

# 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. Isoflurane.
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<sup>3</sup>/ul

**Description:** white\_blood\_cell\_count

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# Red blood cell count HAS\_HEM\_002\_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: 10<sup>6</sup>/ul

Description: red\_blood\_cell\_count

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# Hemoglobin HAS\_HEM\_003\_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: g/dl

Description: hemoglobin

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# Hematocrit HAS\_HEM\_004\_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

Description: hematocrit

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**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

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**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

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**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

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## Platelets count HAS\_HEM\_008\_001 | v1.0

simpleParameter

**Req. Analysis:** false

**Req. Upload:** true

**Is Annotated:** true

**Unit Measured:** 10<sup>3</sup>/ul

**Description:** platelets\_count

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## Equipment ID HAS\_HEM\_009\_001 | v1.0

procedureMetadata

**Req. Analysis:** false

**Req. Upload:** true

**Is Annotated:** false

**Description:** equipment\_name

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## 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 Isoflurane,  
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,

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## 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,

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## 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

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## 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

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## 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

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## Date of measurement HAS\_HEM\_019\_001 | v1.0

procedureMetadata

**Req. Analysis:** true

**Req. Upload:** true

**Is Annotated:** false

**Description:** date\_of\_measurement

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## 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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