Clinical Chemistry NINGLA_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 animals :** 7M + 7F
- **Age at test:** Week 59
- **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 (160-200l of plasma), for the clinical chemistry analyser being used for assessment, preferably using a tube with an anticoagulant 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 on 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 200l aliquots of control sera frozen at -20°C for up to 1 month.

Metadata and examples

Metadata	**Example**
Equipment ID	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.
Equipment manufacturer	Manufacturer of the equipment. E.g. Olympus Diagnostics.
Equipment model	Model of the equipment. E.g. AU400
Blood collection tubes	The tubes used for blood collection. E.g. Sarstedt Li-Heparin gel tubes or Kabe Labortechnik Lithium heparin coated tubes.
Anaesthesia used for blood collection	The drug used for anaesthesia during blood collection. E. g. Isofluorane.
Method of blood collection	Concise description of the method used for blood collection. E.g. retro-orbital puncture.
Anticoagulant	Anticoagulant drug used for blood collection. E. g. Li-Heparin.
Samples kept on ice between collection and analysis	Yes/No.

Storage temperature from blood collection till measurement	E.g. 2°C
Sample status	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.
Plasma dilution	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.
ID of blood collection SOP	ID of the protocol followed for blood collection. Can be a center specific protocol. E.g. ESLIM_024_001.
Date and time of blood collection	Time of day for collection is in the morning, starting no earlier than 07:30. E.g. Year, month, day, time.
Date of measurement	The day of blood analysis. Year, month, day.
Hemolysis status	If no AU analyser score is provided, indicate here the gauged degree of hemolysis. E.g. slight/moderate/marked.
Blood collection experimenter ID	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.
Blood analysis experimenter ID	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.
Date equipment last calibrated	Most recent date in which the equipment (or any part of) used in the procedure was subject to a calibration event.
Date and time of sacrifice	The date and time when the mouse is sacrified.

Parameters and Metadata

Sample dilution NINGLA_CBC_044_001 | v1.2

procedureMetadata

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

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

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Glycerol NINGLA_CBC_027_001 | v1.4

simpleParameter

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

Unit Measured: mmol/l

Storage temperature from blood collection till measurement NINGLA_CBC_041_001 | v1.3

procedureMetadata

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

Unit Measured: C

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

Lactate dehydrogenase NINGLA_CBC_022_001 | v1.2

simpleParameter

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

Unit Measured: U/l

Total cholesterol NINGLA_CBC_015_001 | v1.4

simpleParameter

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

Unit Measured: mg/dl

Samples kept on ice between collection and analysis NINGLA_CBC_042_001 | v1.1

procedureMetadata

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

Options: No, Yes,

Thyroxine NINGLA_CBC_053_001 | v1.2

simpleParameter

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

Unit Measured: ug/dl

Iron NINGLA_CBC_011_001 | v1.5

simpleParameter

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

Unit Measured: mg/dl

Anesthesia used for blood collection NINGLA_CBC_036_001 | v1.0

procedureMetadata

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

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

Injection narcosis with Ketamine (137mg/kg)/Xylazine (6.6mg/kg),
Injection narcosis with Ketamine (100mg/kg)/ Xylazine (10mg/kg)/Antipamezole (Antisedan, 1mg/kg),
Injection narcosis with Sodium Pentobarbital (Euthatal), No,
Gas anaesthesia with Isofluorane,

Creatinine NINGLA_CBC_005_001 | v1.5

simpleParameter

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

Unit Measured: mg/dl

Magnesium NINGLA_CBC_054_001 | v1.5

simpleParameter

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

Unit Measured: mg/dl

Alkaline phosphatase NINGLA_CBC_014_001 | v1.2

simpleParameter

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

Unit Measured: U/l

Blood collection tubes NINGLA_CBC_039_001 | v1.1

procedureMetadata

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

Options: TERUMO CAPIJECT Lithium heparin coated tubes, Sarstedt Li-Heparin gel tubes, Kabe Labortechnik Lithium heparin coated tubes, BD Microtainer Lithium Heparin Tube, Eppendorf 1.7ml, Kabe Labortechnik 1000ul Lithium Heparin,
BD Microtainer Lithium Heparin/PST Gel Blood Tube,
Greiner MiniCollect Lithium Heparin 1ml,

Alpha-amylase NINGLA_CBC_023_001 | v1.2

simpleParameter

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

Unit Measured: U/l

HDL-cholesterol NINGLA_CBC_016_001 | v1.4

simpleParameter

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

Unit Measured: mg/dl

Equipment model NINGLA_CBC_035_001 | v1.0

procedureMetadata

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

Options: JCA-BM2250 (Advia 2400), Hitachi 917, AU 680, 7020, AU 400, UniCel 600 Pro, Integra 400 Plus, AU 480, JCA-BM6070, Cobas,

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Phosphorus NINGLA_CBC_010_001 | v1.6

simpleParameter

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

Unit Measured: mg/dl

Total protein NINGLA_CBC_006_001 | v1.2

simpleParameter

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

Unit Measured: g/l

Glucose NINGLA_CBC_018_001 | v1.5

simpleParameter

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

Unit Measured: mg/dl

ID of blood collection SOP NINGLA_CBC_045_001 | v1.1

procedureMetadata

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

Options: sop.inv.063, PHENO_CBC, sop.inv.019, ESLIM_024_001, RIKENMPP_004a_003,

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Lipase NINGLA_CBC_021_001 | v1.1

simpleParameter

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

Unit Measured: U/l

Sodium NINGLA_CBC_001_001 | v1.3

simpleParameter

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

Unit Measured: mmol/l

C-reactive protein NINGLA_CBC_032_001 | v1.0

simpleParameter

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

Unit Measured: mg/l

Aspartate aminotransferase NINGLA_CBC_012_001 | v1.2

simpleParameter

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

Unit Measured: U/l

Uric acid NINGLA_CBC_029_001 | v1.2

simpleParameter

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

Unit Measured: umol/l

Hemolysis status NINGLA_CBC_048_001 | v1.1

procedureMetadata

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

Options: Moderate, Slight, None, Marked,

Albumin NINGLA_CBC_007_001 | v1.2

simpleParameter

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

Unit Measured: g/l

Total bilirubin NINGLA_CBC_008_001 | v1.4

simpleParameter

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

Unit Measured: mg/dl

Date equipment last calibrated NINGLA_CBC_050_001 | v1.2

procedureMetadata

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

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Sample status NINGLA_CBC_043_001 | v1.1

procedureMetadata

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

Options: Frozen, Fresh and frozen, Fresh,

Date and time of blood collection NINGLA_CBC_046_001 | v1.2

procedureMetadata

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

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Difficult bleed NINGLA_CBC_055_001 | v1.0

procedureMetadata

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

Options: Yes, No,

Free fatty acids NINGLA_CBC_026_001 | v1.4

simpleParameter

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

Unit Measured: mmol/l

Fasting NINGLA_CBC_057_001 | v1.0

procedureMetadata

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

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

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Creatine kinase NINGLA_CBC_028_001 | v1.2

simpleParameter

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

Unit Measured: U/l

Blood analysis experimenter ID NINGLA_CBC_051_001 | v1.0

procedureMetadata

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

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UIBC (unsaturated iron binding capacity) NINGLA_CBC_024_001 | v1.0

simpleParameter

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

Unit Measured: umol/l

Ferritin NINGLA_CBC_030_001 | v1.3

simpleParameter

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

Unit Measured: ng/ml

Alanine aminotransferase NINGLA_CBC_013_001 | v1.2

simpleParameter

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

Unit Measured: U/l

Triglycerides NINGLA_CBC_017_001 | v1.4

simpleParameter

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

Unit Measured: mg/dl

Transferrin NINGLA_CBC_031_001 | v1.2

simpleParameter

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

Unit Measured: mg/dl

Blood collection experimenter ID NINGLA_CBC_049_001 | v1.1

procedureMetadata

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

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Potassium NINGLA_CBC_002_001 | v1.3

simpleParameter

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

Unit Measured: mmol/l

LIH (Hemolysis Severity - available on AU analysers) NINGLA_CBC_019_001 | v1.3

simpleParameter

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

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LDL-cholesterol NINGLA_CBC_025_001 | v1.3

simpleParameter

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

Unit Measured: mg/dl

Urea (Blood Urea Nitrogen - BUN) NINGLA_CBC_004_001 | v1.5

simpleParameter

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

Unit Measured: mg/dl

Date and time of sacrifice NINGLA_CBC_040_001 | v1.1

procedureMetadata

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

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Method of blood collection NINGLA_CBC_037_001 | v1.0

procedureMetadata

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

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

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Calcium NINGLA_CBC_009_001 | v1.5

simpleParameter

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

Unit Measured: mg/dl

Anticoagulant NINGLA_CBC_038_001 | v1.1

procedureMetadata

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

Options: Lithium Heparin, Sodium Heparin, Heparine, No,

Cholesterol ratio NINGLA_CBC_058_001 | v1.0

simpleParameter

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

Derivation: div('NINGLA_CBC_015_001', 'NINGLA_CBC_016_001')

Equipment manufacturer NINGLA_CBC_034_001 | v1.0

procedureMetadata

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

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

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Fructosamine NINGLA_CBC_020_001 | v1.2

simpleParameter

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

Unit Measured: umol/l

Reagent manufacturer NINGLA_CBC_059_001 | v1.0

procedureMetadata

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

Options: Microgenics, Wako and Sekisui, Beckman Coulter,

Chloride NINGLA_CBC_003_001 | v1.4

simpleParameter

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

Unit Measured: mmol/l

Glycosilated hemoglobin A1c (HbA1c) NINGLA_CBC_052_001 | v1.3

simpleParameter

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

Unit Measured: %

Sample type NINGLA_CBC_056_001 | v1.0

procedureMetadata

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

Options: Serum, Plasma,

Equipment ID NINGLA_CBC_033_001 | v1.0

procedureMetadata

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

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