Standard Operating Procedure	Title: Analysis of Haematology samples	
	Doc. Number: ESLIM_016_001	Date Issued: 01/06/09

1. Purpose:

For the determination of blood cell counts (white blood cells, red blood cells, platelets), haemoglobin measurement and the calculation of haematological indexes (mean cell volume, mean corpuscular haemoglobin and mean cell haemoglobin concentration). Haematology measurements are obtained using either the Beckman Coulter AcT Diff or Siemens Advia 2120.

2. Associated Documents:

ESLIM_027_001_blood_ sample_ handling_haematology

Beckman Coulter A^{C}T Diff operator manual

Siemens Advia 2120 operator manual

3. Notes:

3.1. The validity of results obtained from metabolic studies is largely dependent on methods of animal husbandry. It is of vital importance that individuals following this procedure are experienced and aware of the animal's welfare, and are familiar with the animal being tested, in order to reduce the anxiety levels of the animal prior to testing.

3.2. The majority of mouse metabolic studies are age/sex/strain dependent. It is important to keep these parameters comparable throughout a single experiment.

3.3. It is recommended that all metabolic experimentation is conducted at approximately the same time of day because physiological and biochemical parameters change throughout the day.

3.4. All samples should be considered as potentially hazardous

4. Quality Control:

For the Beckman Coulter Act Diff see 4.1-4.5:

4.1. Each morning, all parameters are tested with blood "4C –ES Cell" control.

The 3 levels include:
Abnormal Low
Normal
Abnormal High

4.2. Controls are gently inverted eight times according to the manufacturer's instruction before use.

4.3. Control values must be within three standard deviations, otherwise the measurement has to be repeated.

4.4. Controls can be stored at +4°C.

4.5. Control:

All control data are managed using the Biorad Unity Plus software that provides graphical reports (Levey-Jennings graphs, Youden diagram, and monthly cumulative histograms).

For the Siemens Advia 2120 Analyzer see 4.6-4.9

4.6. Each morning, all parameters are tested with fixed Testpoint control blood samples.

The 3 levels include:
Abnormal Low
Normal
Abnormal High

4.7. Controls are stored at 2-8ºC and brought to room temperature on a roller mixer before use

4.8. Control values must be within the target range specified in the Advia 2120 software, otherwise the measurement has to be repeated.

4.9. All control data are managed using the Advia 2120 software that provides graphical reports.

5. Equipment:

5.1. A^{C}T diff (Beckman Coulter) or

5.2. Siemens Advia 2120 with multispecies software (Siemens Medical Solutions Diagnostics)

6. Supplies:

For the Beckman Coulter Act Diff see sections 6.1-6.2

6.1. Reagents:
A^{C}T Pak (ref 8448322 Beckman Coulter, France):
Reagent 1 = Diluent (balanced electrolyte solution)
Reagent 2 = Lytic reagent

6.2. Quality control:
Blood "4C –ES Cell" control (ref 7547114 Beckman Coulter) with 3 levels (abnormal low, normal and abnormal high).

For the Siemens Advia 2120 Analyzer see 6.3-6.4

6.3. Reagents:
Complete blood count timepac, Differential timepac, Perox sheeth solution, Universal rinse, Defoamer, Ez clean, Wash solution (Siemens Medical Solutions Diagnostics)

6.4. Quality control:
Testpoint controls (Siemens Medical Solutions Diagnostics) with 3 levels (Low, Normal and High).

7. Procedure:

Summary of protocol:

- **Sample collection and storage**
- **Sample preparation**
- **Analysis**

7.1. **Sample collection and storage**:

7.1.1. Collect samples according to the blood and sampling procedures (refer to ESLIM_027_001).

7.1.2. Samples should be analysed within 2 hours after collection.

7.1.3. Volume:

12µl for the Beckman Coulter

200µl for the Siemens Advia 2120

7.1.4. Refer to section 4 to perform QC check

7.2. **Sample preparation**:

Immediately following sample collection put the blood samples (EDTA Microvette tubes) on a rotary agitator.

7.3. **Analysis**:

To perform the analysis: follow either the Siemens Advia 2120 operator manual or the Beckman ACT diff operator manual (pages 3-2 to 3-8)

8. Parameters recorded:

The following parameters are required.

- White blood cell-count
- Red Blood Cell-count
- Haemoglobin
- Haematocrit
- Mean-cell-volume
- Mean-corpuscular-haemoglobin
- Mean-cell-haemoglobin-conc
- Platelets-count

9. Metadata recorded:

The following metadata is required.

- Equipment name (e.g. Haematology analyser)
- Equipment manufacturer (e.g. Siemens Medial Solutions Diagnostics)
- Equipment model (e.g. ADVIA 2120)
- Method of blood collection (e.g. retro-orbital)
- Date/Time of blood collection
- Anaesthesia used for blood collection (e.g. isoflourane)
- Day of measurement

The following metadata is optional.

- EMPReSSID for blood collection SOP
- Chip Card (Beckman analyser only)

10. Supporting information:

There is no supporting information available for this SOP

11. History Review:

There is no history review available for this SOP