

# Hematology IMPC\_HEM\_001

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## 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.

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
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.
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). E.g. 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

	Version	Type	Req. Upload	Req. Analysis	Annotation	Increment	Option	Ontology Options	Derived	Unit	Data Type
<a href="#">White blood cell count</a> <a href="#">IMPC_HEM_001_001</a>	1.3	simpleParameter	✓		✓					10 <sup>3</sup> /ul	FLOAT
<a href="#">Red blood cell count</a> <a href="#">IMPC_HEM_002_001</a>	1.3	simpleParameter	✓		✓					10 <sup>6</sup> /ul	FLOAT
<a href="#">Hemoglobin</a> <a href="#">IMPC_HEM_003_001</a>	1.2	simpleParameter	✓		✓					g/dl	FLOAT
<a href="#">Hematocrit</a> <a href="#">IMPC_HEM_004_001</a>	1.0	simpleParameter	✓		✓					%	FLOAT
<a href="#">Mean cell volume</a> <a href="#">IMPC_HEM_005_001</a>	1.2	simpleParameter	✓		✓					fl	FLOAT
<a href="#">Mean corpuscular hemoglobin</a> <a href="#">IMPC_HEM_006_001</a>	1.1	simpleParameter	✓		✓					pg	FLOAT
<a href="#">Mean cell hemoglobin concentration</a> <a href="#">IMPC_HEM_007_001</a>	1.2	simpleParameter	✓		✓					g/dl	FLOAT
<a href="#">Platelet count</a> <a href="#">IMPC_HEM_008_001</a>	1.3	simpleParameter	✓		✓					10 <sup>3</sup> /ul	FLOAT

	Version	Type	Req. Upload	Req. Analysis	Annotation	Increment	Option	Ontology Options	Derived	Unit	Data Type
Mean platelet volume IMPC_HEM_019_001	1.2	simpleParameter			✓					fl	FLOAT
Red blood cell distribution width IMPC_HEM_027_001	1.2	simpleParameter			✓					%	FLOAT

## Metadata

	Version	Type	Req. Upload	Req. Analysis	Annotation	Increment	Option	Ontology Options	Derived	Unit	Data Type
Equipment ID IMPC_HEM_009_001	1.1	procedureMetadata	✓	✓							TEXT
Equipment manufacturer IMPC_HEM_010_001	1.0	procedureMetadata	✓	✓			Scil animal care company Gmbh Drew Scientific Instrument Beckman Coulter Siemens Medical Solutions Diagnostics Siemens Healthcare Diagnostics Ltd Sysmex Deutschland GmbH Abbot Laboratories				TEXT
Equipment model IMPC_HEM_011_001	1.0	procedureMetadata	✓	✓			Advia 120 Advia 2120 Scil Vet abc Hemavet 950 FS Ac-T diff Analyzer XT-2000iV CELL-DYN 3700 Scil Vet abc Plus+				TEXT
Anesthesia used for blood collection IMPC_HEM_012_001	1.0	procedureMetadata	✓	✓			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) Injection narcosis with Ketamine (137mg/kg)/Xylazine (6.6mg/kg) No anaesthesia Injection narcosis with Tribromoethanol (Avertin)				TEXT
Method of blood collection IMPC_HEM_013_001	1.0	procedureMetadata	✓	✓			Cardiac puncture Retro-orbital puncture Tail vein Saphenous vein				TEXT
Anticoagulant IMPC_HEM_014_001	1.1	procedureMetadata	✓				EDTA K(1)-EDTA K(2)-EDTA K(3)-EDTA No				TEXT
Blood collection tubes IMPC_HEM_015_001	1.2	procedureMetadata					Kabe Labortechnik 200ul EDTA Kabe Labortechnik 1ml EDTA Drummond EDTA Microcaps Microvette 500 K3E Eppendorf 1.7ml				TEXT
Date and time of sacrifice IMPC_HEM_016_001	1.3	procedureMetadata	✓								DATETIME
Blood analysis experimenter ID IMPC_HEM_017_001	1.0	procedureMetadata	✓								TEXT
ID for blood collection SOP IMPC_HEM_020_001	1.1	procedureMetadata	✓				ESLIM_024_001 sop.inv.019 RIKENMPP_003a_003 PHENO_CBC sop.inv.063				TEXT
Samples kept on ice between collection and analysis IMPC_HEM_018_001	1.2	procedureMetadata	✓	✓			Yes No				TEXT

	Version	Type	Req. Upload	Req. Analysis	Annotation	Increment	Option	Ontology Options	Derived	Unit	Data Type
Date and time of blood collection IMPC_HEM_021_001	1.2	procedureMetadata	✓								DATETIME
Date of measurement IMPC_HEM_022_001	1.2	procedureMetadata	✓								DATE
Chip card number IMPC_HEM_023_001	1.1	procedureMetadata		✓			C57/BL6 chip card Mouse Card (E0510051710) Mouse Card (E0401091230) No chip card No chip card (Advia analyser)				TEXT
Blood collection experimenter ID IMPC_HEM_024_001	1.1	procedureMetadata	✓								TEXT
Date equipment last calibrated IMPC_HEM_025_001	1.2	procedureMetadata									DATE
Storage temperature from blood collection until measurement IMPC_HEM_026_001	1.3	procedureMetadata	✓	✓			4 18-22 22 25 23			C	TEXT