**Insulin Blood Level IMPC_INS_003**

**Purpose**

The insulin concentration in the blood is an important indicator of diabetes.

Ontological description: abnormal circulating insulin level [MP:0001560]; increased circulating insulin level [MP:0002079]; decreased circulating insulin level [MP:0002727].

**Experimental Design**

- **Minimum number of animals**: 7M + 7F
- **Age at test**: Week 16
- **Sex**: We would expect the results of this test to show sexual dimorphism

**Equipment**

1. ELISA plate reader / MSD Sector Imager
2. Vortex
3. Refrigerated centrifuge
4. Eppendorf tubes
5. Calibrated Pipettes

**Procedure**

1. Blood is collected by the relevant blood collection procedure (see IMPC protocol "Blood collection by retro-orbital puncture"). Blood is collected in lithium heparin tubes and the samples are kept on ice for a maximum of 2 hours prior to isolation of the plasma.
2. Blood samples are centrifuged at 5,000 x g for 10 minutes at 8°C and the plasma removed and aliquotted for analysis or for freezing (-70°C).
3. Plasma samples are subsequently defrosted and the required amount of sample is used to perform the analysis (e.g. by ELISA or MSD).

**Notes**

Blood collection for Insulin Blood Level is performed as a non-fasting, terminal procedure.

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. Plasma samples must be free of Fibrin clots in order to be analysed.
2. Badly hemolysed samples should not be included in the analysis.
<table>
<thead>
<tr>
<th>Metadata</th>
<th>Example</th>
<th>Required for data upload</th>
<th>Required for data analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of kit</td>
<td>The kit used for analysing the blood samples. E.g. Mouse Insulin kit</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Kit manufacturer</td>
<td>Manufacturer of the kit. E.g. MORINAGA (Yokohama, Japan)</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Kit lot number</td>
<td></td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Equipment ID</td>
<td>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.</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Equipment manufacturer</td>
<td>Manufacturer of the equipment. E.g. Thermo scientific.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Equipment model</td>
<td>Model of the equipment. E.g. Multiskan JX.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Blood collection tubes</td>
<td>The tubes used for blood collection. E.g. Sarstedt Li-Heparin gel tubes or Kabe Labortechnik Lithium heparin coated tubes.</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Anesthesia used for blood collection</td>
<td>The anesthetic used during blood collection. E.g. Isofluorane.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Method of blood collection</td>
<td>Concise description of the method used for blood collection. E.g. retro-orbital puncture.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Anticoagulant</td>
<td>Anticoagulant used for blood collection. E.g. Li-Heparin.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td></td>
<td>Time of day for collection is in the morning, starting no earlier than 07:30. E.g. Year, month, day, time.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>-------------------------</td>
<td>---------------------------------------------------------------------------------------------------------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>Date of measurement</td>
<td>The day of blood analysis. Year, month, day.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Sample status</td>
<td>Indicate if the sample were frozen (analysis on the same day of collection not possible) or fresh (analysis on the same day of collection). E.g. Fresh/Frozen.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Samples kept on ice between collection and analysis</td>
<td>Yes/No.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Plasma dilution</td>
<td>Dilution is highly discouraged but if necessary indicate here. E.g. &quot;No dilution&quot; or 1:2. Note that results submitted to DCC are assumed to be already corrected for any dilutions made.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Replicates</td>
<td>Please specify whether samples were measured once, in duplicate or in triplicate. E.g. 1 or 2 or 3.</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>ID of blood collection SOP</td>
<td>ID of the protocol followed for blood collection. Can be a centre specific protocol. E.g. ESLIM_024_001.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>Hemolysis status</td>
<td>The gauged degree of hemolysis. E.g. slight/moderate/marked.</td>
<td>NO</td>
<td>YES</td>
</tr>
<tr>
<td>Blood collection experimenter ID</td>
<td>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.</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>Blood analysis experimenter ID</td>
<td>procedure and for all procedures indicating the experimenter who analyzed the blood. E.g. Harw_001, or 1/2/3.</td>
<td>YES</td>
<td>NO</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>--------------------------------------------------------------------------------------------------</td>
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<td>----</td>
</tr>
<tr>
<td>Date equipment last calibrated</td>
<td>Most recent date in which the equipment (or any part of) used in the procedure was subject to a calibration event.</td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>

**Parameters and Metadata**

**Insulin** IMPC_INS_001_001 | v1.3

- **simpleParameter**
- **Req. Analysis:** false
- **Req. Upload:** true
- **Is Annotated:** true
- **Unit Measured:** pg/ml
- **Description:** insulin

**Type of kit** IMPC_INS_002_001 | v1.0

- **procedureMetadata**
- **Req. Analysis:** false
- **Req. Upload:** true
- **Is Annotated:** false
- **Description:** type_of_kit
- **Options:** Mouse Insulin kit, K152BZC, Ultrasensitive Mouse Insulin ELISA, MSD PANEL2, MSD PANEL3,
**Kit manufacturer** IMPC_INS_003_001 | v1.0

**Procedure Metadata**

- **Req. Analysis:** false
- **Req. Upload:** true
- **Is Annotated:** false

**Description:** kit_manufacturer

**Options:** MORINAGA (Yokohama, Japan), Meso Scale Discovery, Mercodia, MSD HMGU Custom,

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**Kit lot number** IMPC_INS_004_001 | v1.0

**Procedure Metadata**

- **Req. Analysis:** false
- **Req. Upload:** true
- **Is Annotated:** false

**Description:** kit_lot_number

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**Equipment ID** IMPC_INS_005_001 | v1.0

**Procedure Metadata**

- **Req. Analysis:** false
- **Req. Upload:** true
- **Is Annotated:** false

**Description:** equipment_id

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**Equipment manufacturer** IMPC_INS_006_001 | v1.0

**Procedure Metadata**

- **Req. Analysis:** true
- **Req. Upload:** true
- **Is Annotated:** false
Description: equipment_manufacturer

Options: Thermo scientific, Meso Scale Discovery, Tecan,

Equipment model IMPC_INS_007_001 | v1.0

procedureMetadata


Description: equipment_model

Options: Multiskan JX, SECTOR Imager 2400, SECTOR Imager 6000, Genios Pro, MESO QuickPlex SQ 120,

Blood collection tubes IMPC_INS_008_001 | v1.1

procedureMetadata


Description: blood_collection_tubes

Options: Sarstedt Li-Heparin gel tubes, Kabe Labortechnik Lithium heparin coated tubes, BD Microtainer silicone and micronized silica coated serum separator tubes,

Anesthesia used for blood collection IMPC_INS_009_001 | v1.0

procedureMetadata

Description: anesthesia_used_for_blood_collection

Options: Isofluorane, None,
Injection narcosis with Ketamine (100mg/kg)/Xylazine (10mg/kg),
Injection narcosis with Tribromoethanol (Avertin),
Injection narcosis with Ketamine (137mg/kg)/Xylazine (6.6mg/kg),

Method of blood collection IMPC_INS_010_001 | v1.0

Description: method_of_blood_collection

Options: Retro-orbital puncture, Cardiac puncture,

Anticoagulant IMPC_INS_011_001 | v1.1

Description: anticoagulant

Options: Li-Heparin, No,

Date and time of blood collection IMPC_INS_012_001 | v1.3
**Sample status**  IMPC_INS_014_001 | v1.1

Description: sample_status

Options: Fresh, Frozen,

**Samples kept on ice between collection and analysis**  IMP C_INS_015_001 | v1.0

Description: samples_kept_on_ice_between_collection_and_analysis

Options: Yes, No,

**Sample dilution**  IMPC_INS_016_001 | v1.2
Description: sample_dilution

Options: Neat plasma, 1:2, Neat serum,

Replicates IMPC_INS_017_001 | v1.0

procedureMetadata


Description: replicates

Options: 1, 2, 3,

ID of blood collection SOP IMPC_INS_018_001 | v1.1

procedureMetadata


Description: id_of_blood_collection_sop

Options: ESLIM_024_001, PHENO_CBC, sop.inv.019,

Hemolysis status IMPC_INS_019_001 | v1.1

procedureMetadata


Description: hemolysis_status
Options: Slight, Moderate, Marked,

Blood collection experimenter ID  IMPC_INS_020_001  |  v1.0

procedureMetadata


Description: blood_collection_experimenter_id

Blood analysis experimenter ID  IMPC_INS_021_001  |  v1.0

procedureMetadata


Description: blood_analysis_experimenter_id

Date equipment last calibrated  IMPC_INS_022_001  |  v1.1

procedureMetadata


Description: date_equipment_last_calibrated
Sample type: IMPC_INS_023_001 | v1.0

**Options:** Serum, Plasma,