\*Title: **Clinical chemistry**

\*Centre: IMPC

\*Date\_modified: 12-03-2012

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\*Version: 1

{Sections:}

## \*1. Purpose:

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

Ontological description: MP:0001545 – blood physiology abnormalities.

## \*2. Experimental Design

Minimum number of mutant animals: must maintain ≥ 7 size for male and female.

Age of animals: 16 weeks

Sexual dimorphism: Yes for some of the parameters.

## \*3. Equipment

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

## \*4. Procedure

1. Set up the clinical chemistry analyser and perform QC analyses of the control reagents in accordance with the equipment guidelines.
2. **Sample collection and preparation:**
3. Collect the appropriate volume of blood required (160-200μl of plasma), for the clinical chemistry analyser being used for assessment, in a Sarstedt 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.
4. Keep whole blood samples on ice until centrifugation. If plasma samples cannot be analysed immediately, keep them in the fridge until analysis.
5. 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.
6. Use plasma samples undiluted or diluted to a ratio of 1:2 with deionised water if the volume is insufficient.
7. Vortex all plasma samples and briefly centrifuge them at ~5000 x g for 2 -3 minutes. If necessary, remove fibrin clots using a wooden applicator.
8. **Analysis:**
9. 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.

## \*5. Notes

### Blood collection for Clinical Chemistry and Hematology is performed as a non-fasting, terminal procedure, with some mice being used for subsequent gross pathology and other clinic-specific parameters 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. 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.

### 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 200μl aliquots at -20°C for up to 1 week.

## \*6 . Measured Parameters - list

{Placed in Parameters spreadsheet}

## \*7. MetaData Parameters - list

|  |  |  |  |
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| **Metadata** | **Example** | **Required for data upload** | **Required for data analysis** |
| Equipment ID |  | YES | NO |
| Equipment manufacturer | Beckman Coulter | YES | YES |
| Equipment model | AU680 | YES | YES |
| Date equipment last calibrated |  | NO | NO |
| Anaesthesia used for blood collection | Isofluorane | YES | YES |
| Method of blood collection | retro-orbital puncture | YES | YES |
| Anticoagulant | Li-Heparin | YES | YES |
| Samples kept on ice between collection and analysis | Yes/No | YES | YES |
| Sample status | Fresh/Frozen | YES | YES |
| Plasma dilution | No/1:2 | YES | YES |
| ID of blood collection SOP | ESLIM\_024\_001 | YES | YES |
| Date and time of blood collection | Year, month, day, time | YES | YES |
| Date of measurement | Year, month, day | YES | YES |
| Haemolysis status of sample (LIH score from AU analyser) | +++ | NO | YES |