TOTAL PROTEIN (Biuret Method)

INTENDED USE:
The reagent kit is intended for "in vitro" quantitative determination of Total Protein in serum/plasma.

CLINICAL SIGNIFICANCE:
Proteins are constituents of muscle, enzymes, hormones and several other key functional and structural entities in the body. They are involved in the maintenance of the normal distribution of water between blood and the tissues. Consisting mainly of albumin and globulin in the fractions vary independently and widely in diseases. Increased levels are found mainly in dehydration. Decreased levels are found mainly in malnutrition, impaired synthesis, protein losses as in hemorrhage or excessive protein catabolism.

PRINCIPLE:
Proteins, in an alkaline medium, bind with the cupric ions present in the biuret reagent to form a blue-violet coloured complex. The intensity of the colour formed is directly proportional to the amount of proteins present in the sample.

REACTION:
Total Protein + Cu$^{+}$ → Violet complex.

CONTENTS:
Reagent 1 : Biuret Reagent
Reagent 2 : Protein Standard 6 g/dl

MATERIALS REQUIRED BUT NOT PROVIDED:
- Clean & Dry Glassware.
- Laboratory Glass Pipettes or Micropipettes & Tips.
- Colorimeter or Bio-Chemistry Analyzer.

SAMPLES:
Serum, Heparinized/EDTA Plasma. Proteins are reported to be stable in the sample for 6 days at 2-8°C.

PREPARATION OF REAGENT & STABILITY:
All reagents are stable till the expiry date mentioned on the label at room temperature.
Standard vial once opened should be stored at 2-8°C, it is stable till the expiry date mentioned on the vial.
All reagents are in ready to use form.

GENERAL SYSTEM PARAMETERS:
- Reaction type: End point
- Wave length: 546 nm (530 - 570 nm)
- Temperature: Room temperature
- Incubation: 5 minutes
- Reagent volume: 1.0 ml
- Sample volume: 10 µl
- Standard concentration: 6 gm/dl.
- Zero setting: Reagent blank
- Light path: 1 cm

PROCEDURE:
Pipette into clean dry test tube labeled as Blank (B), Standard (S) and Test (T):

<table>
<thead>
<tr>
<th>Addition sequence</th>
<th>B</th>
<th>S</th>
<th>T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biuret Reagent</td>
<td>1ml</td>
<td>1ml</td>
<td>1ml</td>
</tr>
<tr>
<td>Standard</td>
<td>-</td>
<td>10 µl</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>10 µl</td>
</tr>
</tbody>
</table>

Mix well, Incubate for 5 minutes at Room temperature. Measure the absorbance of the standard Abs. S and sample Abs. T against the reagent blank, within 60 minutes.

CALCULATION:
Total Protein Conc. (gm/dl) = \( \frac{\text{Abs. T}}{\text{Abs. S}} \) x 6

NORMAL VALUE:
Serum: 6.0 - 8.0 gm/dl
It is recommended that each laboratory establish its own normal range.

LINEARITY:
This procedure is linear up to 10 gm/dl. Samples above this concentration should be diluted with normal saline and the results should be multiplied by the dilution factor.

QUALITY CONTROL:
For accuracy it is necessary to run known controls with every assay.

LIMITATION & PRECAUTIONS:
1. Storage condition mentioned on the kit must be adhered.
2. Do not in any case freeze or expose reagent to high temperature as it may effect the performance of the kit.
3. Before the assay bring all the reagents to room temperature.
4. Avoid contamination of the reagents during the assay process.
5. Use clean glassware free from dust or debris

BIBLIOGRAPHY:

CODE NO. PACK SIZE Reagent 1 Reagent 2
Z17 1 x 50 ml 1 x 50 ml 3.0 ml
Z17A 1 x 100 ml 1 x 100 ml 3.0 ml

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