**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 aliquoted 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.

### Example Metadata

<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><strong>Anticoagulant</strong></td>
<td>Anticoagulant used for blood collection. E.g. Li-Heparin.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------------------------------------------------</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td><strong>Date and time of blood collection</strong></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><strong>Date of measurement</strong></td>
<td>The day of blood analysis. Year, month, day.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Sample status</strong></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><strong>Samples kept on ice between collection and analysis</strong></td>
<td>Yes/No.</td>
<td>YES</td>
<td>YES</td>
</tr>
<tr>
<td><strong>Plasma dilution</strong></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><strong>Replicates</strong></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><strong>ID of blood collection SOP</strong></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><strong>Hemolysis status</strong></td>
<td>The gauged degree of hemolysis. E.g. slight/moderate/marked.</td>
<td>NO</td>
<td>YES</td>
</tr>
</tbody>
</table>

An ID of any format to be used coherently both inside the same
Blood collection experimenter ID | procedure and for all procedures indicating the experimenter who collected the blood. E.g. Harw_001, or 1/2/3. | YES | NO

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. | YES | NO

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. | NO | NO

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,

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**Kit manufacturer** IMPC_INS_003_001 | v1.0

**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

**Description**: kit_lot_number

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**Equipment ID** IMPC_INS_005_001 | v1.0
**Description:** equipment_id

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**Equipment manufacturer** IMPC_INS_006_001 | v1.0

*procedureMetadata*

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

**Description:** equipment_manufacturer

**Options:** Thermo scientific, Meso Scale Discovery, Tecan,

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**Equipment model** IMPC_INS_007_001 | v1.0

*procedureMetadata*

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

**Description:** equipment_model

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

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**Blood collection tubes** IMPC_INS_008_001 | v1.1

*procedureMetadata*

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

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

**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

**procedureMetadata**

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

**Description**: method_of_blood_collection

**Options**: Retro-orbital puncture, Cardiac puncture,

**Anticoagulant**  IMPC_INS_011_001 | v1.1
**procedureMetadata**

**Description**: anticoagulant

**Options**: Li-Heparin, No,

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**Date and time of blood collection** IMPC_INS_012_001 | v1.3

**procedureMetadata**

**Description**: date_and_time_of_blood_collection

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**Sample status** IMPC_INS_014_001 | v1.1

**procedureMetadata**

**Description**: sample_status

**Options**: Fresh, Frozen,

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**Samples kept on ice between collection and analysis** IMPC_INS_015_001 | v1.0
**samples_kept_on_ice_between_collection_and_analysis**

**Options:** Yes, No,

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**Sample dilution** IMPC_INS_016_001 | v1.2

**Description:** sample_dilution

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

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**Replicates** IMPC_INS_017_001 | v1.0

**Description:** replicates

**Options:** 1, 2, 3,
**ID of blood collection SOP**  IMPC_INS_018_001 | v1.1

**Description:** id_of_blood_collection_sop

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

**Hemolysis status**  IMPC_INS_019_001 | v1.1

**Description:** hemolysis_status

**Options:** Slight, Moderate, Marked,

**Blood collection experimenter ID**  IMPC_INS_020_001 | v1.0

**Description:** blood_collection_experimenter_id

**Blood analysis experimenter ID**  IMPC_INS_021_001 | v1.0
**blood_analysis_experimenter_id**

Description: blood_analysis_experimenter_id

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**Date equipment last calibrated** IMPC_INS_022_001 | v1.1

Description: date_equipment_last_calibrated

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**Sample type** IMPC_INS_023_001 | v1.0

Options: Serum, Plasma,