

Insulin is measured at the Clinical Chemistry Laboratory at Fletcher Allen Health Care, an affiliate of the University of Vermont. Insulin levels are determined using a microparticle Enzyme Immunoassay, the Immulite Insulin Reagent from Diagnostic Products Corporation (Los Angeles, CA). The reference range for this assay is 6-27 uIU/mL with a median of 11.9 uIU/mL. The CV is approximately 6%.

# IMMULITE<sup>®</sup> Insulin

## English

**Intended Use:** For *in vitro* diagnostic use with the IMMULITE Analyzer — for the quantitative measurement of insulin in, serum or heparinized plasma, for the management of diabetes.

Catalog Number: **LKIN1** (100 tests), **LKIN5** (500 tests)

Test Code: **INS** Color: **Orange**

## Summary and Explanation

Human insulin is a polypeptide hormone originating in the beta cells of the pancreas and serving as a principal regulator for the storage and production of carbohydrates. Its secretion is normally stimulated by increases in the amount of glucose in circulation. This leads to higher insulin levels and more rapid tissue-assimilation of glucose — followed by a decline in the insulin level as the glucose level subsides.

In a number of conditions, notably insulinoma and diabetes, this relationship is impaired. Insulin tends to circulate at inappropriately high levels in patients with insulin-secreting pancreatic tumors; such tumors can thus be a cause of hypoglycemia. Accordingly, insulin immunoassays — used sometimes in connection with provocative doses of tolbutamide or calcium — play an essential role in the identification (and localization) of insulinomas. The finding of fasting hypoglycemia in association with an *inappropriately high* serum insulin concentration is considered diagnostic.

Insulin levels do not figure in the subclassification of diabetes worked out by the National Diabetes Data Group. Nevertheless, when obtained in the course of a glucose tolerance test, they appear to be of some prognostic value in predicting the benefits of insulin therapy and the likelihood of progression to insulin-dependence and the complications (such as retinopathy) characteristic of diabetes.

The application of insulin immunoassay to patients already undergoing insulin therapy is complicated by the fact that such therapy typically leads to the formation of anti-insulin antibodies capable of interfering with the assay. Some investigators have sought therefore to measure insulin in urine, or in serum samples subjected to column chromatography or PEG precipitation. But the measurement of "free" insulin remains of limited interest as a technique for monitoring insulin therapy in the absence of statistics establishing therapeutic or toxic ranges. So far it appears that glucose control in diabetics cannot in general be achieved by normalizing the insulin profile. Nor is it known at what point abnormally high insulin levels become dangerous.

## Principle of the Procedure

Immunoassay.

**Incubation Cycles:** 1 × 60 minutes.

## Specimen Collection

**EDTA tubes** should *not* be used in the IMMULITE Insulin procedure.

In collecting samples for insulin determinations, it is important to avoid hemolysis, which can lead to spuriously low values.<sup>22,23</sup>

Centrifuging samples before a complete clot forms may result in the presence of fibrin. To prevent erroneous results due to the presence of fibrin, ensure that complete clot formation has taken place prior to centrifugation of samples. Some samples, particularly those from patients receiving anticoagulant therapy, may require increased clotting time.

**Volume Required:** 100  $\mu$ L serum or heparinized plasma. (Sample cup must contain at least 250  $\mu$ L more than the total volume required.)

**Storage:** 7 days at 2–8°C, or 3 months at –20°C.<sup>23</sup>

## Warnings and Precautions

For *in vitro* diagnostic use.

**Reagents:** Store at 2–8°C. Dispose of in accordance with applicable laws.

Follow universal precautions, and handle all components as if capable of transmitting infectious agents. Source materials derived from human blood were tested and found nonreactive for syphilis; for antibodies to HIV 1 and 2; for hepatitis B surface antigen; and for antibodies to hepatitis C.

Sodium azide, at concentrations less than 0.1 g/dL, has been added as a preservative. On disposal, flush with large volumes of water to prevent the buildup of potentially explosive metal azides in lead and copper plumbing.

**Chemiluminescent Substrate:** Avoid contamination and exposure to direct sunlight. (See insert.)

**Water:** Use distilled or deionized water.

## Materials Supplied

Components are a matched set. The barcode labels are needed for the assay.

### Insulin Test Units (LIN1)

Each barcode-labeled unit contains one bead coated with monoclonal murine anti-insulin. Stable at 2–8°C until expiration date.

**LKIN1:** 100 units. **LKIN5:** 500 units.

Allow the Test Unit bags to come to room temperature before opening. Open by cutting along the top edge, leaving the ziplock ridge intact. Reseal the bags to protect from moisture.

### Insulin Reagent Wedge (LIN2)

With barcode. 6.5 mL alkaline phosphatase (bovine calf intestine) conjugated to polyclonal chicken anti-insulin in buffer. Store capped and refrigerated: stable at 2–8°C until expiration date. Recommended usage is within 30 days after opening when stored as indicated.

**LKIN1:** 2 wedges. **LKIN5:** 10 wedges.

### Insulin Adjustors (LINL, LINH)

Two vials (Low and High) of lyophilized insulin in a nonhuman serum matrix. At least 30 minutes before use, reconstitute

each vial by adding 4.0 mL distilled or deionized water. Mix by *gentle swirling* or inversion. Aliquot and freeze: stable at –20°C for 60 days after reconstitution. **LKIN1:** 1 set. **LKIN5:** 2 sets.

### Insulin Controls (LINC1, LINC2)

Two vials of lyophilized insulin in a nonhuman serum matrix. Reconstitute each vial by adding 4.0 mL distilled or deionized water. Mix by *gentle swirling* or inversion. Aliquot and freeze: stable at –20°C for 60 days after reconstitution. Refer to Insulin Control insert for the respective concentrations in  $\mu$ IU/mL. **LKIN1:** 1 set. **LKIN5:** 2 sets.

## Kit Components Supplied Separately

### Insulin Sample Diluent (LINZ)

For the manual dilution of samples. One vial with 25 mL of ready-to-use, insulin-free nonhuman serum matrix. Stable at 2–8°C for 30 days after opening, or for 6 months (aliquotted) at –20°C.

**LSUBX:** Chemiluminescent Substrate

**LPWS2:** Probe Wash Module

**LKPM:** Probe Cleaning Kit

**LCHx-y:** Sample Cup Holders (barcoded)

**LSCP:** Sample Cups (disposable)

**LSCC:** Sample Cup Caps (optional)

Also Required

Sample transfer pipets, distilled or deionized water.

## Assay Procedure

See the IMMULITE Operator's Manual for: preparation, setup, dilutions, adjustment, assay and quality control procedures.

**Adjustment Interval:** 2 weeks.

**Quality Control Samples:** Use Insulin Controls supplied with the kit.

Each Sample cup holder can be followed by up to four test units.

## Expected Values

A study performed on 52 fasting, apparently healthy laboratory volunteers yielded a median of 11.9  $\mu$ IU/mL and a central 95% range of 6 – 27  $\mu$ IU/mL.

Consider these limits as *guidelines* only. Each laboratory should establish its own reference ranges.

## Limitations

Circulating anti-insulin antibodies are often found in patients who have been treated with nonhuman forms of insulin. If present, these antibodies may interfere with the assay.

For individuals who are significantly overweight, fasting insulin levels are typically somewhat higher than for adults of normal weight.

EDTA plasma has an effect on the measurement of insulin in the IMMULITE Insulin procedure.

Heterophilic antibodies in human serum can react with the immunoglobulins included in the assay components causing interference with *in vitro* immunoassays. [See Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988;34:27-33.] Samples from patients routinely exposed to animals or animal serum products can demonstrate this type of interference potentially causing an anomalous result. These reagents have been formulated to minimize the risk of interference; however, potential interactions between rare sera and test components can occur. For diagnostic purposes, the results obtained from this assay should always be used in combination with the clinical examination, patient medical history, and other findings.

## Performance Data

See Tables and Graphs for data representative of the assay's performance. Results are expressed in  $\mu\text{IU/mL}$ . (Unless otherwise noted, all were generated on serum samples collected in tubes without gel barriers or clot-promoting additives.)

**Calibration Range:** Up to 400  $\mu\text{IU/mL}$ .

**Analytical Sensitivity:** 2  $\mu\text{IU/mL}$ .

**High-dose Hook Effect:**

None up to 14,220  $\mu\text{IU/mL}$ .

**Precision:** Samples were assayed in duplicate over the course of 20 days, two runs per day, for a total of 40 runs and 80 replicates. (See "Precision" table.)

**Linearity:** Samples were assayed under various dilutions. (See "Linearity" table for representative data.)

**Recovery:** Samples spiked 1 to 19 with three insulin solutions (354, 712 and 1473  $\mu\text{IU/mL}$ ) were assayed. (See "Recovery" table for representative data.)

**Specificity:** The antibody is specific for insulin. (See "Specificity" table.) The assay reacts on an equimolar basis with porcine, bovine and human insulin.

**Bilirubin:** Severe icterus (bilirubin up to 200  $\text{mg/L}$ ) may cause a depression of values.

**Effect of Anticoagulants:** Blood was collected from 11 laboratory volunteers into plain, heparinized and EDTA vacutainer tubes. All samples were spiked with insulin and then assayed by the IMMULITE Insulin procedure, with the following results.

(Heparin) = 0.92 (Serum) + 2.9  $\mu\text{IU/mL}$   $r = 0.988$

(EDTA) = 0.41 (Serum) - 2.8  $\mu\text{IU/mL}$   $r = 0.983$

Means:

123  $\mu\text{IU/mL}$  (Serum)

116  $\mu\text{IU/mL}$  (Heparin)

48  $\mu\text{IU/mL}$  (EDTA)

These results show that EDTA interferes with the IMMULITE Insulin assay.

**Method Comparison:** The assay was compared to DPC's Coat-A-Count Insulin on 96 samples. (Concentration range: approximately 5.6 to 96  $\mu\text{IU/mL}$ . See graph.) By linear regression:

(IML) = 1.03 (CAC) - 0.31  $\mu\text{IU/mL}$

Means:

41.5  $\mu\text{IU/mL}$  (IMMULITE)

$r = 0.964$

40.6  $\mu\text{IU/mL}$  (CAC)

## References

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## Technical Assistance

In the United States, contact DPC's Technical Services department.

Tel: 800.372.1782 or 973.927.2828

Fax: 973.927.4101. Outside the United States, contact your National Distributor.

The Quality System of Diagnostic Products Corporation is registered to ISO 9001:1994.

## Tables and Graphs

### Precision ( $\mu\text{IU/mL}$ )

	Mean <sup>3</sup>	Within-Run <sup>1</sup>			Total <sup>2</sup>	
		SD <sup>4</sup>	CV <sup>5</sup>	SD	CV	
1	10.7	0.51	4.8%	0.62	5.8%	
2	16.1	0.69	4.3%	0.77	4.8%	
3	29.5	1.31	4.4%	1.73	5.9%	
4	41.0	2.21	5.4%	3.10	7.6%	
5	66.4	2.40	3.6%	2.91	4.4%	
6	177	6.81	3.8%	7.40	4.2%	
7	439	16.5	3.8%	21.0	4.8%	

### Linearity ( $\mu\text{IU/mL}$ )

	Dilution <sup>1</sup>	Observed <sup>2</sup>	Expected <sup>3</sup>	%O/E <sup>4</sup>
1	4 in 4 <sup>5</sup>	8.0	—	—
	2 in 4	4.7	4.0	118%
	1 in 4	2.4	2.0	120%
2	4 in 4	12.5	—	—
	2 in 4	6.9	6.3	110%
	1 in 4	3.5	3.1	113%
3	8 in 8	21	—	—
	4 in 8	11	10	110%
	2 in 8	5.2	5.1	102%
	1 in 8	3.2	2.5	123%
4	8 in 8	124	—	—
	4 in 8	69	62	111%
	2 in 8	30	31	97%
	1 in 8	14.1	15.5	91%
5	8 in 8	159	—	—
	4 in 8	89	80	111%
	2 in 8	44	40	110%
	1 in 8	19.4	19.9	97%
6	8 in 8	378	—	—
	4 in 8	197	189	104%
	2 in 8	95	95	100%
	1 in 8	43	47	91%

### Recovery (µIU/mL)

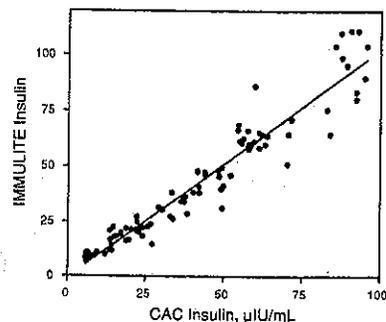
Solution <sup>1</sup>	Observed <sup>2</sup>	Expected <sup>3</sup>	%O/E <sup>4</sup>
1	8.0	—	—
A	27	25	108%
B	46	43	107%
C	82	81	101%
2	12.5	—	—
A	29	30	97%
B	48	47	102%
C	80	86	93%
3	21	—	—
A	38	38	100%
B	53	56	95%
C	100	94	106%
4	40	—	—
A	54	56	96%
B	69	74	93%
C	96	112	86%
5	124	—	—
A	146	136	107%
B	158	153	103%
C	193	191	101%
6	159	—	—
A	191	169	113%
B	205	187	110%
C	235	225	104%

### Specificity

Compound <sup>1</sup>	ng/mL Added <sup>2</sup>	% Cross-reactivity <sup>3</sup>
C-Peptide	12.6	ND
Glucagon	12.6	ND
Proinsulin	12.6	ND

ND: not detectable.<sup>4</sup>

### Method Comparison



**Deutsch. Precision:** <sup>1</sup>Intra-Assay, <sup>2</sup>Gesamt, <sup>3</sup>Mittelwert, <sup>4</sup>SD (Standardabweichung), <sup>5</sup>CV (Variationskoeffizient). **Linearity:** <sup>1</sup>Verdünnung, <sup>2</sup>Beobachtet (B), <sup>3</sup>Erwartet (E), <sup>4</sup>% B/E, <sup>5</sup>8 in 8. **Recovery:** <sup>1</sup>Probe, <sup>2</sup>Beobachtet (B), <sup>3</sup>Erwartet (E), <sup>4</sup>% B/E. **Specificity:** <sup>1</sup>Verbindung, <sup>2</sup>zugesetzte Menge, <sup>3</sup>% Kreuzreaktivität, <sup>4</sup>NN: Nicht nachweisbar. **Method Comparison:** Insulin: Insulin.

**Español. Precision:** <sup>1</sup>Intraensayo, <sup>2</sup>Total, <sup>3</sup>Media, <sup>4</sup>DS, <sup>5</sup>CV. **Linearity:** <sup>1</sup>Dilución, <sup>2</sup>Observado (O), <sup>3</sup>Esperado (E), <sup>4</sup>%O/E, <sup>5</sup>8 en 8. **Recovery:** <sup>1</sup>Solución, <sup>2</sup>Observado (O), <sup>3</sup>Esperado (E), <sup>4</sup>%O/E. **Specificity:** <sup>1</sup>Compuesto, <sup>2</sup>Cantidad añadida, <sup>3</sup>% Reacción cruzada, <sup>4</sup>ND: no detectable. **Method Comparison:** Insulin: Insulina.

**Français. Precision:** <sup>1</sup>Intraessai, <sup>2</sup>Total, <sup>3</sup>Moyenne, <sup>4</sup>SD, <sup>5</sup>CV. **Linearity:** <sup>1</sup>Dilution, <sup>2</sup>Observé (O), <sup>3</sup>Attendu (A), <sup>4</sup>%O/A, <sup>5</sup>8 dans 8. **Recovery:** <sup>1</sup>Solution, <sup>2</sup>Observé (O), <sup>3</sup>Attendu (A), <sup>4</sup>%O/A. **Specificity:** <sup>1</sup>Composé, <sup>2</sup>ajouté, <sup>3</sup>Réaction croisée %, <sup>4</sup>ND: non détectable. **Method Comparison:** Insulin: insuline.

**Italiano. Precision:** <sup>1</sup>Intra-serie, <sup>2</sup>Totale, <sup>3</sup>Media, <sup>4</sup>SD (Deviazione Standard), <sup>5</sup>CV (Coefficiente di Variazione). **Linearity:** <sup>1</sup>Diluzione, <sup>2</sup>Osservato (O), <sup>3</sup>Atteso (A), <sup>4</sup>%O/A, <sup>5</sup>8 in 8. **Recovery:** <sup>1</sup>Soluzione, <sup>2</sup>Osservato (O), <sup>3</sup>Atteso (A), <sup>4</sup>%O/A. **Specificity:** <sup>1</sup>Composto, <sup>2</sup>quantità aggiunta, <sup>3</sup>Percentuale di Crossreattività, <sup>4</sup>ND: non determinabile. **Method Comparison:** Insulin: Insulina.

**Português. Precision:** <sup>1</sup>Entre-ensaios, <sup>2</sup>Total, <sup>3</sup>Média, <sup>4</sup>Desvio padrão, <sup>5</sup>Coefficiente de variação. **Linearity:** <sup>1</sup>Diluição, <sup>2</sup>Observado (O), <sup>3</sup>Esperado (E), <sup>4</sup>%O/E, <sup>5</sup>8 em 8. **Recovery:** <sup>1</sup>Solução, <sup>2</sup>Observado (O), <sup>3</sup>Esperado (E), <sup>4</sup>%O/E. **Specificity:** <sup>1</sup>Composto, <sup>2</sup>Quantidade adicionada, <sup>3</sup>Percentagem de reação cruzada, <sup>4</sup>ND: não detectável. **Method Comparison:** Insulin: insulina.