Insulin Blood Level KMPCLA_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 60

• 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

- Plasma samples must be free of Fibrin clots in order to be analysed.
 Badly hemolysed samples should not be included in the analysis.

Example Metadata

Metadata	Example	Required for data upload	Required for data analysis
Type of kit	The kit used for analysing the blood samples. E.g. Mouse Insulin kit	YES	NO
Kit manufacturer	Manufacturer of the kit. E.g. MORINAGA (Yokohama, Japan)	YES	NO
Kit lot number		YES	NO
Equipment ID	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.	YES	NO
Equipment manufacturer	Manufacturer of the equipment. E.g. Thermo scientific.	YES	YES
Equipment model	Model of the equipment. E.g. Multiskan JX.	YES	YES
Blood collection tubes	The tubes used for blood collection. E.g. Sarstedt Li-Heparin gel tubes or Kabe Labortechnik Lithium heparin coated tubes.	NO	YES
Anesthesia used for blood collection	The anesthetic used during blood collection. E. g. Isofluorane.	YES	YES
Method of blood collection	Concise description of the method used for blood collection. E.g. retro-orbital puncture.	YES	YES

Anticoagulant	Anticoagulant used for blood collection. E.g. Li-Heparin.	YES	YES
Date and time of blood collection	Time of day for collection is in the morning, starting no earlier than 07: 30. E.g. Year, month, day, time.	YES	YES
Date of measurement	The day of blood analysis. Year, month, day.	YES	YES
Sample status	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.	YES	YES
Samples kept on ice between collection and analysis	Yes/No.	YES	YES
Plasma dilution	Dilution is highly discouraged but if necessary indicate here. E.g. "No dilution" or 1:2. Note that results submitted to DCC are assumed to be already corrected for any dilutions made.	YES	YES
Replicates	Please specify whether samples were measured once, in duplicate or in triplicate. E.g. 1 or 2 or 3.	YES	NO
ID of blood collection SOP	ID of the protocol followed for blood collection. Can be a centre specific protocol. E.g. ESLIM_024_001.	YES	YES
Hemolysis status	The gauged degree of hemolysis. E. g. slight/moderate/marked.	NO	YES
	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

Hemolysis status KMPCLA_INS_019_001 | v1.1

procedureMetadata

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

Options: Moderate, Slight, Marked,

.....

Blood collection tubes KMPCLA_INS_008_001 | v1.1

procedureMetadata

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

Options: Sarstedt Li-Heparin (BD Microtainer silicone and mi		
Anticoagulant KMPC procedureMetadata	LA_INS_011_001 v1.1	
Req. Analysis: false	Req. Upload: true	Is Annotated: false
Options: No, Li-Heparin,		
Replicates KMPCLA_IN procedureMetadata	NS_017_001 v1.0	
Req. Analysis: false	Req. Upload: true	Is Annotated: false
Options: 1, 3, 2,		
Kit lot number KMPC procedureMetadata	CLA_INS_004_001 v1.0	
Req. Analysis: false	Req. Upload: true	Is Annotated: false

Blood analysis experimenter ID KMPCLA_INS_021_001 | v1.0

procedureMetadata

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

Sample type KMPCLA_INS_023_001 | v1.0

procedureMetadata

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

Options: Plasma, Serum,

Method of blood collection KMPCLA_INS_010_001 | v1.0

procedureMetadata

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

Options: Cardiac puncture, Retro-orbital puncture,

Type of kit KMPCLA_INS_002_001 | v1.0

procedureMetadata

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

Options: MSD PANEL2, Ultra MSD PANEL3, K152BZC,	asensitive Mouse Insulin ELISA	, Mouse Insulin kit,
Insulin KMPCLA_INS_00 simpleParameter	01_001 v1.3	
Req. Analysis: false	Req. Upload: true	Is Annotated: true
Unit Measured: pg/ml		
Sample dilution KM procedureMetadata	PCLA_INS_016_001 v1.2	
Req. Analysis: false	Req. Upload: true	Is Annotated: false
Options: Neat plasma, Neat s	serum, 1:2,	
Equipment ID KMPC	LA_INS_005_001 v1.0	
Req. Analysis: false	Req. Upload: true	Is Annotated: false

Sample status KMPCLA_INS_014_001 | v1.1

procedureMetadata

Req. Analysis: false **Req. Upload:** true Is Annotated: false Options: Frozen, Fresh, Anesthesia used for blood collection KMPCLA_INS_009_001 | v1.0 procedureMetadata Req. Analysis: true Req. Upload: true Is Annotated: false **Options:** None, Isofluorane, Injection narcosis with Tribromoethanol (Avertin), Injection narcosis with Ketamine (100mg/kg)/Xylazine (10mg/kg), Injection narcosis with Ketamine (137mg/kg)/Xylazine (6.6mg/kg), Blood collection experimenter ID KMPCLA_INS_020_001 | v1.0 procedureMetadata Req. Analysis: false Req. Upload: true Is Annotated: false

Date equipment last calibrated KMPCLA_INS_022_001 | v1.1

procedureMetadata

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

ID of blood collection SOP KMPCLA_INS_018_001 | v1.1 procedureMetadata Req. Analysis: false Req. Upload: true Is Annotated: false Options: ESLIM_024_001, PHENO_CBC, sop.inv.019, Equipment manufacturer KMPCLA_INS_006_001 | v1.0 procedureMetadata Req. Analysis: true Req. Upload: true Is Annotated: false Options: Tecan, Meso Scale Discovery, Thermo scientific, Kit manufacturer KMPCLA INS 003 001 | v1.0 procedureMetadata Req. Analysis: false Req. Upload: true Is Annotated: false Options: MORINAGA (Yokohama, Japan), MSD HMGU Custom, Meso Scale Discovery,

Mercodia,

Equipment model KMPCLA_INS_007_001 | v1.0

procedureMetadata

Req. Analysis: true Req. Upload: true Is Annotated: false Options: Genios Pro, SECTOR Imager 2400, SECTOR Imager 6000, Multiskan JX, MESO QuickPlex SQ 120, Date and time of blood collection KMPCLA INS 012 001 I v1.3 procedureMetadata Req. Analysis: false Req. Upload: true Is Annotated: false Samples kept on ice between collection and analysis KMPC LA_INS_015_001 | v1.0 procedureMetadata Req. Analysis: true Req. Upload: true Is Annotated: false Options: No, Yes,