Hematology NINGLA_HEM_002

Purpose

Hematological assessment of blood determines blood cell counts (white blood cells, red blood cells, hemoglobin, and platelets) and additional hematological parameters (hematocrit, mean cell volume, mean corpuscular hemoglobin, mean cell hemoglobin concentration) can be derived using these indices. These tests will indicate abnormalities in the production of blood and its components (blood cells and hemoglobin) as well as in the associated blood-forming organs.

Ontological description: MP:0002429 - abnormal blood cell morphology/development.

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. Hematology automated analyzers (e.g. Beckman Coulter AcT Diff , Siemens Advia 2120 or Hemavet Multispecies Hematology Analyzer HV950FS Drew Scientific, CT, U. S.A.)
2. Rotary agitator

Procedure

Set up the hematological analyser and perform QC analyses of the control reagents in accordance with the guidelines provided by the manufacturer.

Sample collection and preparation:

a. Collect the appropriate volume of blood required for the hematology analyser being used for assessment (~200µl), in an EDTA coated tube with the relevant blood collection procedure (see IMPC protocol Blood collection by retro-orbital puncture) or according to the manufacturer guidance. The time of day for collection is in the morning, starting no earlier than 07:30.

b. Mix the blood sample on a rotary mixer immediately following collection for a minimum of 30 minutes and keep the sample at room temperature (for no more than 2 hours) pending analysis. Samples must *not* be frozen at this stage.
c. Analysis of samples is optimally done on the day of collection, ideally within 2 hours after collection, but latest within 6 hours after collection. When not possible the blood samples can be stored at 2-8°C for up to 24 hours. Long term storage of whole blood is not recommended. All samples are allowed to come to room temperature prior to analysis.

Analysis:

a. Perform hematological assessment of each sample including: white and red blood cell counts, hemoglobin and platelets in accordance with the analyser being used.
b. Derive additional parameters for the sample that may be estimated from the initial assessment such as: hematocrit, mean cell volume, mean corpuscular hemoglobin and mean cell hemoglobin concentration.

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. 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.

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").

Data QC

1. Sample must be free of blood clots in order to be analyzed.
2. Some results from hemolysed samples should not be reported.
3. Perform routinely and immediately prior to sample analysis:

a. assessment of control samples with different levels of hematology phenotypes (abnormally low; normal; abnormally high).
b. analysis of the graphical reports generated for each control level to ensure that they lie within their respective ranges.

Metadata and examples

Metadata	**Example**
	ID of the machine used when more than 1 is used having same model and manufacturer.

Equipment ID	E.g. machine 1, machine 2, machine Minnie, machine Mickey Mouse, etc.
Equipment manufacturer	Manufacturer of the equipment. E.g. SIEMENS.
Equipment model	Model of the equipment. E.g. ADVIA120.
Blood collection tubes	The tubes used for blood collection. E.g. Sarstedt Li-Heparin gel tubes or Kabe Labortechnik Lithium heparin coated tubes.
Method of blood collection	Concise description of the method used for blood collection. E.g. Retro-orbital puncture.
Anesthesia used for blood collection	The drug used for anaesthesia during blood collection. E. g. Isofluorane.
Anticoagulant	Anticoagulant drug used for blood collection. E.g. EDTA.
Samples kept on ice between collection and analysis?	Yes/No
Storage temperature from blood collection till measurement	E.g. 2°C
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. E.g. Year, month, day.
ID for blood collection SOP	ID of the protocol followed for blood collection. Can be a center specific protocol. E.g. ESLIM_024_001

Chip card	The chip card contains the settings and thresholds that are used to calculate the numbers of cell types in a blood sample. As the blood cell sizes differ between the species, there are different thresholds for the categorization and therefore there are different chip cards for different species (mouse strains). Eg. C57BL/6 chip card. The chip cards really look like a chip card. You put them into a slot on the haematology device and then you start measuring the haematological parameters of the corresponding blood samples.
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 sacrificed.

Parameters and Metadata

Hematocrit NINGLA_HEM_004_001 | v1.0

simpleParameter

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

Unit Measured: %

Method of blood collection NINGLA_HEM_013_001 | v1.0

procedureMetadata

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

Options: Tail vein, Saphenous vein, Retro-orbital puncture, Cardiac puncture,

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Mean corpuscular hemoglobin NINGLA_HEM_006_001 | v1.1

simpleParameter

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

Unit Measured: pg

Mean cell hemoglobin concentration NINGLA_HEM_007_001 | v1.2

simpleParameter

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

Unit Measured: g/dl

Service-related calibration start date NINGLA_HEM_042_001 | v1.0

procedureMetadata

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

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Equipment model NINGLA_HEM_011_001 | v1.0

procedureMetadata

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

Options: Hemavet 950 FS, CELL-DYN 3700, Ac-T diff Analyzer, Advia 2120, Advia 120, Scil Vet abc, Scil Vet abc Plus+, XT-2000iV, ProCyte Dx,

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Blood collection experimenter ID NINGLA_HEM_024_001 | v1.1

procedureMetadata

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

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ID for blood collection SOP NINGLA_HEM_020_001 | v1.1

procedureMetadata

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

Options: sop.inv.019, RIKENMPP_003a_003, ESLIM_024_001, sop.inv.063, PHENO_CBC,

Date equipment last calibrated NINGLA_HEM_025_001 | v1.2

procedureMetadata

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

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White blood cell count NINGLA_HEM_001_001 | v1.3

simpleParameter

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

Unit Measured: 10^3/ul

Blood analysis experimenter ID NINGLA_HEM_017_001 | v1.0

procedureMetadata

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

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Storage temperature from blood collection until measurement NINGLA_HEM_026_001 | v1.3

procedureMetadata

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

Unit Measured: C

Options: 25, 4, 22, 23, 18-22,

Equipment ID NINGLA_HEM_009_001 | v1.1

procedureMetadata

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

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Hemoglobin NINGLA_HEM_003_001 | v1.2

simpleParameter

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

Unit Measured: g/dl

Basophil cell count NINGLA_HEM_037_001 | v1.1

simpleParameter

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

Unit Measured: 10^3/ul

Basophil differential count NINGLA_HEM_038_001 | v1.0

simpleParameter

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

Unit Measured: %

Mean cell volume NINGLA_HEM_005_001 | v1.2

simpleParameter

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

Unit Measured: fL

Samples kept on ice between collection and analysis NINGLA_HEM_018_001 | v1.2

procedureMetadata

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

Options: Yes, No,

Neutrophil cell count NINGLA_HEM_030_001 | v1.3

simpleParameter

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

Unit Measured: 10^3/ul

Large Unstained Cell (LUC) differential count NINGLA_HEM_040_001 | v1.0

simpleParameter

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

Unit Measured: %

Date and time of blood collection NINGLA_HEM_021_001 | v1.2

procedureMetadata

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

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Red blood cell count NINGLA_HEM_002_001 | v1.3

simpleParameter

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

Unit Measured: 10^6/ul

Date and time of sacrifice NINGLA_HEM_016_001 | v1.3

procedureMetadata

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

Platelet count NINGLA_HEM_008_001 | v1.3

simpleParameter

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

Unit Measured: 10^3/ul

LIH (Hemolysis Severity - available on AU analysers) NINGLA_HEM_043_001 | v1.0

simpleParameter

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

Neutrophil differential count NINGLA_HEM_029_001 | v1.3

simpleParameter

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

Unit Measured: %

Monocyte differential count NINGLA_HEM_033_001 | v1.2

simpleParameter

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

Unit Measured: %

Monocyte cell count NINGLA_HEM_034_001 | v1.3

simpleParameter

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

Unit Measured: 10^3/ul

Lymphocyte cell count NINGLA_HEM_032_001 | v1.3

simpleParameter

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

Unit Measured: 10^3/ul

Sample clotted NINGLA_HEM_041_001 | v1.1

simpleParameter

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

Options: Yes, No,

Mean platelet volume NINGLA_HEM_019_001 | v1.2

simpleParameter

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

Unit Measured: fL

Blood collection tubes NINGLA_HEM_015_001 | v1.2

procedureMetadata

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

Options: Microvette 500 K3E, Kabe Labortechnik 1ml EDTA, Drummond EDTA Microcaps, Eppendorf 1.7ml, Kabe Labortechnik 200ul EDTA,

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Eosinophil cell count NINGLA_HEM_036_001 | v1.3

simpleParameter

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

Unit Measured: 10^3/ul

Eosinophil differential count NINGLA_HEM_035_001 | v1.2

simpleParameter

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

Unit Measured: %

Anesthesia used for blood collection NINGLA_HEM_012_001 | v1.0

procedureMetadata

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

Options:
Injection narcosis with Ketamine (100mg/kg)/Xylazine (10mg/kg)/Antipamezole (Antisedan, 1mg/kg),
No anesthesia, Gas anaesthesia with Isofluorane,
Injection narcosis with Ketamine (100mg/kg)/Xylazine (10mg/kg),
Injection narcosis with Ketamine (137mg/kg)/Xylazine (6.6mg/kg),
Injection narcosis with Tribromoethanol (Avertin),
Injection narcosis with Ketamine (110mg/kg)/Xylazine (11mg/kg),
Injection narcosis with Ketamine (110mg/kg)/Xylazine (11mg/kg)/ Antipamezole (Antisedan, 1mg/kg),

Equipment manufacturer NINGLA_HEM_010_001 | v1.0

procedureMetadata

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

Options: Sysmex Deutschland GmbH, Abbot Laboratories, Beckman Coulter, Scil animal care company Gmbh, Siemens Healthcare Diagnostics Ltd, Siemens Medical Solutions Diagnostics, Drew Scientific Instrument, IDEXX,

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Fight wounds NINGLA_HEM_028_001 | v1.0

procedureMetadata

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

Options: Yes, No,

Large Unstained Cell (LUC) count NINGLA_HEM_039_001 | v1.0

simpleParameter

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

Unit Measured: 10^3/ul

Anticoagulant NINGLA_HEM_014_001 | v1.1

procedureMetadata

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

Options: K(1)-EDTA, K(3)-EDTA, EDTA, K(2)-EDTA, No,

Chip card number NINGLA_HEM_023_001 | v1.1

procedureMetadata

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

Options: Mouse Card (E0510051710), No chip card (Advia analyser), No chip card, Mouse Card (E0401091230), C57/BL6 chip card,

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Lymphocyte differential count NINGLA_HEM_031_001 | v1.2

simpleParameter

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

Unit Measured: %

Red blood cell distribution width NINGLA_HEM_027_001 | v1.2

simpleParameter

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

Unit Measured: %