

# Hematology HRWLLA\_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). 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 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   |
|--------------|---|
| 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   |
|  | 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 |

|                                  |  |
|----------------------------------|--|
| Chip card                        | <p>categorization and therefore there are different chip cards for different species (mouse strains). Eg. C57BL/6 chip card.</p> <p>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.</p> |
| 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

### Eosinophil cell count HRWLLA\_HEM\_036\_001 | v1.3

simpleParameter

**Req. Analysis:** false

**Req. Upload:** false

**Is Annotated:** true

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

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## Mean cell hemoglobin concentration HRWLLA\_HEM\_007\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: g/dl

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## Neutrophil differential count HRWLLA\_HEM\_029\_001 | v1.3

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

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## Service-related calibration start date HRWLLA\_HEM\_042\_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: false

Is Annotated: false

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## Chip card number HRWLLA\_HEM\_023\_001 | v1.1

procedureMetadata

Req. Analysis: true

Req. Upload: false

Is Annotated: false

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

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## **Equipment ID** HRWLLA\_HEM\_009\_001 | v1.1

procedureMetadata

**Req. Analysis:** true

**Req. Upload:** true

**Is Annotated:** false

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## **Fight wounds** HRWLLA\_HEM\_028\_001 | v1.0

procedureMetadata

**Req. Analysis:** false

**Req. Upload:** false

**Is Annotated:** false

**Options:** Yes, No,

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## **Equipment model** HRWLLA\_HEM\_011\_001 | v1.0

procedureMetadata

**Req. Analysis:** true

**Req. Upload:** true

**Is Annotated:** false

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

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## Hemoglobin HRWLLA\_HEM\_003\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: g/dl

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## Lymphocyte cell count HRWLLA\_HEM\_032\_001 | v1.3

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured:  $10^3/\text{ul}$

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## Samples kept on ice between collection and analysis HRWLLA\_HEM\_018\_001 | v1.2

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Options: No, Yes,

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## Date equipment last calibrated HRWLLA\_HEM\_025\_001 | v1.2

procedureMetadata

Req. Analysis: false

Req. Upload: false

Is Annotated: false

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## Monocyte cell count HRWLLA\_HEM\_034\_001 | v1.3

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured:  $10^3/\text{ul}$

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## Basophil cell count HRWLLA\_HEM\_037\_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured:  $10^3/\text{ul}$

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## Mean cell volume HRWLLA\_HEM\_005\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: fL

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## Eosinophil differential count HRWLLA\_HEM\_035\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

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## Mean corpuscular hemoglobin HRWLLA\_HEM\_006\_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: pg

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## Blood analysis experimenter ID HRWLLA\_HEM\_017\_001 | v1.0

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

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## ID for blood collection SOP HRWLLA\_HEM\_020\_001 | v1.1

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

Options: RIKENMPP\_003a\_003, sop.inv.063, PHENO\_CBC, sop.inv.019, ESLIM\_024\_001,

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## Method of blood collection HRWLLA\_HEM\_013\_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

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

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## Mean platelet volume HRWLLA\_HEM\_019\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: fL

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

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured: %

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## Anesthesia used for blood collection HRWLLA\_HEM\_012\_001 | v1.

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procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

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

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## Monocyte differential count HRWLLA\_HEM\_033\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

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## Basophil differential count HRWLLA\_HEM\_038\_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

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## Red blood cell count HRWLLA\_HEM\_002\_001 | v1.3

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured:  $10^6/\text{ul}$

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## Date and time of blood collection HRWLLA\_HEM\_021\_001 | v1.2

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

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## LIH (Hemolysis Severity - available on AU analysers) HRWL

LA\_HEM\_043\_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: false

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## Date and time of sacrifice HRWLLA\_HEM\_016\_001 | v1.3

procedureMetadata

Req. Analysis: false

Req. Upload: false

Is Annotated: false

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## Platelet count HRWLLA\_HEM\_008\_001 | v1.3

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured:  $10^3/\text{ul}$

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## Sample clotted HRWLLA\_HEM\_041\_001 | v1.1

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: false

Options: No, Yes,

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## Large Unstained Cell (LUC) count HRWLLA\_HEM\_039\_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured:  $10^3/\text{ul}$

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## Blood collection experimenter ID HRWLLA\_HEM\_024\_001 | v1.1

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

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## Anticoagulant HRWLLA\_HEM\_014\_001 | v1.1

procedureMetadata

Req. Analysis: false

Req. Upload: true

Is Annotated: false

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

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## Blood collection tubes HRWLLA\_HEM\_015\_001 | v1.2

procedureMetadata

Req. Analysis: false

Req. Upload: false

Is Annotated: false

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

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## Neutrophil cell count HRWLLA\_HEM\_030\_001 | v1.3

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

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

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## Storage temperature from blood collection until measurement

HRWLLA\_HEM\_026\_001 | v1.3

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

Unit Measured: C

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

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## Red blood cell distribution width

HRWLLA\_HEM\_027\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

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## Large Unstained Cell (LUC) differential count

HRWLLA\_HEM\_040\_001 | v1.0

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

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## White blood cell count HRWLLA\_HEM\_001\_001 | v1.3

simpleParameter

Req. Analysis: false

Req. Upload: true

Is Annotated: true

Unit Measured:  $10^3/\text{ul}$

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## Lymphocyte differential count HRWLLA\_HEM\_031\_001 | v1.2

simpleParameter

Req. Analysis: false

Req. Upload: false

Is Annotated: true

Unit Measured: %

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## Equipment manufacturer HRWLLA\_HEM\_010\_001 | v1.0

procedureMetadata

Req. Analysis: true

Req. Upload: true

Is Annotated: false

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

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