1. NAME AND INTENDED USE
INSULIN-CT is a radioimmunoassay kit for the quantitative determination of Insulin in human serum and plasma (EDTA).

2. INTRODUCTION
Insulin is a polypeptide hormone of M.W 5800 composed of two peptide chains, A and B, linked by two disulphide bonds and synthesized by the beta-cells of the islets of Langerhans of the pancreas.
Insulin influences most of the metabolic functions of the body. Its best known action is to lower the blood glucose concentration by increasing the rate at which glucose is converted to glycogen in the liver and muscles and to fat in adipose tissue, by stimulating the rate of glucose metabolism and by depressing gluconogenesis.
Insulin stimulates the synthesis of proteins and promotes the uptake of aminoacids and their incorporation into the cell. It increases the uptake of glucose in adipose tissue and its conversion into fat, and inhibits lipolysis. Insulin’s primary action is on the cell membrane, where it probably facilitates the transport of glucose and aminoacids into the cells.
At the same time it may activate intracellular enzymes such as glycogen synthetase, involved in glycogen synthesis.
The main clinical uses of insulin assays are the following:
- Determination of the \(\beta\) -cell reserve during oral glucose tolerance test or after a carbohydrate rich meal, as a guide for the start of insulin therapy.
- Contribution to the diagnosis of insulin and non-insulin dependent diabetes.
- Characterisation and follow-up of states of glucose intolerance.
- Diagnosis and study of cases of insulin resistance (obesity, various endocrinopathies, insulin receptor defects, anti-receptor antibodies).
- Diagnosis of insulinoma and other causes of hypoglycemia.

3. PRINCIPLE
The principle of the assay is based on the competition between the labelled insulin contained in standards or specimens to be assayed for a fixed and limited number of antibody binding sites bound on the solid phase (coated tubes). After the incubation, the unbound tracer is easily removed by a washing step. The amount of labelled insulin bound to the antibody is inversely related to the amount of unlabelled insulin present in the sample.

4. REAGENTS
Each kit contains enough reagents for 100 tubes. The expiry date is given on the external label.

<table>
<thead>
<tr>
<th>REAGENTS</th>
<th>QUANTITY</th>
<th>STORAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>COATED TUBES:</td>
<td>ready-to-use.</td>
<td>2-8°C until the expiration date.</td>
</tr>
<tr>
<td>Guinea pig anti-porcine insulin serum coated on the bottom of the tube.</td>
<td>100 tubes (4 x 25)</td>
<td>Unused coated tubes must be stored in the plastic bag supplied, with dessicant, until the expiration date.</td>
</tr>
<tr>
<td>(^{125})I – INSULIN:</td>
<td>lyophilised.</td>
<td>2-8°C until the expiration date.</td>
</tr>
<tr>
<td>(125)I labelled porcine insulin, phosphate buffer, bovine albumin and sodium azide.</td>
<td>1 vial reconstitute with 5 mL distilled water</td>
<td>After reconstitution and dilution: 7 days at 2-8°C or until the expiration date at –20°C.</td>
</tr>
<tr>
<td>STANDARDS 1 to 7:</td>
<td>ready to use.</td>
<td>2-8°C until the expiration date.</td>
</tr>
<tr>
<td>Porcine insulin, buffer, EDTA, bovine albumin and preservative.</td>
<td>2 mL vials</td>
<td></td>
</tr>
<tr>
<td>0 - 5.5 - 15 - 35 - 70 -175 - 300 µIU/mL (*).</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CONTROLS 1 and 2:</td>
<td>ready to use.</td>
<td>2-8°C until the expiration date.</td>
</tr>
<tr>
<td>Insulin, buffer, EDTA, bovine albumin and preservative.</td>
<td>2 mL vials</td>
<td></td>
</tr>
<tr>
<td>BUFFER SOLUTION:</td>
<td>ready to use.</td>
<td>2-8°C until the expiration date.</td>
</tr>
<tr>
<td>Buffer, horse serum and sodium azide.</td>
<td>107 mL vial</td>
<td></td>
</tr>
</tbody>
</table>

(*) The true values of the reagent concentrations are printed on the labels. Standards and controls are calibrated against the WHO IRP 66/304.

5. PRECAUTIONS FOR USE
5.1. Safety measures
Raw materials of human origin contained in the reagents of this kit have been tested with licensed kits and found negative for the anti-HIV 1, anti-HIV 2, anti-HCV antibodies and the HBs antigen. However as it is impossible to strictly guarantee that such products will not transmit hepatitis, the HIV virus, or any other viral infection, all raw materials of human origin including the samples to be assayed must be treated as potentially infectious.
Do not pipette by mouth.
Do not smoke, eat or drink in areas in which specimens or kit reagents are handled.
Wear disposable gloves while handling kit reagents or specimens and wash hands thoroughly afterwards.
Avoid splashing.
Decontaminate and dispose of specimens and all potentially contaminated materials as if they contained infectious agents. The recommended method of doing this is autoclaving for a minimum of one hour at 121.5°C.
Sodium may react with lead or copper piping to form highly explosive metal azides. During waste disposal, flush the drains thoroughly to prevent a build-up of these products.
5.2. Basic radioprotection rules
This radioactive product may only be received, purchased, stored or used by persons so authorized, and by laboratories covered by such authorization. The solution should under no circumstance be administered to humans or to animals.
The purchase, storage, use or exchange of radioactive products are subject to the laws in force in the user's country.
The enforcement of the basic rules for handling radioactive products ensures adequate security.
A summary of these is given below:
Radioactive products must be stored in their original containers in a suitable area.
A record of the reception and storage of radioactive products must be kept up to date.
Handling of radioactive products should take place in a suitably-equipped area with restricted access (controlled zone).
Do not eat drink, smoke or apply cosmetics in a controlled zone. Do not mouth-pipette radioactive solutions.
Avoid any direct contact with all radioactive products by using laboratory coats and protective gloves.
Contaminated laboratory equipment and glassware must be disposed of immediately after contamination to prevent cross-contamination of different isotopes.
Any contamination or radioactive substance loss should be dealt with in accordance with the established procedures.
All radioactive waste disposal must be carried out according to the regulations in force.

5.3. Handling precautions
Do not use kit components beyond their expiry date.
Do not mix reagents from different batches.
Avoid any microbial contamination of the reagents or of the water used for washing.
Fully respect the incubation times and the washing instructions indicated.

6. SPECIMEN COLLECTION AND PREPARATION

The assay is performed on sera or EDTA-plasma. Haemolyzed or hyperlipemic samples should not be used. If the test is to be carried out within 24 hours, the samples must be refrigerated at 2-8°C. Otherwise, they should be divided into aliquots, deep frozen (-20°C) until needed (maximum 6 months), and must be thawed only just before using and used thoroughly. Specimens should not be frozen more than once.

Dilutions
If elevated insulin levels are suspected, standard 1 should be used for dilution. It is recommended that disposable plastic tubes be used when carrying out the dilutions.

7. ASSAY PROCEDURE

7.1. Material required
Precision micropipettes or similar, with disposable tips, capable of dispensing 100 µL, 1 mL, 4 mL and 5 mL. Their calibrations should be checked regularly. Distilled water. Vortex type mixer. Parafilm. Absorbant paper. Disposable plastic test-tubes. Clean volumetric laboratory glassware (125 mL). Aspirating device. Gamma scintillation counter calibrated for 125 iodine.

7.2. Reconstitution and dilution of the tracer

Reconstitute the tracer with 5 mL of distilled water. Recap the vial and mix gently by inversion to assure complete dissolution of the lyophilized material.
N.B.: The reconstituted Tracer solution should stand at least 30 minutes after reconstitution before being used.
Pour the reconstituted Insulin Tracer solution directly into a clean 125 mL Erlenmeyer glass flask. Wash out the Tracer solution vial three times with Buffer solution, adding these washes to the Erlenmeyer flask. Add remaining buffer solution. Mix thoroughly.

7.3. Protocol

All reagents should be brought to room temperature (18-25°C) at least 30 minutes before their use.
Dispensing of reagents into the tubes is also carried out at room temperature.
The assay requires the following groups of tubes:
T group, for the total activity determination.
Standard groups, to establish the standard curve.
Reference group for the controls.
Sx groups, for the test samples.

It is recommended to perform the assay in duplicate for the standard groups, controls and samples (serum or plasma EDTA). It has been noted that heparin-plasma results give higher insulin values.
Strictly respect the order in which reagents are to be added:

Dispense 100 µL of standards, controls and samples to be assayed into appropriately labelled coated tubes.
Add 900 µL of prediluted 125I-Insulin to each tube (and T group).
Mix each tube gently with a Vortex-type mixer.
Incubate 18 hours at 18-25°C (cover tubes with plastic film).
Aspirate liquid from each assay tube (except T tubes).
Rinse each coated tube with 4 mL of distilled water (except T tubes).
Aspirate liquid (except T tubes).
Measure the remaining radioactivity bound to the tubes with a gamma scintillation counter calibrated for 125 iodine.
8. QUALITY CONTROL
Good laboratory practices require the use of quality control samples in each series of assays to check the quality of the results obtained. All specimens should be treated identically, and results analysed using the appropriate statistical methods is recommended.

9. RESULTS
For each duplicate, compute the mean counts and draw up the standard curve by plotting the standards, mean cpm against their concentration. Read sample values directly from the standard curve, and correct the read value for the dilution factor, if necessary.

Typical standard curve (example only): this data must not be substituted for results obtained in the laboratory.

<table>
<thead>
<tr>
<th>GROUPS OF TUBES</th>
<th>Mean CPM</th>
<th>B/Bo x 100</th>
<th>Concentration µIU/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>33807</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Standard 1</td>
<td>10 µIU/mL</td>
<td>14548</td>
<td>100</td>
</tr>
<tr>
<td>Standard 2</td>
<td>5.5 µIU/mL</td>
<td>11556</td>
<td>79.4</td>
</tr>
<tr>
<td>Standard 3</td>
<td>15 µIU/mL</td>
<td>9317</td>
<td>64.0</td>
</tr>
<tr>
<td>Standard 4</td>
<td>35 µIU/mL</td>
<td>6742</td>
<td>46.3</td>
</tr>
<tr>
<td>Standard 5</td>
<td>70 µIU/mL</td>
<td>5081</td>
<td>34.9</td>
</tr>
<tr>
<td>Standard 6</td>
<td>175 µIU/mL</td>
<td>2832</td>
<td>19.5</td>
</tr>
<tr>
<td>Standard 7</td>
<td>310 µIU/mL</td>
<td>2036</td>
<td>14.0</td>
</tr>
<tr>
<td>Control 1</td>
<td>7559</td>
<td>51.9</td>
<td>28</td>
</tr>
<tr>
<td>Control 2</td>
<td>4016</td>
<td>27.6</td>
<td>105</td>
</tr>
</tbody>
</table>

10. PROCEDURAL LIMITATIONS
Strict adherence to the exact procedures described in this package insert and special care should be taken when performing the assay, in order to obtain reliable results with the INSULIN-CT kit.
It has been noted that heparin-plasma results give higher insulin values than serum or EDTA-plasma. The presence of circulating anti-insulin antibodies in the plasma or serum of insulin treated diabetics, may interfere in the assay (values artificially elevated). Hemolysis releases proteases, which quickly damage the insulin; samples (even minimally hemolysed) will give in clinically unreliable levels.
Drugs that may increase the circulating Insulin level in serum or plasma:
- Calcium Gluconate
- Chlorpropamide
- Glipizide
- Glisoxepide
- Calcium Gluconate
- Chlorpropamide
- Glipizide
- Glisoxepide

Drugs that may decrease the circulating Insulin level in serum or plasma:
- Chlorpromazine
- Chlorpromazine
- Diphenhydantoin
- Chlorpromazine
- Diphenhydantoin
- Chlorpromazine
- Diphenhydantoin
- Chlorpromazine

Insulin specimens obtained via a glucose tolerance study on a single patient should be run within the same assay to ensure best results.

11. EXPECTED VALUES
Each laboratory must establish its own range of normal values. The values given below are only to serve as an indicator. Insulin levels from healthy, fasting individuals obtained utilizing the INSULIN-CT kit yielded a range of 4.3 – 19.9 µIU/mL (mean = 9.6, n = 24).
A 1:1 correlation was found between serum and EDTA plasma samples from the same individuals (n = 26, slope = 0.99, r² = 0.99).
12. SPECIFIC CHARACTERISTICS OF THE ASSAY

12.1. Precision
This was evaluated with 3 samples with different concentrations assayed either 10 times in the same run or once in 9 different runs.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Within-run</th>
<th>Between-run</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean µIU/mL</td>
<td>CV (%)</td>
</tr>
<tr>
<td>1</td>
<td>10.7</td>
<td>12.2</td>
</tr>
<tr>
<td>2</td>
<td>206</td>
<td>3.2</td>
</tr>
<tr>
<td>3</td>
<td>91.4</td>
<td>4.3</td>
</tr>
</tbody>
</table>

12.2. Recovery test
Known quantities of Insulin (WHO 66/304) were added to different plasma and serum base pools, including both normal male and normal female samples. The recovery percentage of Insulin was obtained between 95% and 115%.

12.3. Specificity
Determined from equivalent displacement measurements at 50% binding. The antiserum used in the test shows the following cross-reactions:

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cross reactivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Insulin</td>
<td>100</td>
</tr>
<tr>
<td>C-Peptide</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Glucagon</td>
<td>&lt;0.2</td>
</tr>
</tbody>
</table>

12.4. Detection limit
The detection limit is defined as being the smallest concentration different from zero with a probability of 95%. It has been determined as being 4.6 µIU/mL, based on 95% B/BO.

ASSAY FLOW-CHART

<table>
<thead>
<tr>
<th>TUBES</th>
<th>Standards Controls Samples µL</th>
<th>(^{125}\text{I}\	ext{Insulin (*) µL}</th>
<th>Distilled water µL</th>
</tr>
</thead>
<tbody>
<tr>
<td>T</td>
<td>-</td>
<td>900</td>
<td>-</td>
</tr>
<tr>
<td>Standards</td>
<td>100</td>
<td>900</td>
<td>4</td>
</tr>
<tr>
<td>Controls and Samples</td>
<td>100</td>
<td>900</td>
<td>4</td>
</tr>
</tbody>
</table>

(*) After reconstitution with 5 mL distilled water and dilution in Buffer solution.