated tubes for analyzing are available at your animal phenotyping platform!

Sample Volumes:

It is recommended to have a personal consultation with the animal phenotyping platform staff to determine the sample volume needed for your requested measurement.

Clinical Chemistry

Analyses of clinical chemistry are performed on the AU480 Analyser (Beckman Coulter). The AU480 is a fully-automated system for the assessment of blood parameters.

Due to this automating, you have to add an initial dead volume of approximately 20µl that will be kept for technical reasons during the entire measurement.

Depending on the parameters to be measured, different sample volumes are needed. A detailed list of sample volumes needed for different parameters can be seen on the intranet.

For example: **5 parameters** to be measured in plasma:

-	Creatinine	→	6µl
-	AST	→	5µl
-	ALT	→	5µl
-	LDH	→	3µl
-	TRIG	→	2µl
-	integrity test (LIH)	→	5µl
-	dead vol.	→	20µl
			46µl

Mathematically, 46µl would be sufficient to measure these 5 parameters one-time. But for several reasons a multiple measurement approach might be necessary. Please take account of handing in more than the absolute necessary minimum of sample volume if possible.

Hematology

Analyses of hematology will be carried out on the ProCyte Dx analyser (Idexx).

For the measurement and determination of blood counts, a sample volume of 60µl anti-coagulant treated whole blood is needed. EDTA-blood is the recommended sample type.

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**!