Insulin and C-peptide analysis at Manchester University NHS Foundation Trust

Insulin

Indications	Investigation of hypoglycaemia Assessment of insulin resistance (fasting/glucose tolerance test)
Sample type	Lithium heparin plasma, plain serum, gel serum
Sample volume	100µL plasma/ serum
Stability	Whole blood: 4 hours at room temperature. Separate and freeze plasma/serum. Transport frozen. Plasma/ serum: 7 days at 2-8°C.
Turnaround time	14 days. Urgent samples within 2 working days by arrangement, if laboratory glucose collected at the same time is <3.0 mmol/L.
Haemolysis	Negative interference even at very low levels of haemolysis. This is due to release of insulinases from red blood cells which degrade insulin. The degradation is dependent on time, temperature and the haemoglobin concentration. If insulin ≥12 pmol/L, result reported as a comment: "Insulin *X* pmol/L, which is an underestimation due to the presence of haemolysis".
Methods	Direct sandwich ELISA: Mercodia Insulin ELISA. Typical analytical range: 18-1200 pmol/L. Results below analytical range reflexed to ultrasensitive assay. 120 minute post glucose tolerance test samples diluted 1 in 2. Mercodia Insulin ELISA Ultrasensitive. Typical analytical range: 0.9-120 pmol/L.
Method Cross-reactivity	Mercodia Insulin ELISA:

Substance	Cross-reaction	Concentration Tested
C-peptide	n.d	662-310000 pM
Proinsulin	n.d	220-111000 pM
Proinsulin des (31-32)	n.d	110-2202 pM
Proinsulin split (32-33)	n.d	126-2510 pM
Proinsulin des (64-65)	98%	37.5-149 pM
Proinsulin split (65-66)	56%	117-2333 pM
Insulin Aspart (Novorapid)	4%	30-90 mg/L
Insulin Detemir (Levemir)	n.d	3906-14200000 mg/L
Insulin Glargine (Lantus)	24%	2.5-20 mg/L
Insulin Glulisine (Apidra)	n.d	0.003-3490000 mg/L
Insulin Lispro (Humalog®)	n.d	3906-3500000 mg/L
IGF-1	n.d	7.8-1000 µg/L
IGF-2	n.d	31-1000 µg/L
Mouse insulin	n.d	0.20-6.5 µg/L
Rat insulin	0.70%	0.31-40 µg/L
Porcine insulin	101%	2.3-173 mU/L

Ovine insulin	65%	0.1-2.5 µg/L
Bovine insulin	38%	0.25-6.0 µg/L

Mercodia Insulin ELISA Ultrasensitive:

Substance	**Cross-reaction**
C-peptide	<0.01%
Proinsulin	<0.01%
Proinsulin des (31-32)	<0.5%
Proinsulin split (32-33)	<0.5%
Proinsulin des (64-65)	98%
Proinsulin split (65-66)	56%
Insulin Aspart (Novorapid)	3%
Insulin Glargine (Lantus)	12%
Insulin Detemir (Levemir)	n.d
Insulin Lispro (Humalog®)	n.d
Insulin Glulisine (Apidra)	n.d
IGF-1	<0.02%
IGF-2	<0.02%
Rat insulin	0.7%
Mouse insulin	0.3%
Porcine insulin	93%
Ovine insulin	48%
Bovine insulin	31%

n.d=not detected

Telephone number for discussion of results/ requests for urgent analysis	0161 701 2265
Reference Ranges	Fasting adult insulin reference range 12-150 pmol/L Pre-pubertal insulin reference range <60 pmol/L Glucose tolerance test – interpretative comments Hypoglycaemia - interpretative comments
Vetting	Internal requests on adult patients undergoing prolonged fasts/ investigation of hypoglycaemia are analysed provided there is a lab glucose of <3 mmol/L collected at the same time as the insulin sample.
Advantages of method	Well-suited to analysis in neonates as assay volume small (25µL, no dead volume), no cross-reactivity with proinsulin and low limit of quantification. The ultrasensitive assay allows measurement of low but inappropriate insulin concentrations with high precision.

C-peptide

Indications	Investigation of hypoglycaemia Investigation of diabetes type/ endogenous insulin deficiency
Sample type	Lithium heparin plasma, plain serum, gel serum
Sample volume	100µL plasma/ serum
Stability	Whole blood: 24 hours at room temperature. Plasma/ serum: stable for 24 hours at room temperature, 7 days at 2-8°C.
Turnaround time	14 days. Urgent samples within 2 working days by arrangement, if laboratory glucose collected at the same time is <3.0 mmol/L.
Haemolysis	C-peptide is unaffected by haemolysis up to an approximate haemoglobin concentration of 750 mg/dL.
Method	Direct sandwich ELISA: Mercodia C-peptide ELISA Typical analytical range 93-4000pmol/L.
Method Cross-reactivity	See table below
Telephone number for discussion of results/ requests for urgent analysis	0161 701 2265
Reference range	Fasting C-peptide range 343-1803 pmol/L

Substance	Cross-reaction
Insulin	<0.0006%
Proinsulin	2%
Proinsulin des (31-32)	3%
Proinsulin split (32-33)	2%
Proinsulin des (64-65)	74%
Proinsulin split (65-66)	10%