Appendixes
Blood glucose measurement by Accutrend Sensor Comfort
Blood Glucose monitor (Roche Diagnostics, Indianapolis)
(Reproduced from the manual)

**Test principle:** Bioamperometry: Glucose dehydrogenase in the strip converts the glucose in the blood sample to gluconolactone. This reaction creates a harmless electrical current that meter interprets as blood sugar.

**Operation of meter:** Press the button of meter to show the display, in few seconds display asks for insertion of test strip. Remove the new test strip from the vial. Insert the test strip with silver bars up into test strip slot of meter. The display asks for placing a drop of blood.

**Blood collection:** 1. Warm the tail of the animal to increase the blood flow 2. Cut the tip of the tail gently with small scissors 3. Wipe out the first drop of blood 4. Allow the second drop to touch the edge of the of the strip within the strip and blood occupy completely the yellow window of the test strip. A beep sound indicates beginning of the test. Test result will appear in 26 seconds with beep sound in mg/dL. Remove the test strip from the meter and discard.

**Normal values:** fasting blood glucose 70-105 mg/dL

**Accuracy:** The majority of slopes obtained in external studies ranged between 0.96 and 1.03

**Repeatability:** The repeatability using venous whole blood is <4.0% CV (coefficient of variation) at a level of 164 mg/dL and <2.9 mg/dL SD at a level of 60 mg/dL.

**Reproducibility:** The reproducibility using controls is <3.1% CV at a level of 130 mg/dL and <2.9 mg/dL SD at a level of 60 mg/dL.

**Detection limit:** 10 –600 mg/dL for the test strip.
Radioimmunoassay Kit for Insulin (Code: RIAK-1) BARC, BOMBAY
(Reproduced from the manual)

Intended use: RIAK-1 kit should be used for the quantitative determination of insulin in serum by radioimmunoassay (RIA). Each kit has reagents sufficient for 100 tubes.

Principle of the assay: RIA method is based upon the competition of unlabelled insulin in the standard or samples and radioiodinated (I-125) insulin for the limited binding sites on the specific antibody. At the end of incubation, the antibody bound and free insulin are separated by the second antibody-polyethylene glycol (PEG) aided separation method. Insulin concentration of samples is quantitated by measuring the radioactivity associated with the bound fraction of sample and standards.


Specimen collection: Collect 5 ml of blood in a glass vial or tube. Allow the blood to clot at room temperature. Rin the clot, centrifuge and collect the serum. Haemolysed, lipemic or turbid samples should not be analysed.

RIA procedure: Reconstitute the reagents as shown in the following table and vortex. Carry out the assay as shown in the insulin as flow chart (page no. iii). By interpolation on the standard curve, the sample values are found to be 29 µU/ml and 125 µU/ml of insulin.
### ASSAY FLOW CHART:

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>Assay Buffer</th>
<th>Insulin Standard (ml)</th>
<th>Serum Sample (ml)</th>
<th>Insulin free Serum (ml)</th>
<th>Insulin anti Serum (ml)</th>
<th>Insulin 1-25 (ml)</th>
<th>Insulin 1-125 (ml)</th>
<th>Second antibody (ml)</th>
<th>PEG (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1, 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</table>

### MODEL CALCULATION

<table>
<thead>
<tr>
<th>Description</th>
<th>Tube No.</th>
<th>Control/Actual counts</th>
<th>Avg. counts</th>
<th>Corrected Avg. counts</th>
<th>% R</th>
<th>% B</th>
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<tbody>
<tr>
<td>Background</td>
<td></td>
<td>150</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Total</td>
<td></td>
<td>294725</td>
<td>29579</td>
<td>29664</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blank</td>
<td></td>
<td>636</td>
<td>488</td>
<td>542</td>
<td>1.8%</td>
<td></td>
</tr>
<tr>
<td>Zero standard</td>
<td></td>
<td>14031</td>
<td>13981</td>
<td>13867</td>
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<tr>
<td>7.5 µu/ml</td>
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<td>12827</td>
<td>12770</td>
<td>12745</td>
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<tr>
<td>12.5 µu/ml</td>
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<td>12993</td>
<td>12973</td>
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<tr>
<td>25 µu/ml</td>
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<td>12241</td>
<td>12091</td>
<td>12081</td>
<td>38.8</td>
<td>96.5</td>
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<tr>
<td>50 µu/ml</td>
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<td>12323</td>
<td>12321</td>
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<tr>
<td>100 µu/ml</td>
<td></td>
<td>10955</td>
<td>10905</td>
<td>10766</td>
<td>10224</td>
<td>34.4</td>
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<tr>
<td>200 µu/ml</td>
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<td>15357</td>
<td>12.0</td>
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<td>Sample - 2</td>
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<td>7064</td>
<td>7024</td>
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<td>73.1</td>
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<td>Sample - 3</td>
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<td>5635</td>
<td>5545</td>
<td>5553</td>
<td>3011</td>
<td>16.3</td>
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</tbody>
</table>

By interpolation on the standard curve, the sample values are found to be 29 µu/ml and 125 µu/ml of insulin.

**CAUTION:** The data produced above must not be employed in lieu of data obtained by the user in his laboratory with the insulin Radioimmunoassay kit.
**Calculation:**

1. Subtract counter background from all the counts to get actual counts.
2. Average all the duplicates.
3. Average count of tubes 1 and 2 is called 'Total Count'.
4. Average count of tubes 3 and 4 is called 'Blank Count'.

\[
\text{Blank Count} = \frac{\text{Calculation of Blank}}{\text{Total Count}} \times 100
\]

5. Subtract blank count from the average of all the remaining duplicates. This is called corrected average count.

6. Calculate zero standard binding or Bo as follows:

\[
\% \text{Bo} = \frac{\text{Corrected average count of tubes 5 & 6}}{\text{Total Count}} \times 100
\]

7. Calculate percentage binding (%B/T) and percentage B/Bo of all standards and samples.

\[
\% (B/T) = \frac{\text{Corrected average count of standard or samples}}{\text{Total count}} \times 100
\]

\[
\% \text{B/Bo} = \frac{\text{Corrected average count of standard or samples}}{\text{Corrected average count of tubes 5 and 6}} \times 100
\]

8. Plot the standard curve. (a) % B/Bo (or %B/T) against µU/ml of insulin on linear graph paper. (b) B/Bo on the logit and µU/ml of insulin on logarithmic scale of logit-log graph sheet.

9. Read the sample value from the standard curve obtained above as µU of insulin per ml directly.

**Note:** If the sample value higher than 200 µU/ml is obtained, the sample should be diluted with insulin free serum and reassayed. However, extrapolation up to 300 µU/ml is permitted in logit-log curve.

**Expected values:** The normal fasting levels of insulin obtained by analysing 150 samples ranged from 0-30 µU/ml. This range may be used as a guide line until the laboratory has established its own range by analyzing sufficient number of normal samples.
C-Peptide of Insulin Radioimmunoassay (Diagnostic Systems Laboratories, Inc. Texas, USA) (Revision date: March 8, 2000)
(Reproduced from the manual)

Intended use: The DSL-7000 C-Peptide Radioimmunoassay Kit provides materials for the quantitative measurement of in vitro C-peptide in serum, plasma or urine.

Principle of the test: The procedure follows the basic principle of radioimmunoassay where there is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites. The amount of (I-125)-labeled C-peptide bound to the antibody is inversely proportional to the concentration of the unlabeled C-peptide present. The separation of free and bound antigen is easily and rapidly achieved using a double antibody system.

Reagents supplied: The DSL-7000 C-peptide RIA Kit contains sufficient reagents for 100 tubes. Each kit contains the following reagents:

A. C-peptide Standards: (Lyophilized) One vial, labeled A, containing 0 ng/ml, and five vials, labeled B - F, containing approximate concentrations of 0.1, 0.5, 1.5, 5 and 20 ng/ml (0.033 – 6.66 n mol/L) of C-peptide in protein-based buffer. Reconstitute Standard A with 5 ml deionized water and Standards B-F with 1.0 ml deionized water. Use immediately upon reconstitution.

B. C-peptide [I-125] Reagents: (Lyophilized) (Red) One vial containing <5 μ Ci (185 kBq) of [I-125]-labeled C-peptide in a protein-based buffer. Reconstitute with 11 ml of deionized water.

C. C-peptide Antiserum: (Lyophilized) (Blue) One vial containing guinea pig anti-C-peptide serum in a protein-based buffer. Reconstitute with 10 ml deionized water.
D. Precipitating Reagents: (Blue) One bottle, 105 ml, containing goat anti-guinea pig gamma globulin serum in a buffered solution with polyethylene glycol as a precipitating aid.

E. C-peptide Controls: (Lyophilized) Two vials, Levels I and II, containing a low and high level of C-peptide. Reconstitute each vial with 1.0 ml deionized water.

VI. Specimen collection and preparation – Serum or plasma: Patients should fast for 12 h prior to sampling. Serum or EDTA plasma should be used and the usual precautions for venipuncture should be observed. The specimens may be stored at 2-8°C for up to 24 h and should be frozen at -20°C or lower for longer periods. Do not use hemolyzed or lipemic specimens.

VIII. Test procedure

A. Materials required: 1. 12 x 75 mm plastic or glass test tubes 2. Test tube rack for 12 x 75 mm tubes. 3. Precision pipette to deliver 50 µl and 100 µl 4. Precision repeating pipette to deliver 1 ml 5. Vortex mixer 6. Centrifuge (1500 x g) 7. Sponge test tube rack for decantation 8. Absorbent material for blotting tubes 9. Gamma counter 10. Semi-log graph paper.

B. Assay procedure: Allow all reagents to reach room temperature and mix thoroughly before using standards. Controls and unknown should be assayed in duplicate.

Overnight assay procedure: 1. Label and arrange test tubes in duplicate 2. Add 50 µl of the Standards, Controls or unknowns to the appropriate tubes. To NSB tubes add 150 µl of 0 ng/ml Standard 3. Add 100 µl of the C-peptide Antiserum to all tubes except NSB and Total count tubes 4. Add 100 µl of the C-Peptide [I-125] Reagents to all tubes 5. Vortex all 6. Incubate at 2-8°C for 16-24 h 7. Add 1 ml of precipitating reagent to all tubes except total count tubes. THIS REAGENT SHOULD BE MIXED THOROUGHLY BEFORE USE 8. Vortex all tubes 9. Incubate at room temperature for 15-20 min 10. Centrifuge all tubes for 20 min at 1500 x g 11. Aspirate or decant all tubes, except total count tubes, by simultaneous inversion with a sponge rack into a radioactive waste
receptacle. Allow them to drain on absorbent material for 15 – 30 sec and gently blot
the tubes to remove any droplets adhering to the rim before returning them to the
upright position. Failure to blot tubes adequately may result in poor replication and
spurious values 12. Count all tubes in a gamma counter for one min.

IX. Results: 1. Calculate the mean counts per min (cpm) for each standard, control and
unknown. Subtract the mean cpm of the NSB tubes from all counts to obtain corrected
counts. Calculate the %B/T or %B/Bo for each standard, control and unknown as fol-

\[
\frac{\text{Mean sample counts} - \text{NSB counts}}{\text{Mean total counts}} \times 100
\]

\[
\frac{\text{Mean sample counts} - \text{NSB counts}}{\text{Mean counts of 0 ng/ml standard} - \text{NSB counts}} \times 100
\]

B. Plot a curve of %B/T or %B/Bo for each of the Standard (y-axis) against the
C-peptide concentration (x-axis) on semi-long graph paper. Draw a standard
curve through the mean of the duplicate points.

C. Determine the C-peptide concentration from the means of the duplicate counts
of each control and unknown from the standard curve.

D. Any sample reading greater than the higher standard should be diluted ap-
propriately with the 0 ng/ml C-peptide standard and reassayed.

E. Any sample reading less than the lowest standard should be reported as
such.
A. Materials Supplied:

Materials supplied in the DSL C-Peptide RIA Kit, Catalog No. DSL-7000:

<table>
<thead>
<tr>
<th>MATERIAL</th>
<th>QUANTITY</th>
<th>CATALOG NO</th>
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<tbody>
<tr>
<td>C-peptide Standard A</td>
<td>One Vial</td>
<td>DSL-7001</td>
</tr>
<tr>
<td>C-peptide Standard B</td>
<td>One Vial</td>
<td>DSL-7002</td>
</tr>
<tr>
<td>C-peptide Standard C</td>
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<td>DSL-7003</td>
</tr>
<tr>
<td>C-peptide Standard D</td>
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<td>DSL-7004</td>
</tr>
<tr>
<td>C-peptide Standard E</td>
<td>One Vial</td>
<td>DSL-7005</td>
</tr>
<tr>
<td>C-peptide Standard F</td>
<td>One Vial</td>
<td>DSL-7006</td>
</tr>
<tr>
<td>C-peptide Antiserum</td>
<td>One Vial</td>
<td>DSL-7010</td>
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<tr>
<td>C-peptide [1-125I] Reagent</td>
<td>One Vial</td>
<td>DSL-7020</td>
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<tr>
<td>Precipitating Reagent</td>
<td>One Bottle</td>
<td>DSL-2430</td>
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<td>C-peptide Control Level I</td>
<td>One Vial</td>
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<td>C-peptide Control Level II</td>
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TYPICAL C-PEPTIDE STANDARD CURVE DATA

<table>
<thead>
<tr>
<th>TUBE NO.</th>
<th>TUBE LABEL</th>
<th>BOUND (kpm)</th>
<th>MEAN (kpm)</th>
<th>R/T (%)</th>
<th>R/B (%)</th>
<th>C-Peptide (ng/mL)</th>
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<tr>
<td>1.2</td>
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<td>38632</td>
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<tr>
<td>3.4</td>
<td>NSB</td>
<td>1026</td>
<td>997</td>
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<td>STANDARDS</td>
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<td>A</td>
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<td>4259</td>
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<td>20.6</td>
<td>5.43</td>
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</table>

CAUTION: The above data must not be employed in lieu of data obtained by the user in the laboratory.
Composition of Tyrode solution (per litre)


<table>
<thead>
<tr>
<th>Component</th>
<th>Quantity</th>
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</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8.0 g</td>
</tr>
<tr>
<td>KCl</td>
<td>0.2 g</td>
</tr>
<tr>
<td>CaCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>1.8 g</td>
</tr>
<tr>
<td>MgCl&lt;sub&gt;2&lt;/sub&gt;</td>
<td>0.01 g</td>
</tr>
<tr>
<td>NaHCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>1.0 g</td>
</tr>
<tr>
<td>NaH&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt;</td>
<td>0.05 g</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0 g</td>
</tr>
<tr>
<td>Distilled water (q.s)</td>
<td>1 litre</td>
</tr>
</tbody>
</table>

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   7. Effect of prokinetic drug-induced (metoclopramide) alterations of SIT on BG
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Papers presented at Conferences

1. PMK Reddy and S. Ramaswamy
   Relationship between changes in blood glucose levels and small intestinal transit.

2. PRMK Reddy, Ranganathan P, Shashindran CH and Ramaswamy S
   Role of Insulin on the changes in small intestinal transit elicited by physiological changes in blood glucose.

   Further studies on effect of Insulin on small intestinal transit in mice.
   33rd Annual Conference of Indian Pharmacological Society, December 28-30, 2000, Gandhi Nagar, Gujarat.

4. PMK Reddy, Ranganathan P, Shewade DG and Ramaswamy S
   Association between changes in the glycemic state, small intestinal transit and C-peptide level in non-diabetic mice.