Hormones related to Glucose Metabolism

(Clinical Utility)

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Clinical Utility of Insulin Assay

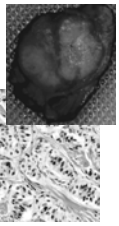
- Evaluation of fasting hypoglycemia
- Evaluation of the poly cystic ovary syndrome
- Classification of diabetes mellitus
- Predict diabetes mellitus
- Assessment of β-cell activity
- Select optimal therapy for diabetes
- Investigation of insulin resistance
- Predict the development of coronary artery disease

Insulinoma

Pancreatic and peripancreatic neuroendocrine tumors Neuroendocrine tumors:

- Insulinomas (55%)
- Gastrinomas (36%)
- VIPomas (vasoactive intestinal polypeptide tumor) (5%)
- Glucagonomas (3%).[1]

J Gastrointest Surg. Sep-Oct 1998;2(5):472-82



- About 85% of patients present with symptoms of hypoglycemia that include diplopia, blurred vision, palpitations, or weakness.
- Other symptoms include confusion, abnormal behavior, unconsciousness, or amnesia.
- About 12% of patients have grand mal seizures.

A study from Iran found 68 cases in a time span of 20 years in a university in Tehran

Larijani B, Aghakhani S, Lor SS, Farzaneh Z, Pajouhi M, Bastanhagh MH. Insulinoma in Iran: a 20-year review. *Ann Saudi Med*. Nov-Dec 2005;25(6):477-80

Insulinoma

- The calculation of ratios of insulin (µU/mL) to plasma glucose (mg/dL) is diagnostic.
- Healthy patients maintain a rate of less than 0.25.
- Obese patients may have a slightly higher rate.
- In patients with insulinoma, the ratio rises during fasting.

Whipple triad (75%)**:**

1. Presence of symptoms of hypoglycemia
2. Documented low blood sugar at the time symptoms are present
3. Reversal of symptoms by glucose administration.

In a study from the Netherlands, a positive Whipple triad on a prolonged fasting test, in combination with an insulin/C-peptide ratio < 1, had a sensitivity of 88.9% and a specificity of 100% for the diagnosis of insulinoma.

Neth J Med. Jul-Aug 2009;67(7):274-8

Insulin, Serum

Useful For

- Diagnosing insulinoma, when used in conjunction with proinsulin and C-peptide measurements
- Management of diabetes mellitus

Reference Values 2.6-24.9 µIU/mL

Method Name Electrochemiluminescence Sandwich Immunoassay

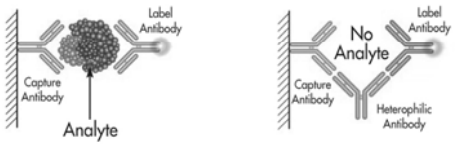
Interpretation

- In normal individuals, insulin levels parallel blood glucose levels.
- During prolonged fasting, when the patient's glucose level is reduced to <40 mg/dL, elevated insulin level plus elevated levels of proinsulin and C-peptide suggest insulinoma.
- Insulin levels generally decline in patients with type 1 diabetes mellitus.
- In the early stage of type 2 diabetes, insulin levels are either normal or elevated.
- In the late stage of type 2 diabetes, insulin levels decline.
- To compare insulin and C-peptide concentrations (ie, insulin to C-peptide ratio):
  -Convert insulin to pmol/L: insulin concentration in µIU/mL x 6.945 = insulin concentration in pmol/L.
  -Convert C-peptide to pmol/L: C-peptide concentration in ng/mL x 331 = C-peptide concentration in pmol/L.

Cautions

- Human anti-mouse antibodies (HAMA) may interfere with the assay.
- Patients on insulin therapy may develop anti-insulin antibodies. These antibodies may interfere in the assay system, causing inaccurate results. In such individuals, measurement of free insulin should be performed.
- This assay has 100% cross-reactivity with recombinant human insulin (Novolin R and Novolin N).
- It does not recognize other commonly used analogues of injectable insulin (ie, insulin lispro, insulin aspart, and insulin glargine).

Insulin Assay Interference
(Heterophilic Antibodies)



http://scantibodies.com/hbr.html

Insulin, Serum

Specimen Type
Serum

Specimen Required
Collection Container/Tube:
Preferred: Red top
Acceptable: Serum gel
Submission Container/Tube: Plastic vial
Specimen Volume: 1 mL
Collection Instructions:
1. Fasting. Non-fasting specimens are accepted for special studies.
2. Avoid hemolysis.
3. Label specimens with corresponding draw times.
Additional Information: If multiple specimens are drawn, send separate order for each specimen

Specimen Minimum Volume
0.5 mL

Reject Due To

Hemolysis	Mild reject; Gross reject
Lipemia	Mild OK; Gross OK
Icterus	NA
Other	Autopsy specimen

Specimen Stability Information

Specimen Type	Temperature	Time
Serum	Frozen (preferred)	180 days
	Refrigerated	7 days

Insulin, Free, Serum

Useful For
- the patient who has known insulin autoantibodies.

Reference Values 1.4-14.0 µIU/mL

Method Name Automated Chemiluminescent Immunoenzymatic Assay

Interpretation
- Insulin autoantibodies may develop in patients who have been injecting non human insulin for treatment of insulin-dependent diabetes.
- These antibodies will directly bind to insulin, making it unavailable for metabolic activity.
- The antibodies may also adversely affect the binding characteristics of insulin in immunoassays, making reliable quantitation difficult.

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Cautions.
No significant cautionary statements (PEG Percipitation)

Insulin, Free, Serum

Specimen Type
Serum

Specimen Required
Collection Container/Tube:
Preferred: Red top
Acceptable: Serum gel
Submission Container/Tube: Plastic vial
Specimen Volume: 1 mL
Collection Instructions:
1. Fasting. Non-fasting specimens are accepted for special studies.
2. Label specimens with corresponding draw time.
Additional Information: If multiple specimens are drawn, send separate order for each specimen.

Specimen Minimum Volume
0.4 mL

Reject Due To

Hemolysis	Mild reject; Gross reject
Lipemia	Mild OK; Gross OK
Icterus	Mild OK; Gross OK
Other	NA

Specimen Stability Information

Specimen Type	Temperature	Time
Serum	Frozen (preferred)	14 days
	Refrigerated	7 days

Insulin Antibodies (IgG, IgM), Serum

Useful For
- Predicting the future development of type 1 diabetes in asymptomatic children, adolescents, and young adults [1]
- Differential diagnosis of type 1 versus type 2 diabetes
- Evaluating diabetics with insulin resistance in patients with established diabetes (type 1 or type 2)
- Investigation of hypoglycemia in nondiabetic subjects

Reference Values < or =0.02 nmol/L (Reference values apply to all ages)
Method Name Radioimmunoassay (RIA)

Interpretation
- Seropositivity (> or =0.03 nmol/L) in a patient never treated with insulin is consistent with **predisposition** to type 1 diabetes [2]
- In patients presenting with **hypoglycemia**, the presence of insulin autoantibodies may indicate surreptitious insulin administration or, rarely, insulin autoantibody-related hypoglycemia [3].

Cautions:
This test should not be requested in patients who have recently received radioisotopes, therapeutically or diagnostically, because of potential assay interference

Insulin Antibodies (IgG, IgM), Serum

Specimen Type
Serum

Specimen Required
Container/Tube:
Preferred: Red top
Acceptable: Serum gel
Specimen Volume: 1 mL

Specimen Minimum Volume
0.3 mL

Reject Due To

Hemolysis	Mild OK; Gross reject
Lipemia	Mild OK; Gross reject
Icterus	Mild OK; Gross reject
Other	NA

Specimen Stability Information

Specimen Type	Temperature	Time
Serum	Refrigerated (preferred)	14 days
	Ambient	72 hours
	Frozen	

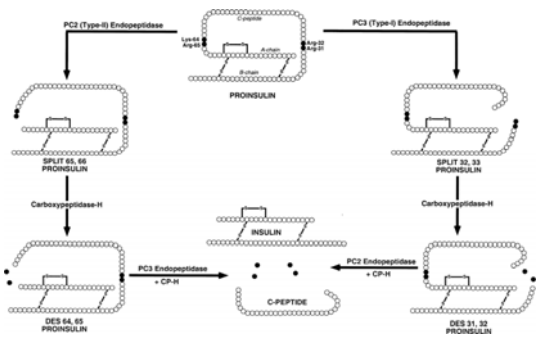
Clinical Utility of Proinsulin, C-Peptide, Glucagon Assay

Proinsulin
- Diagnosis of β-cell tumors
- Familial hyperproinsulinemia
- Cross-reactivity of insulin assays

C-Peptide
- Evaluation of fasting hypoglycemia
- β -cell tumors
- Factitious
- Classification of diabetes mellitus
- Assessment of β -cell activity
- Obtain insurance coverage for insulin pump
- Monitoring therapy
- Pancreatectomy
- Transplant (pancreas-islet cell)

Glucagon
- Diagnosis of α-cell tumors

Processing of Proinsulin



Proinsulin, Plasma

Useful For

- As part of the diagnostic workup of suspected **insulinoma**
- As part of the diagnostic workup of patients with suspected **PC1/3 deficiency**
- As part of the diagnostic workup of patients with **suspected proinsulin mutations**

Reference Values 3-20 pmol/L

Method Name Immunochemiluminescent Assay

Interpretation

- Normal individuals will have proinsulin concentrations below the upper limit of the normal fasting reference range (20 pmol/L) when hypoglycemic (blood glucose <45-60 mg/dL). Conversely, most (>80%) insulinoma patients will have proinsulin concentrations above the upper limit of the reference range [1]
- Patients with PC1/3 deficiency have low, or sometimes undetectable, insulin levels and substantially elevated proinsulin levels, exceeding the upper limit of the reference range substantially in the fasting state and rising even higher after food intake [3].

Cautions

To avoid misdiagnoses, all proinsulin measurements used in the diagnostic workup of patients with hypoglycemia must be interpreted in the context of coexisting illnesses, the blood glucose concentration at the time of sampling, and other test results (ie, insulin, C-peptide, beta-hydroxybutyrate, and sulfonylurea drug screen) [4].

Proinsulin, Plasma

Specimen Type
Plasma EDTA

Specimen Required
Collection Container/Tube: Ice-cooled, lavender top (EDTA)
Submission Container/Tube: Plastic vial
Specimen Volume: 1.5 mL
Collection Instructions:
1. Fasting
2. After draw, chill the whole blood on ice for at least 10 minutes, then spin down in a refrigerated centrifuge.

Specimen Minimum Volume
0.65 mL

Reject Due To

Hemolysis	Mild OK; Gross reject
Lipemia	Mild OK; Gross reject
Icterus	Mild OK; Gross OK
Other	NA

Specimen Stability Information

Specimen Type	Temperature	Time
Plasma EDTA	Frozen (preferred)	14 days
	Refrigerated	7 days

Clinical Utility of Proinsulin, C-Peptide, Glucagon Assay

Proinsulin
- Diagnosis of β-cell tumors
- Familial hyperproinsulinemia
- Cross-reactivity of insulin assays

C-Peptide
- Evaluation of fasting hypoglycemia
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Glucagon
- Diagnosis of α-cell tumors

C-Peptide, Serum

Useful For

1. Diagnostic work-up of hypoglycemia:
   - Diagnosis of factitious hypoglycemia due to surreptitious administration of insulin
   - Evaluation of possible insulinoma
   - Surrogate measure for the absence or presence of physiological suppressibility of endogenous insulin secretion during diagnostic insulin-induced hypoglycemia (C-peptide suppression test)
2. Assessing insulin secretory reserve in selected diabetic patients (as listed below) who either have insulin autoantibodies or who are receiving insulin therapy:
   - Assessing residual endogenous insulin secretory reserve
   - Monitoring pancreatic and islet cell transplant function
   - Monitoring immunomodulatory therapy aimed at slowing progression of preclinical, or very early stage type 1 diabetes mellitus

Reference 1.1-4.4 ng/mL

Method Name Electrochemiluminescence Immunoassay

Interpretation

- Factitious hypoglycemia due to surreptitious insulin administration results in elevated serum insulin levels and low or undetectable C-peptide levels, with a clear reversal of the[1] physiological molar insulin to C-peptide ratio (< or =1) to an insulin to C-peptide ratio of >1.
- In patients with insulin autoantibodies, the insulin to C-peptide ratio may be reversed to >1, because of the prolonged half-life of autoantibody-bound insulin.[3].

Cautions.

Hemolysed samples not accepted. significant (>20%) cross-reactivity between C-peptide Sand proinsulin. Hook effect ((>180 ng/mL). Heterophile antimouse antibodies (HAMA).

C-Peptide, Serum

Specimen Type

Serum

Specimen Required

Collection Container/Tube:
Preferred: Red top
Acceptable: Serum gel
Submission Container/Tube: Plastic vial
Specimen Volume: 0.5 mL
Collection Instructions: Fasting

Specimen Minimum Volume

0.4 mL

Reject Due To

Hemolysis	Mild OK; Gross reject
Lipemia	Mild OK; Gross OK
Icterus	NA
Other	Autopsy specimen

Specimen Stability Information

Specimen Type	Temperature	Time
Serum	Frozen (preferred)	30 days
	Refrigerated	7 days

Clinical Utility of Proinsulin, C-Peptide, Glucagon Assay

Proinsulin
- Diagnosis of β-cell tumors
- Familial hyperproinsulinemia
- Cross-reactivity of insulin assays

C-Peptide
- Evaluation of fasting hypoglycemia
- β -cell tumors
- Factitious
- Classification of diabetes mellitus
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- Monitoring therapy
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- Transplant (pancreas-islet cell)

Glucagon
- Diagnosis of α-cell tumors

Glucagonoma

4D syndrome consists of
1. diabetes,
2. dermatitis,
3. DVT (deep vein thrombosis),
4. depression

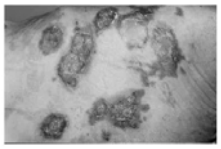
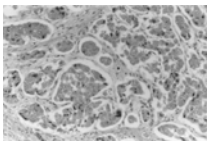


Fig. 2 - NME. Necrolytic migratory erythema.



Glucagon, Plasma

Useful For
- Diagnosis and follow-up of Glucagonomas and other glucagon-producing tumors
- Assessing diabetic patients with problematic hyper- or hypoglycemic episodes (extremely limited utility)
- Glucagon is routinely measured along with serum glucose, insulin, and C-peptide levels, during the mixed-meal test employed in the diagnostic workup of suspected postprandial hypoglycemia. However, it plays only a minor role in the interpretation of this test.

Reference Values
1-2 days: 70-450 pg/mL
2-4 days: 100-650 pg/mL
4-14 days: declining gradually to adult levels
>14 days: < or =80 pg/mL

Method Name Immunoassay Following Ethanol Extraction

Interpretation
- Glucagon is a single-chain polypeptide of 29 amino acids that is derived from a larger precursor peptide (big plasma glucagon), which is cleaved upon secretion.
- The main sites of glucagon production are the hypothalamus and pancreatic alpha-islet cells.
- Elevated glucagon levels in the absence of hypoglycemia may indicate the presence of a glucagon-secreting tumor.
- Inappropriate elevations in glucagon levels in hyperglycemic type I diabetic patients indicate that paradoxical glucagon release may contribute to disease severity.
- In diabetic patients, low glucagon levels (undetectable or in the lower quartile of the normal range) in the presence of hypoglycemia indicate impairment of hypoglycemic counter-regulation.

Cautions.
- Results obtained with different glucagon assays can differ substantially.
- All immunometric assays can, on rare occasions, be subject to hooking at extremely high analyte concentrations (false-low results), heterophilic antibody interference (false-high results), or autoantibody interference (unpredictable effects).

Glucagon, Plasma

Specimen Type
Plasma EDTA

Specimen Required
Collection Container/Tube: Lavender top (EDTA)
Submission Container/Tube: Plastic vial
Specimen Volume: 2 mL
Collection Instructions:
1. Fasting.
2. Prechill tube at 4 degrees C before drawing the specimen.
3. Draw the prechilled tube, and process as follows:
a. After drawing specimen, chill tube in wet ice for 10 minutes.
b. Centrifuge in a refrigerated centrifuge or in chilled centrifuge cup.
c. Immediately after centrifugation, remove plasma, place in a plastic transport vial (Supply T465), and freeze.

Specimen Minimum Volume
0.45 mL

Reject Due To

Hemolysis	Mild OK; Gross reject
Lipemia	Mild OK; Gross OK
Icterus	Mild OK; Gross OK
Other	NA

Gastric Inhibitory Polypeptide (GIP)

Clinical Information
- Gastric Inhibitory Peptide is a 43 amino acid peptide structurally related to Glucagon and Secretin and is found in the mucosa of upper intestine produced by K cells.
- GIP was originally detected as a factor inhibiting the secretion of gastric acid and Gastrin secretion.
- Its major action has now been determined to be a potent stimulant of B cells to release Insulin and is also known as Glucose-Dependent Insulinotropic Peptide.
- Exaggerated increases in GIP are noted after glucose administration to patients with Pancreatitis. This increase is also seen in patients with Diabetes Mellitus.
- GIP levels are decreased by Calcitonin.
- Elevated levels are present in cases of Verner-Morrison Syndrome (VIPoma).

Reference
Fasting: Up to 50 pg/ml
Postprandial: 110 - 720 pg/ml

Method Name Direct Radioimmunoassay (RIA)

Cautions.
No significant cautionary statements.

Gastric Inhibitory Polypeptide (GIP)

Specimen Type
GI Plasma

Specimen Required
Collect 10 mL of blood in special tube containing G.I. Preservative (MML supply number T125).
Specimen should be separated and 3 mL plasma frozen as soon as possible.
Patient preparation:
1. Patient should be fasting 10-12 hours prior to collection.
2. Antacid medication or medications that affect intestinal motility or Insulin secretion should be discontinued, if possible, for at least 48 hours prior to specimen collection.

Specimen Minimum Volume
1 mL

Reject Due To

Specimens other than	Plasma
Anticoagulants other than	Special GI preservative (MML supply T125)
Hemolysis	NA
Thawing	Warm reject; Cold reject
Lipemia	NA
Icteric	NA

Specimen Stability Information

Specimen Type	Temperature	Time
GI Plasma	Frozen	180 days


Thank you for your Attention
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