	Title: Clinical chemistry (fasted) – pipeline 1	
Standard Operating Procedure	Doc. Number: ESLIM_021_001	Date Issued: 02/06/09

1. Purpose:

For the determination of biochemical parameters in plasma, total cholesterol, HDL cholesterol, non-HDL cholesterol, triglycerides, glucose, free fatty acids and glycerol using an Olympus AU400 analyser (Olympus Diagnostics).

2. Associated Documents:

ESLIM_024_001: Blood collection by retro-orbital puncture

ESLIM_025_001: Blood collection by tail venipuncture

ESLIM_026_001: Blood sample handling Clinical chemistry

ESLIM_015_001_Annex_1: Clinical chemistry reagents

ESLIM_015_001_Annex_2: Clinical chemistry calibrators

ESLIM_015_001_Annex_3: Clinical chemistry controls

Olympus AU400 analyser 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.

4. Quality Control:

4.1. Each morning, all parameters are tested with control sera (see ESLIM_015_001_Annex_3: Clinical chemistry controls). Some parameters are tested with control serum level 1 (Olympus System Reagent, ODC0003) and control serum level 2 (Olympus System Reagent, ODC0004), which consists of lyophilised human plasma with a normal and a pathological concentration. Other parameters are tested with specific controls from other suppliers.

4.2. Controls are thawed and vortexed before utilisation and loaded according to the analyser's display. Control values must lie within the acceptable range indicated by the manufacturer, otherwise the specific tests must be recalibrated and specific measurements repeated. Controls can be stored in 200µl aliquots at -20°C for up to 1 week.

5. Equipment:

5.1. Olympus AU400 analyser (Olympus Diagnostics)

5.2. Vortex

5.3. Refrigerated centrifuge

5.4. Eppendorf tubes

5.5. Pipettes (200-1000µl)

6. Supplies:

6.1. Deionised water

6.2. Reagents:

All reagents for Olympus AU400 from Olympus Diagnostics and other suppliers (see ESLIM_015_001_Annex_1: Clinical chemistry reagents

6.3. Calibrators:

All calibrators for Olympus AU400 from Olympus Diagnostics and other suppliers (see ESLIM_015_001_Annex_2: Clinical chemistry calibrators

6.4. Quality control:

All quality controls for Olympus AU400 from Olympus Diagnostics and other suppliers (see ESLIM_015_001_Annex_3: Clinical chemistry controls

7. Procedure:

Summary of protocol:

- Fasting
- Collection and storage
- Calibration
- Sample preparation
- Analysing results

7.1. **Fasting**

7.1.1. Fast animals overnight prior to blood sampling and record period of fasting (see metadata parameter in section 9).

7.2. **Collection and storage:**

7.2.1. Collect blood samples according to the blood sample collection and handling SOPs
(see
ESLIM_024_001 Blood collection retro-orbital puncture,
ESLIM_025_001 Blood collection tail venipuncture,
ESLIM_026_001 Blood sample handling clinical chemistry).

7.2.2. Keep whole blood samples on wet ice until centrifugation and then keep plasma samples on wet ice or in the fridge until analysis (allowing them to reach room temperature prior to analysis). Record whether samples are kept on ice between collection and analysis (see metadata parameter in Section 9).

7.2.3. Stability during storage varies between plasma parameters (see ESLIM_015_001_Annex_1: Clinical chemistry reagents. If analyses are not performed on the day of collection, store plasma samples at minus 20°C.

7.2.4. Volume required: 60-80µl.

7.2.5. Exclusion criteria: severe haemolysis.

7.3. **Calibration:**

7.3.1. Frequency of calibration varies between tests and depends on the workflow, (see operator manual and ESLIM_015_001_Annex_2: Clinical chemistry calibrators

7.3.2. Calibration is required when an existing calibration expires, when reagents are replaced and when control results fall outside specified acceptable ranges.

7.3.3. Most of the parameters are calibrated using the Olympus system calibrator. Parameters that cannot be calibrated with the Olympus system calibrator need additional calibration material - see ESLIM_015_001_Annex_2: Clinical chemistry calibrators

7.4. **Sample preparation:**

7.4.1. Prepare the plasma samples collected on the same day of the measurement (see section 4.0) or thaw frozen samples.

7.4.2. Use plasma samples undiluted or diluted to a ratio of 1:2 with deionised water if the volume is insufficient.

7.4.3. Vortex all plasma samples and briefly centrifuge them at ~5000 x g for 2 -3 minutes.

7.4.4. If necessary, remove fibrinogen clots using a wooden applicator.

7.4.5. Load the racks according to the work lists.

7.5. **Analysing results:**

7.5.1. Samples that produce results that lie outside the linear range for a specific assay have to be re-tested. In some cases it may be necessary to dilute samples with water to bring test results into range.

7.5.2. Validate the data.

7.5.3. Transfer the data to the database

8. Parameters recorded:

The following parameters are required.

- Glucose
- Total cholesterol
- Triglycerides
- Free fatty acids
- HDL-cholesterol

The following parameters are optional.

- LDL-cholesterol

9. Metadata recorded:

The following metadata is required.

• Equipment name	(e.g. Clinical chemistry analyzer)
• Equipment manufacturer	(e.g. Olympus Diagnostics)
• Equipment model	(e.g. AU400)
• Method of blood collection	(e.g. retro-orbital)
• Date/Time of blood collection	
• Fasting prior to experiment	should be yes
• Period of fasting	fasting will be entered as an approximate period, e.g. 14 hours
• Moved from cage for fasting	
• Plasma dilution	(e.g. neat)
• Sample Status	(e.g. fresh)
• Anaesthesia used for blood collection	(e.g. isofluorane)
• Samples kept on ice between collection and analysis	should be recorded as yes or no

The following metadata is optional.

- EMPReSSID for blood collection SOP
- Day of measurement

10. Supporting information:

There is no supporting information available for this SOP.

11. History Review:

There is no history review available for this SOP.