Hematology HAS_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 mutant animals: 7 mice for each sex.

Age of animals: 16 weeks (fixed).

Sexual dimorphism: yes for some of the parameters.

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). 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. 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 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. 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**
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. 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, time
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

White blood cell count HAS_HEM_001_001 | v1.0

simpleParameter

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

Unit Measured: 10^3/ul

Description: white_blood_cell_count

Red blood cell count HAS_HEM_002_001 | v1.0

simpleParameter

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

Unit Measured: 10^6/ul

Description: red_blood_cell_count

Hemoglobin HAS_HEM_003_001 | v1.0

simpleParameter

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

Unit Measured: g/dl

Description: hemoglobin

Hematocrit HAS_HEM_004_001 | v1.0

simpleParameter

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

Unit Measured: %

Description: hematocrit

Mean cell volume HAS_HEM_005_001 | v1.0

simpleParameter

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

Unit Measured: fL

Description: mean_cell_volume

Mean corpuscular hemoglobin HAS_HEM_006_001 | v1.0

simpleParameter

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

Unit Measured: pg

Description: mean_corpuscular_hemoglobin

Mean cell hemoglobin concentration HAS_HEM_007_001 | v1.0

simpleParameter

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

Unit Measured: g/dl

Description: mean_cell_hemoglobin_concentration

Platelets count HAS_HEM_008_001 | v1.0

simpleParameter

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

Unit Measured: 10^3/ul

Description: platelets_count

Equipment ID HAS_HEM_009_001 | v1.0

procedureMetadata

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

Description: equipment_name

Equipment manufacturer HAS_HEM_010_001 | v1.0

procedureMetadata

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

Description: equipment_manufacturer

Options: Siemens, Scil animal care company Gmbh, Drew Scientific Instrument, Beckmann Coulter,

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Equipment model HAS_HEM_011_001 | v1.0

procedureMetadata

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

Description: equipment_model

Options: Advia 120, Advia 2120, Scil Vet abc, Hemavet 950 FS, Ac-T diff Analyzer,

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Anesthesia used for blood collection HAS_HEM_012_001 | v1.0

procedureMetadata

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

Description: anesthesia_used_for_blood_collection

Options: Gas anaesthesia with Isofluorane,
Injection narcosis with Ketamine (100mg/kg)/Xylazine (10mg/kg),
Injection narcosis with Ketamine(100mg/kg)/ Xylazine (10mg/kg)/Antipamezole (Antisedan, 1mg/kg),
Injection narcosis with Ketamine (110mg/kg)/Xylazine (11mg/kg),
Injection narcosis with Ketamine (110mg/kg)/Xylazine (11mg/kg)/ Antipamezole (Antisedan, 1mg/kg),

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Method of blood collection HAS_HEM_013_001 | v1.0

procedureMetadata

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

Description: method_of_blood_collection

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

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Anticoagulant HAS_HEM_014_001 | v1.0

procedureMetadata

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

Description: anticoagulant

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

Samples kept on ice between collection and analysis? HAS_HEM_015_001 | v1.0

procedureMetadata

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

Description: samples_kept_on_ice_between_collection_and_analysis_

Options: Yes, No,

ID for blood collection SOP HAS_HEM_016_001 | v1.0

procedureMetadata

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

Description: id_for_blood_collection_sop

Storage temperature from blood collection till measurement HAS_HEM_017_001 | v1.0

procedureMetadata

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

Unit Measured: C

Date and time of blood collection HAS_HEM_018_001 | v1.0

procedureMetadata

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

Description: date_and_time_of_blood_collection

Date of measurement HAS_HEM_019_001 | v1.0

procedureMetadata

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

Description: date_of_measurement

Chip card number HAS_HEM_020_001 | v1.0

procedureMetadata

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

Description: chip_card_number

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

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Blood collection experimenter ID HAS_HEM_021_001 | v1.0

procedureMetadata

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

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Date equipment last calibrated HAS_HEM_022_001 | v1.0

procedureMetadata

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

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Blood collection tubes HAS_HEM_023_001 | v1.0

procedureMetadata

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

Options: Sarstedt Li-Heparin gel tubes, Kabe Labortechnik Lithium heparin coated tubes,

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Date and time of sacrifice HAS_HEM_024_001 | v1.2

procedureMetadata

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

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Blood analysis experimenter ID HAS_HEM_025_001 | v1.0

procedureMetadata

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

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Barcode HAS_HEM_026_001 | v1.0

simpleParameter

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

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Procedural comments HAS_HEM_027_001 | v1.0

simpleParameter

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

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