Clinical Chemistry IMPC_CBC_003

Purpose

Clinical chemistry determines biochemical parameters in plasma including enzymatic activity, specific substrates and electrolytes.


Experimental Design

- Minimum number of animals: 7M + 7F
- Age at test: Week 16
- Sex: We would expect the results of this test to show sexual dimorphism

Equipment

1. Clinical chemistry analyser
2. Vortex
3. Refrigerated centrifuge
4. Eppendorf tubes
5. Pipettes (200-1000 ul)

Procedure

Set up the clinical chemistry analyser and perform QC analyses of the control reagents in accordance with the equipment guidelines.

Sample collection and preparation:

a. Collect the appropriate volume of blood required (160-200 μl of plasma), for the clinical chemistry analyser being used for assessment, in gel tube containing lithium Heparin with the relevant blood collection procedure (see IMPC protocol Blood collection by retro-orbital puncture). Time of day for collection is in the morning, starting no earlier than 07:30.

b. Keep whole blood samples in a bag on wet ice until centrifugation. Centrifuge for 10 minutes at 5000 x g in a refrigerated centrifuge set at 8°C. If plasma samples cannot be analysed immediately, keep them in the fridge until analysis.

c. Analysis of samples is optimally done on the day of collection. When not possible the plasma samples can be stored at 2-8°C. If samples require storage for > 48 hours, freeze plasma at -20 °C in single aliquots. All samples are allowed to come to room temperature prior to analysis.
d. Use plasma samples undiluted or diluted to a ratio of 1:2 with deionised water if the volume is insufficient.

e. Plasma samples that were frozen or stored in the fridge should be vortexed briefly and centrifuged again at ~5000 x g for 2-3 minutes immediately prior to analysis. If necessary, remove fibrin clots using a wooden applicator.

Analysis:

Samples that produce results that lie outside the linear range for a specific assay have to be re-tested. In some cases it may be necessary to dilute samples with water to bring test results into range.

Notes

Blood collection for Clinical Chemistry and Hematology is usually performed as a non-fasting, terminal procedure but can be performed as a non-terminal procedure under certain circumstances. Mice from the terminal procedure may be used for subsequent gross pathology and other procedures included in terminal assessments. Whole blood (for Hematology) and plasma (for Clinical Chemistry) require different collection tubes so two independent samples are required from each mouse.

The information about the date of the experiment, that is the date when the measurement is performed, is an important parameter which is to be submitted in the Experiment xml file (dateOfExperiment="2013-02-28").

Dilution. Dilution of blood is highly discouraged, but is allowed when the total necessary amount is not obtained. If dilution is necessary then the assays should be done in one run.

Hemolysis. Two fields currently exist to capture metadata information about the hemolysis status in the clinical chemistry plasma samples. The first is the LIH Hemolysis severity score which can only be performed by clinics who run one of the Beckman Coulter AU-series of analysers. Such clinics are encouraged to capture and submit the hemolysis score of the LIH test in this field. Clinics who do not have an AU analyser are encouraged to use the second/alternative field which is simply titled Hemolysis. Simply enter “slight”, “moderate”, or “marked” based on whether the sample is visibly haemolysed or not. Provision of this information is not compulsory and it is suggested that any clinic completes at least one field or the other (not both).

Data QC

1. Plasma samples must be free of Fibrin clots in order to be analysed.
2. Badly haemolysed samples should be discarded.
3. Each morning, all parameters are tested with control sera (see ESLIM_015_001_Annex_3: Controls for biochemistry on AU400). Some parameters are tested with control serum level 1 (Beckman Coulter System Reagent, ODC0003) and control serum level 2 (Beckman Coulter System Reagent, ODC0004), which consists of lyophilised human plasma with a normal and a pathological concentration. Other parameters are tested with specific controls from other suppliers.
4. Controls are thawed and vortexed before utilisation and loaded according to the analyser’s display. Control values must lie within the acceptable range indicated by the manufacturer, otherwise the specific tests must be recalibrated and specific
measurements repeated. Controls can be stored in 200 l aliquots at -20°C for up to 1 week.

## Metadata and examples

<table>
<thead>
<tr>
<th>Metadata</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Equipment ID</td>
<td>ID of the machine used when more than 1 is used having same model and manufacturer. E.g. machine 1, machine 2, machine Minnie, machine Mickey Mouse, etc.</td>
</tr>
<tr>
<td>Equipment manufacturer</td>
<td>Manufacturer of the equipment. E.g. Olympus Diagnostics.</td>
</tr>
<tr>
<td>Equipment model</td>
<td>Model of the equipment. E.g. AU400</td>
</tr>
<tr>
<td>Blood collection tubes</td>
<td>The tubes used for blood collection. E.g. Sarstedt Li-Heparin gel tubes or Kabe Labortechnik Lithium heparin coated tubes.</td>
</tr>
<tr>
<td>Anaesthesia used for blood collection</td>
<td>The drug used for anaesthesia during blood collection. E.g. Isofluorane.</td>
</tr>
<tr>
<td>Method of blood collection</td>
<td>Concise description of the method used for blood collection. E.g. retro-orbital puncture.</td>
</tr>
<tr>
<td>Anticoagulant</td>
<td>Anticoagulant drug used for blood collection. E.g. Li-Heparin.</td>
</tr>
<tr>
<td>Samples kept on ice between collection and analysis</td>
<td>Yes/No.</td>
</tr>
<tr>
<td>Storage temperature from blood collection till measurement</td>
<td>E.g. 2°C</td>
</tr>
<tr>
<td>Sample status</td>
<td>Indicate if the sample were frozen (analysis on the same day of collection not possible) or fresh (analysis on the same day of collection). E.g Fresh/Frozen.</td>
</tr>
<tr>
<td>Parameter</td>
<td>Description</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Plasma dilution</td>
<td>Dilution is highly discouraged but if necessary indicate here. E.g. “No dilution” or 1:2. Note that results submitted to DCC are assumed to be already corrected for any dilutions made.</td>
</tr>
<tr>
<td>ID of blood collection SOP</td>
<td>ID of the protocol followed for blood collection. Can be a center specific protocol. E.g. ESLIM_024_001.</td>
</tr>
<tr>
<td>Date and time of blood collection</td>
<td>Time of day for collection is in the morning, starting no earlier than 07:30. E.g. Year, month, day, time.</td>
</tr>
<tr>
<td>Date of measurement</td>
<td>The day of blood analysis. Year, month, day.</td>
</tr>
<tr>
<td>Hemolysis status</td>
<td>If no AU analyser score is provided, indicate here the gauged degree of hemolysis. E.g. slight/moderate/marked.</td>
</tr>
<tr>
<td>Blood collection experimenter ID</td>
<td>An ID of any format to be used coherently both inside the same procedure and for all procedures indicating the experimenter who collected the blood. E.g. Harw_001, or 1/2/3.</td>
</tr>
<tr>
<td>Blood analysis experimenter ID</td>
<td>An ID of any format to be used coherently both inside the same procedure and for all procedures indicating the experimenter who analyzed the blood. E.g. Harw_001, or 1/2/3.</td>
</tr>
<tr>
<td>Date equipment last calibrated</td>
<td>Most recent date in which the equipment (or any part of) used in the procedure was subject to a calibration event.</td>
</tr>
<tr>
<td>Date and time of sacrifice</td>
<td>The date and time when the mouse is sacrificed.</td>
</tr>
</tbody>
</table>

**Parameters and Metadata**

**Fasting** IMPC_CBC_057_001 | v1.0

procedureMetadata
Options: No, Four hours before bleeding, Sixteen hours before bleeding,

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**Albumin** IMPC_CBC_007_001 | v1.2
simpleParameter

Unit Measured: g/l

---

**Date and time of sacrifice** IMPC_CBC_040_001 | v1.1
procedureMetadata

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**Magnesium** IMPC_CBC_054_001 | v1.5
simpleParameter

Unit Measured: mg/dl

---

**Glycerol** IMPC_CBC_027_001 | v1.4
**Potassium** IMPC_CBC_002_001 | v1.3

- **Unit Measured:** mmol/l

**Uric acid** IMPC_CBC_029_001 | v1.2

- **Unit Measured:** umol/l

**Calcium** IMPC_CBC_009_001 | v1.5

- **Unit Measured:** mg/dl
**Alpha-amylase**  IMPC_CBC_023_001 | v1.2

*simpleParameter*

Req. Analysis: false  
Req. Upload: false  
Is Annotated: true

Unit Measured: U/l

---

**Hemolysis status**  IMPC_CBC_048_001 | v1.1

*procedureMetadata*

Req. Analysis: false  
Req. Upload: false  
Is Annotated: false

Options: Slight, Moderate, None, Marked,

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**Fructosamine**  IMPC_CBC_020_001 | v1.2

*simpleParameter*

Req. Analysis: false  
Req. Upload: false  
Is Annotated: true

Unit Measured: umol/l

---

**Lactate dehydrogenase**  IMPC_CBC_022_001 | v1.2

*simpleParameter*

Req. Analysis: false  
Req. Upload: false  
Is Annotated: true
Sample dilution IMPC_CBC_044_001 | v1.2

**procedureMetadata**

**Req. Analysis:** false  
**Req. Upload:** true  
**Is Annotated:** false

**Options:** 1:2, Neat serum, Yes (by Equipment, automatically), Neat plasma, 1:4, Varies, 1:3, 1:5,

Chloride IMPC_CBC_003_001 | v1.4

**simpleParameter**

**Req. Analysis:** false  
**Req. Upload:** false  
**Is Annotated:** true

**Unit Measured:** mmol/l

Anesthesia used for blood collection IMPC_CBC_036_001 | v1.0

**procedureMetadata**

**Req. Analysis:** true  
**Req. Upload:** true  
**Is Annotated:** false

**Options:** Injection narcosis with Sodium Pentobarbital (Euthatal), Injection narcosis with Ketamine (100mg/kg)/ Xylazine (10mg/kg) /Antipamezole (Antisedan, 1mg/kg), Injection narcosis with Ketamine (110mg/kg)/Xylazine (11mg/kg) / Antipamezole (Antisedan, 1mg/kg),
Injection narcosis with Ketamine (100mg/kg)/Xylazine (10mg/kg),
Injection narcosis with Tribromoethanol (Avertin),
Injection narcosis with Sodium Pentobarbital (Pentobarb, 0.1ml),
Injection narcosis with Ketamine (137mg/kg)/Xylazine (6.6mg/kg),
Injection narcosis with Ketamine (110mg/kg)/Xylazine (11mg/kg),
Injection narcosis with Sodium Pentobarbital (Somnopentyl),
Gas anaesthesia with Isofluorane,

Sample type IMPC_CBC_056_001 | v1.0

| Options | Plasma, Serum, |

Iron IMPC_CBC_011_001 | v1.5

| Unit Measured | mg/dl |

Transferrin IMPC_CBC_031_001 | v1.2

| Unit Measured | mg/dl |
Samples kept on ice between collection and analysis

Blood collection experimenter ID

LIH (Hemolysis Severity - available on AU analysers)

Creatine kinase
Unit Measured: U/l

**Creatinine** IMPC_CBC_005_001 | v1.5

*simpleParameter*

Req. Analysis: false  
Req. Upload: true  
Is Annotated: true

Unit Measured: mg/dl

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**UIBC (unsaturated iron binding capacity)** IMPC_CBC_024_001 | v1.0

*simpleParameter*

Req. Analysis: false  
Req. Upload: false  
Is Annotated: true

Unit Measured: umol/l

---

**HDL-cholesterol** IMPC_CBC_016_001 | v1.4

*simpleParameter*

Req. Analysis: false  
Req. Upload: true  
Is Annotated: true

Unit Measured: mg/dl
**Date and time of blood collection**  IMPC_CBC_046_001 | v1.2

*procedureMetadata*

<table>
<thead>
<tr>
<th>Req. Analysis</th>
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</tr>
</thead>
<tbody>
<tr>
<td>false</td>
<td>true</td>
<td>false</td>
</tr>
</tbody>
</table>

**LDL-cholesterol**  IMPC_CBC_025_001 | v1.3

*simpleParameter*

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<tr>
<td>false</td>
<td>false</td>
<td>true</td>
</tr>
</tbody>
</table>

*Unit Measured:* mg/dl

**Aspartate aminotransferase**  IMPC_CBC_012_001 | v1.2

*simpleParameter*

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</thead>
<tbody>
<tr>
<td>false</td>
<td>true</td>
<td>true</td>
</tr>
</tbody>
</table>

*Unit Measured:* U/l

**Blood collection tubes**  IMPC_CBC_039_001 | v1.1

*procedureMetadata*

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</thead>
<tbody>
<tr>
<td>false</td>
<td>false</td>
<td>false</td>
</tr>
</tbody>
</table>

*Options:* TERUMO CAPIJECT Lithium heparin coated tubes, Eppendorf 1.7ml, Kabe Labortechnik 1000ul Lithium Heparin, BD Microtainer Lithium Heparin Tube, Sarstedt Li-Heparin gel tubes, Kabe Labortechnik Lithium heparin coated tubes,
Total protein  IMPC_CBC_006_001 | v1.2

simpleParameter


Unit Measured: g/l

Glycosilated hemoglobin A1c (HbA1c)  IMPC_CBC_052_001 | v1.3

simpleParameter


Unit Measured: %

Glucose  IMPC_CBC_018_001 | v1.5

simpleParameter


Unit Measured: mg/dl
**Equipment ID** IMPC_CBC_033_001 | v1.0

**Difficult bleed** IMPC_CBC_055_001 | v1.0

**Thyroxine** IMPC_CBC_053_001 | v1.2

**Free fatty acids** IMPC_CBC_026_001 | v1.4
C-reactive protein  IMPC_CBC_032_001 | v1.0

Simple Parameter


Unit Measured: mg/l

Total cholesterol  IMPC_CBC_015_001 | v1.4

Simple Parameter


Unit Measured: mg/dl

Sample status  IMPC_CBC_043_001 | v1.1

Procedure Metadata


Options: Fresh, Frozen, Fresh and frozen,

Ferritin  IMPC_CBC_030_001 | v1.3

Simple Parameter

Unit Measured: ng/ml

ID of blood collection SOP  IMPC_CBC_045_001 | v1.1

**procedureMetadata**

**Req. Analysis:** false  **Req. Upload:** true  **Is Annotated:** false

**Options:** sop.inv.063, sop.inv.019, PHENO_CBC, ESLIM_024_001, RIKENMPP_004a_003, Biochem _blood SOP,

---

Equipment manufacturer  IMPC_CBC_034_001 | v1.0

**procedureMetadata**

**Req. Analysis:** true  **Req. Upload:** true  **Is Annotated:** false

**Options:** Cobas, Hitachi, Roche, JEOL (Siemens), Olympus Diagnostics, Beckman Coulter,

---

Equipment model  IMPC_CBC_035_001 | v1.0

**procedureMetadata**

**Req. Analysis:** true  **Req. Upload:** true  **Is Annotated:** false

**Options:** AU 480, UniCel 600 Pro, JCA-BM2250 (Advia 2400), Integra 400 Plus, AU 680, AU 400, 7020, Hitachi 917, JCA-BM6070,
Method of blood collection  IMPC_CBC_037_001  | v1.0

**Options:** Jugular vein, Heart puncture, Tail vein, Cardiac puncture, Retro-orbital puncture,

---

Storage temperature from blood collection till measurement  IMPC_CBC_041_001  | v1.3

**Options:** 4, 2, 18-22, -80,

---

Alkaline phosphatase  IMPC_CBC_014_001  | v1.2

**Unit Measured:** U/l

---

Lipase  IMPC_CBC_021_001  | v1.1

**Unit Measured:** U/l
**Phosphorus** IMPC_CBC_010_001 | v1.6

simpleParameter

**Alanine aminotransferase** IMPC_CBC_013_001 | v1.2

simpleParameter

**Blood analysis experimenter ID** IMPC_CBC_051_001 | v1.0

procedureMetadata

**Cholesterol ratio** IMPC_CBC_058_001 | v1.0
**Simple Parameter**

**Derivation**: \(\text{div('IMPC_CBC_015_001', 'IMPC_CBC_016_001')}\)

---

**Reagent manufacturer** IMPC_CBC_059_001 | v1.0

**Procedure Metadata**

**Options**: Wako and Sekisui, Microgenics, Beckman Coulter,

---

**Urea (Blood Urea Nitrogen - BUN)** IMPC_CBC_004_001 | v1.5

**Simple Parameter**

**Unit Measured**: mg/dl

---

**Anticoagulant** IMPC_CBC_038_001 | v1.1

**Procedure Metadata**

**Options**: Sodium Heparin, Heparine, Lithium Heparin, No,
Date equipment last calibrated  IMPC_CBC_050_001 | v1.2


Triglycerides  IMPC_CBC_017_001 | v1.4


Unit Measured: mg/dl

Total bilirubin  IMPC_CBC_008_001 | v1.4


Unit Measured: mg/dl

Sodium  IMPC_CBC_001_001 | v1.3

Unit Measured: mmol/l