\*Title: **Hematology**

\*Centre: IMPC

\*Date\_modified: 12-03-2012

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\*Version: 1

{Sections:}

## \*1. 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.

## \*2. 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.

## \*3. 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

## \*4. Procedure

1. Set up the hematological analyser and perform QC analyses of the control reagents in accordance with the guidelines provided by the manufacturer.
2. **Sample collection and preparation:**
3. 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.
4. 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.
5. 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.

1. **Analysis:**
2. Perform hematological assessment of each sample including: white and red blood cell counts, hemoglobin and platelets in accordance with the analyser being used.
3. 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.

## \*5. 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:
4. assessment of control samples with different levels of hematology phenotypes (abnormally low; normal; abnormally high).
5. analysis of the graphical reports generated for each control level to ensure that they lie within their respective ranges.

## \*6 . Measured Parameters - list

{Placed in Parameters spreadsheet}

## \*7. MetaData Parameters - list

|  |  |  |  |
| --- | --- | --- | --- |
| **Metadata** | **Example** | **Required for data upload** | **Required for data analysis** |
| Equipment ID |  | YES | NO |
| Equipment manufacturer | SIEMENS | YES | YES |
| Equipment model | ADVIA120 | YES | YES |
| Date equipment last calibrated |  | NO | NO |
| Method of blood collection | retro-orbital puncture | YES | YES |
| Anesthesia used for blood collection | Isofluorane | YES | YES |
| Anticoagulant | EDTA | YES | YES |
| Samples kept on ice between collection and analysis? | Yes | YES | YES |
| Time and storage temperature from blood collection till measurements | 1 day, 2°C | YES | YES |
| Date and time of blood collection | Year, month, day, time | YES | YES |
| Date of measurement | Year, month, day, time | YES | YES |
| ID for blood collection SOP | ESLIM\_024\_001 | YES | YES |
| Chip card | Not used | NO | NO |