MLC Pathology

MRC Harwell

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Clinical Chemistry of Blood & Urine
Guidelines for Sample Collection, Processing & Shipment

This document provides users of the Clinical Pathology Service with important guidelines for sample collection, processing & shipment. The most up to date version of this document is available on our website at:
https://www.har.mrc.ac.uk/resources/clinical-chemistry

Available Assays

We can perform a diverse range of clinical chemistry tests on plasma, serum and urine on board a Beckman Coulter AU680 clinical chemistry analyser.

A table of the plasma/serum and urine tests we have available and their specific volume requirements can also be found on our website. Plasma is generally the preferred sample type for blood tests and suitable for all the tests listed, although serum is also suitable for some of the tests as indicated in the table.

Collection of blood samples from mice

When collecting blood from mice ensure that you comply with Home Office Regulations and ethical practices. Good quality plasma/serum samples are clear/straw coloured (not haemolysed, see below) and are essential to achieve reliable and consistent results. Sample quality can be greatly affected by how blood samples are drawn and processed.

Retro-orbital punctures are usually performed under non-recovery anaesthesia, so are terminal procedures and therefore not suitable for time course studies. It is a suitable technique for rapid collection of a good quality blood sample of reasonable volume.

Tail vein punctures are not terminal procedures and therefore suitable for time course studies. Placing mice in an environment maintained at 38°C, for ±15 minutes prior to sampling can facilitate vasodilation to speed up collection and improve sample quality.

Jugular punctures are performed under non-recovery anaesthesia. The need for dissection can be time consuming, but samples obtained in this way tend to be of good quality and usually of adequate volume.

Cardiac punctures often produce samples that are haemolysed and/or clotted. These problems can be difficult to overcome, but with experience it is possible to obtain large volumes of blood relatively quickly.

Collection Tubes for Blood Samples

Contact us for information on suitable sample types for the assays you require as well as information on the correct tubes required to collect such samples. It is important to collect the correct volume of blood into a tube so that the ratio of sample to anti-coagulant is optimal and remains consistent between samples. There are various paediatric blood collection tubes (typically <1 mL) that are suitable for the small samples obtained from mice. Contact us for more information and details of suggested products and suppliers.

Lithium heparin coated tubes usually have orange (sometimes brown) caps and are used to collect samples to produce heparinised plasma.

EDTA coated tubes usually have red, purple or pink caps and are used to collect samples to produce EDTA plasma.

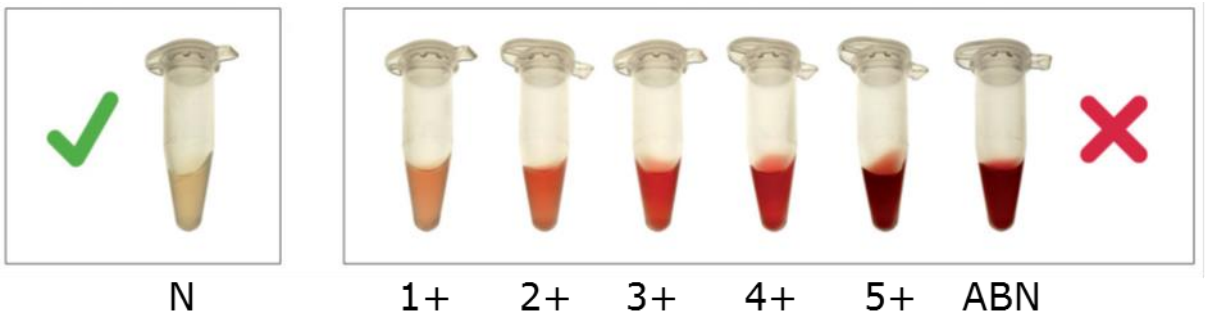
Clot activator tubes usually have clear (sometimes brown) caps and are used to collect samples to produce serum.

Sample processing

It is essential to process all samples in a consistent manner. After collection each sample should be mixed by gently inverting the tube a few times and then kept on ice until centrifugation. Centrifuge samples within 1 hour of collection (the sooner the better) e.g. at 5000 x g, for 10 minutes in a refrigerated centrifuge set at 8°C. Once separated from the cells, plasma / serum should be refrigerated if analysis is delayed. If plasma / serum samples cannot be analysed fresh, they should be frozen as soon as possible at -20°C for short term storage or -70ºC for long term storage. Freeze/thaw cycles should be avoided.

Haemolysis

Samples should be free from haemolysis to avoid artefacts. Reliable and consistent data can only be collected from non-haemolysed samples.



Examples of plasma samples with varying degrees of haemolysis ranging from non-haemolysed (normal) on the left to severely haemolysed (abnormal) on the right.

Examples of plasma test results affected by haemolysis

Due to the interference of haemolysis with various assays including **sodium**, **potassium**, **chloride AST**, **iron**, **bilirubin**, **LDH**, **ALP**, **CK**, **alpha-amylase**, **total protein**, **total cholesterol**, **inorganic phosphate** and **uric acid**, we recommend that the affected results be interpreted with caution depending on the severity of haemolysis. The details of samples and assays affected (if any) will be indicated when results are provided.

Lipaemia

If mice are fed a high fat diet then lipaemia (milky/turbid plasma) can interfere with the analysis and may lead to inaccurate results. Lipaemia can be reduced by fasting experimental animals prior to blood sampling.

Examples of plasma test results affected by lipaemia

Due to the interference of lipaemia with various assays including **iron**, **calcium**, **glucose**, **creatinine**, **LDL-cholesterol**, **albumin**, **inorganic phosphorus**, **uric acid**, **HDL-cholesterol** and **magnesium**, we recommend that the affected results be

interpreted with caution depending on the severity of lipaemia. The details of samples and assays affected (if any) will be indicated when results are provided.

Collection of urine samples from mice

It is desirable to collect urine over a 24 hour period as is possible when mice are temporarily housed in metabolic cages. Data obtained from spot urine collections tend to produce less informative results. Urinary creatinine is often used as an indicator of urinary concentration. Sufficient amounts of the recommended preservative must be added at the point of collection to prevent precipitation or adsorption of analytes. Urine samples should be centrifuged to remove any suspended matter and only aliquots of the clear supernatant fraction should be used for analysis or frozen for storage. When urine samples are frozen the tubes should not be filled by more than 80% to avoid leakage. For certain assays it is recommended to adjust the pH of urine samples (refer to the list of available assays).

Important General Information

Sample containers

Please send plasma, serum and urine samples in 1.5mL micro-centrifuge (eppendorf) tubes only.

Labelling

We require that users follow one of two labelling conventions.

Option 1: Barcode Labels. Use only the labels we supply for larger / high throughput projects - please enquire. Users scan or keep a written record of the barcode numbers they use for specific samples. **Labels should be stuck length ways to 1.5mL micro-centrifuge tubes with one short end of the label against the hinge of the lid**. This is the only orientation in which the barcodes can be read by our analyser.

Option 2: Sequential Numbers. Simply label tubes as 1, 2, 3 etc with a freezer proof lab marker. Do not use any other codes or numbers. Users tend to keep details of their samples (age, sex, strain, study ID, date, genotype etc) and join the information up with the results we provide.

Do not send samples in any other size of micro-centrifuge tube or use any other kind of barcode label or sticker. Failure to comply will result in significant delays in the analysis of your samples.

Numbers of Samples

Many parameters we measure in blood and urine are affected by age, sex, diet, sample collection method, processing and storage. We therefore do not provide reference ranges, but suggest the inclusion of suitable control samples in your study design. We recommend collection of at least 10-15 samples from every age-, strain-, and sex-matched cohort you wish to compare from each genotype. It is often difficult to draw any conclusions from the data obtained from smaller cohorts of samples.

Sample volume

We will not analyse any samples that are less than 20uL in volume but will still charge you for every sample you submit. It is important to calculate the sample volume you require prior to sample collection. Please refer to the sample volumes indicated in the table of tests available from our website. **On board our analyser the dead volume in each sample tube is 40µl so the total volume of the required profile should be added on top of this to calculate the required volume for any plasma / serum / urine profile.** The volume of whole blood required to obtain a specified amount of plasma / serum is approximately more than twice (roughly 2.2 times). If plenty of sample volume is available, it is recommended to send some extra volume (e.g. an extra 20µL) to allow for out of range results to be repeated and to allow for slight variations in dead volume between different makes of 1.5 mL micro-centrifuge tubes. Fibrin clots also occasionally form in plasma samples after they have been thawed and can be of substantial size – these have to be removed prior to analysis and can result in substantial loss of sample volume. The order in which biochemical tests are performed on the analyser cannot be specified, so if the volume is insufficient important assays may be missed.

Note that we are unable to retain and/or return leftover samples to users

Dilutions

By prior agreement, in exceptional circumstances where sample volumes are insufficient for the test profile required, we will be prepared to dilute samples up to 1:3. **Do not dilute samples prior to sending them to us.** It is better for samples to be diluted immediately before they are analysed. If dilutions are required then it needs to be clearly stated in correspondence and it is also recommended to include a reminder note

with the actual samples. **Be aware that levels of analytes in diluted samples may end up falling below the detectable ranges of the assays used**. We will perform dilutions of 1:2 or up to 1:3 but will only apply one (the same) dilution to each batch of samples.

Sample information

Users are asked to supply information about analytes they expect to be exceptionally high or low along with any other information about treatments and sample processing that may affect sample analysis or results on the user request form.

Shipment of plasma / serum for clinical chemistry

Please do not send us samples until we have received a scanned attachment of your purchase order, signed terms and conditions and your completed request form.

- Pack frozen samples in an adequate amount of dry ice.
- **Place samples inside a ziplock bag or sample box - not loose inside the dry ice.**
- Ship samples to the address at the end of this document.
- **Clearly indicate sender details** (your name, address & contact number) - failure to do so could result in significant delays.
- Avoid deliveries on Friday afternoons and over weekends.
- Notify us of shipments by emailing us at clinchem@har.mrc.ac.uk prior to delivery to let us know when to expect your samples and to inform us of the exact test profile you require.

Note that we only analyse external samples in our laboratory on Wednesdays, Thursdays and Fridays.

Pricing

The cost of analysis for any batch of samples depends on the total number of tests ordered, which is calculated as the number of samples x the number of tests requested (e.g. 20 samples submitted for a 6 test profile = 120 tests) and is billed as follows:

Total number of tests ordered	Cost per test
1-30	£5.00
31 - 300	£2.50
301 and more	£1.50

A minimum charge of £5.00 per sample and £50.00 per batch is applied.

Please contact us for a full quote and user request form.
Our Terms & Conditions are available on our website. A copy of these will be emailed to the client together with the full quote and request form.

Request Forms, Purchase Orders and Terms of Conditions

Please address your purchase order to:

Mary Lyon Centre
MRC Harwell
C/o RCUK Shared Services Centre Ltd
North Star House
North Star Avenue
Swindon
SN2 1FF
UK

Before samples are shipped, **please email** your completed request form, purchase order, and signed terms and conditions to clinchem@har.mrc.ac.uk **as pdf attachments.**

If you are VAT exempt, please also provide a copy of your certificate of VAT exemption along with your purchase order. Note that under HM Revenue & Customs legislation we may not be able to fulfil your request.

Payment instructions

Full details for BACS transactions will be given on our invoice.
Cheques should be made payable to MRC
Please contact finance@ssc.rcuk.ac.uk with any queries

Contact & Sample delivery address

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