Insulin (serum, plasma)

1 Name and description of analyte

1.1 Name of analyte
Insulin

1.2 Alternative names
None

1.3 NLMC code

1.4 Description of analyte
Insulin is a 53-amino acid peptide hormone produced by the β-cells of the pancreatic islets of Langerhans. The primary translation product of the gene is a larger peptide called preproinsulin; the N-terminal sequence is removed by proteases to form proinsulin from which a connecting peptide (C-peptide) is removed leaving insulin itself. This comprises two separate chains, the A-chain and the B-chain, linked by two disulphide bonds. Both proinsulin and insulin are secreted into the circulation but proinsulin has only approximately 1% of the biological activity of insulin.

1.5 Function of analyte
Insulin is the body's chief anabolic hormone. It is released after a meal in response to a rise in plasma [glucose]. Its principal action is to promote cellular uptake of glucose, fatty acids and amino acids and their conversion into complex carbohydrate, fat and protein in liver and muscle. It also inhibits glycogenolysis, gluconeogenesis and ketogenesis. Conversely, fasting suppresses insulin release to very low levels. Recombinant insulin, animal insulins or insulin analogues may be administered to patients with diabetes mellitus to control blood glucose concentrations.

2 Sample requirements and precautions

2.1 Medium in which measured
Serum or plasma

2.2 Precautions re sampling, handling etc.
[Insulin] may fall when whole blood samples are stored; it is therefore recommended that serum or plasma be separated within no more than 5 h. Separated serum samples may be stored in a refrigerator for up to 24 h. Once thawed, frozen samples should not be refrozen. Insulin measurements are most useful when performed in the fasting state. For the investigation of hypoglycaemia it is essential that glucose is measured simultaneously.

3 Summary of clinical uses and limitations of measurements

3.1 Uses
1. Identification of the cause of hypoglycaemia.
2. Localisation of an insulinoma.

3. Quantification of insulin resistance.

3.2 Limitations
Insulin resistance is present in many overweight or obese people, in whom [insulin] may be raised even during fasting.

4 Analytical considerations

4.1 Analytical methods
Insulin is usually measured by immunoassay. Many different immunoassays are available, including both manual assays and those performed on automated instruments.
Biological assay of insulin by measuring the effect on plasma [glucose] is now obsolete.

4.2 Reference method
Isotope dilution-liquid chromatography/tandem mass spectrometry.

4.3 Reference materials
WHO reference reagent: Insulin, human for immunoassay.
1st International Reference Preparation. NIBSC code: 66/304. One vial contains 3 International Units by definition.

4.4 Interfering substances
Exogenous insulins and insulin analogues (positive).
Anti-insulin antibodies (positive).

4.5 Sources of error
Haemolysis may result in breakdown of insulin in the sample.

5 Reference intervals and variance

5.1.1 Reference interval (adults)
Varies depending on assay; typically <10-20 mU/L (fasting)
5.1.2 Reference intervals (others)
No major differences, though tends to rise with age.
5.1.3 Extent of variation
5.1.3.1 Interindividual CV: 58.3%
5.1.3.2 Intraindividual CV: 21.1%
5.1.3.3 Index of individuality: 2.8
5.1.3.4 CV of method: typically ~ 5–10%
5.1.3.5 Critical difference: 60.1% (if assay CV 5%)
5.1.4 Sources of variation
Recent food intake (see 1.4), body mass index (see 3.2).

6 Clinical uses of measurement and interpretation of results

6.1 Uses and interpretation
1. Insulin is measured of the investigation of unexplained hypoglycaemia, which usually occurs in infants or young children but may also occur in adults. In a person with normal insulin sensitivity, plasma [insulin] should be totally suppressed in the presence of hypoglycaemia. If

this is not observed, it suggests that insulin is responsible for the hypoglycaemia.

The source of the insulin may be identified by simultaneous measurement of C-peptide: endogenous hyperinsulinism is accompanied by a raised [C-peptide] whereas exogenous insulins do not contain C-peptide.

2. Insulin resistance is best measured by a using a complex euglycaemic clamp technique requiring multiple measurements of both insulin and glucose. However, simpler indices of insulin resistance can be calculated from single fasting insulin and glucose measurements (see section 9.1).

6.2. Confounding factors

Taking blood for insulin measurement during or after treatment for hypoglycaemia rather than while hypoglycaemia is still present can cause problems in interpretation, since an increase in blood [glucose] stimulates insulin secretion.

Inter-assay differences. Insulin assays are poorly standardised, such that different assays may give significantly different results on the same sample. It is therefore advisable to use a single assay and assay-specific reference limits when testing patient samples.

7 Causes of abnormal results

7.1 High values

7.1.1 Causes

1. Hyperinsulinism causing hypoglycaemia may be seen in:
- infants of diabetic mothers
- persistent hyperinsulinaemic hypoglycaemia of infancy
- Beckwith syndrome
- sulphonylurea or insulin overdose
- insulinoma
- the presence of autoantibodies to insulin or the insulin receptor.

2. Hyperinsulinism in the presence of normal or raised plasma [glucose] indicates insulin resistance and is a common finding in overweight people with or without type 2 diabetes.

7.1.2 Investigation

In hyperinsulinaemic hypoglycaemia, further investigations may include measurement of serum C-peptide and serum or urine sulphonyureas, and pancreatic imaging. Further investigation of insulin resistance is generally not required.

7.2 Low values

7.2.1 Causes

1. Low or undetectable [insulin] is an expected finding in the presence of hypoglycaemia or in the fasting state (unless the individual is insulin-resistant; see above).

2. Type 1 diabetes and advanced type 2 diabetes are characterised by insulin deficiency leading to hyperglycaemia. However, insulin measurement is not required to make these diagnoses.

7.2.2 Investigation

Not required.

8 Performance

8.1 Sensitivity, specificity etc. for individual conditions
Not applicable. Insulin measurement is not used alone as a diagnostic test, but must be interpreted together with blood/plasma [glucose] and clinical information.

9 Systematic reviews and guidelines

9.1 Systematic reviews
1. Grant CS. Insulinoma. *Best Pract Res Clin Gastroenterol* 2005;19:783-798. *This describes biochemical diagnosis and localisation of these rare tumours.*
2. Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations and appropriate usage. *Am J Physiol Endocrinol Metab* 2008;294:E15-26. *This review describes a range of strategies for assessing insulin sensitivity and resistance from simple fasting plasma measurements to sophisticated clamp techniques.*

9.2 Guidelines
1. Bowker R, Green A, Bonham JR. Guidelines for the investigation and management of a reduced level of consciousness in children: implications for clinical biochemistry laboratories. Ann Clin Biochem 2007;44:506-511. (Erratum in: *Ann Clin Biochem* 2008;45:227-228). *This guideline describes the appropriate investigation of a child with hypoglycaemia, among other conditions.)*
2. Cryer PE, Axelrod L, Grossmann AB *et al.* Evaluation and management of adult hypoglycaemic disorders: an Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab *2009;94:709-728. Authoritative guidance on the investigation of hypoglycaemia in adults.*

9.3 Recommendations
None

10 Links

10.1 Related analytes
Glucose, C-peptide, sulphonylureas

10.2 Related tests
Not applicable

Author Brona Roberts