



IMPC phenotyping SOPs in JMC

Clinical Blood Chemistry IMPC_CBC_003

Purpose

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

Ontological description: MP:0001545 – blood physiology abnormalities.

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.

Equipment

1. Clinical chemistry analyser
2. Vortex
3. Refrigerated centrifuge
4. Microtubes
5. Pipettes (20-100ul, 50-200ul, 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:

1. Collect the appropriate volume of blood required (70 μ l of plasma), for the clinical chemistry analyser being used for assessment, in lithium Heparin coated tube 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.
2. 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. Transfer the supernatant to a clean tube. Centrifuge the sample again for 5 minutes, 5000x g at 8°C.
3. Collected plasma samples are frozen at -80°C in single aliquots and stored until the day of analysis (usually within 30days). All samples are allowed to come to room temperature prior to analysis.
4. Plasma samples that were frozen should be vortexed briefly and centrifuged again at ~5000 x g for few seconds immediately prior to analysis. If necessary, remove fibrin clots using a tip.

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 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.

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.

Data QC

1. Plasma samples must be free of Fibrin clots in order to be analysed.
2. Data obtained from badly haemolysed samples should be excluded.
3. Each morning, all parameters are tested with control sera (e.g. Liquid control serum I wako C&C). Some 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.

Parameters

	Version	Type	Increment	Option	Derived	Unit	Data Type
Urea (Blood Urea Nitrogen - BUN) IMPC_CBC_004_001	1.5	simpleParameter				mg/dl	FLOAT
Creatinine (enzymatic method preferred) IMPC_CBC_005_001	1.4	simpleParameter				mg/dl	FLOAT
Total protein IMPC_CBC_006_001	1.2	simpleParameter				g/l	FLOAT
Albumin IMPC_CBC_007_001	1.2	simpleParameter				g/l	FLOAT
Total bilirubin IMPC_CBC_008_001	1.4	simpleParameter				mg/dl	FLOAT
Calcium IMPC_CBC_009_001	1.5	simpleParameter				mg/dl	FLOAT
Phosphorus IMPC_CBC_010_001	1.6	simpleParameter				mg/dl	FLOAT
Iron IMPC_CBC_011_001	1.5	simpleParameter				mg/dl	FLOAT

Aspartate aminotransferase IMPC_CBC_012_001	1.2	simpleParameter				U/l	FLOAT
Alanine aminotransferase IMPC_CBC_013_001	1.2	simpleParameter				U/l	FLOAT
Alkaline phosphatase IMPC_CBC_014_001	1.2	simpleParameter				U/l	FLOAT
Total cholesterol IMPC_CBC_015_001	1.4	simpleParameter				mg/dl	FLOAT
HDL-cholesterol IMPC_CBC_016_001	1.4	simpleParameter				mg/dl	FLOAT
Triglycerides IMPC_CBC_017_001	1.4	simpleParameter				mg/dl	FLOAT
Glucose IMPC_CBC_018_001	1.5	simpleParameter				mg/dl	FLOAT
Lactate dehydrogenase IMPC_CBC_022_001	1.2	simpleParameter				U/l	FLOAT
Alpha-amylase IMPC_CBC_023_001	1.2	simpleParameter				U/l	FLOAT
LDL-cholesterol IMPC_CBC_025_001	1.3	simpleParameter				mg/dl	FLOAT
Creatine kinase IMPC_CBC_028_001	1.2	simpleParameter				U/l	FLOAT
Uric acid IMPC_CBC_029_001	1.2	simpleParameter				umol/l	FLOAT
Glycosilated hemoglobin A1c (HbA1c) IMPC_CBC_052_001	1.3	simpleParameter				%	FLOAT

Metadata

	Version	Type	Increment	Option	Derived	Unit	Data Type
Equipment ID IMPC_CBC_033_001	1.0	procedureMetadata					TEXT
Equipment manufacturer IMPC_CBC_034_001	1.0	procedureMetadata		JEOL			TEXT
Equipment model IMPC_CBC_035_001	1.0	procedureMetadata		JCA-BM2250			TEXT
				JCA-BM6070			
Anesthesia used for blood collection IMPC_CBC_036_001	1.0	procedureMetadata		Injection narcosis with Sodium Pentobarbital (Somnopentyl)			TEXT
Anticoagulant IMPC_CBC_038_001	1.1	procedureMetadata		Lithium Heparin			TEXT
Method of blood collection IMPC_CBC_037_001	1.0	procedureMetadata		Retro-orbital puncture			TEXT
Samples kept on ice between collection and	1.1	procedureMetadata		No			TEXT

	Version	Type	Increment	Option	Derived	Unit	Data Type
analysis IMPC_CBC_042_001							
Sample status IMPC_CBC_043_001	1.1	procedureMetadata		Frozen			TEXT
Sample dilution IMPC_CBC_044_001	1.2	procedureMetadata		Yes (by Equipment, automatically)			TEXT
ID of blood collection SOP IMPC_CBC_045_001	1.1	procedureMetadata		RIKENMPP_004a_003			TEXT
Date and time of blood collection IMPC_CBC_046_001	1.2	procedureMetadata					DATETIME
Blood collection experimenter ID IMPC_CBC_049_001	1.1	procedureMetadata					TEXT
Blood collection tubes IMPC_CBC_039_001	1.1	procedureMetadata		TERUMO CAPIJECT Lithium heparin coated tubes			TEXT
Date and time of sacrifice IMPC_CBC_040_001	1.1	procedureMetadata					DATETIME
Storage temperature from blood collection till measurement IMPC_CBC_041_001	1.3	procedureMetadata		-80		C	TEXT
Blood analysis experimenter ID IMPC_CBC_051_001	1.0	procedureMetadata					TEXT
Sample type IMPC_CBC_056_001	1.0	procedureMetadata		Plasma			TEXT
Fasting IMPC_CBC_057_001	1.0	procedureMetadata		No			TEXT