TOTAL PROTEIN

(Biuret method)

INTRODUCTION

Most serum proteins except gamma globulins and hemoglobin are synthesized in the liver. Proteins participate in transport, catalysis and coagulation, act as hormones and receptors, antigens and antibodies, regulate osmotic pressure and play structural functions. Correct serum level of total protein depends mainly on balance between synthesis and degradation of albumin and immunoglobulins. Total protein level abnormalities are caused usually by dehydration, liver or kidney disease and starvation.

METHOD PRINCIPLE

The method is based on the biuret reaction. Protein forms the coloured complex with cupric ions
intensity is proportional to the protein concentration.

KIT CONTENTS

Reagent Name	Pack Size
R1 - Total Protein Reagent	2 x 50 ml
R2 - Standard	2 ml

Refer standard value mentioned in the vial.

WORKING REAGENT PREPARATION AND STABILITY

The reagent is ready to use.
The reagent when stored at 2-8°C is stable up to expiry date printed on the package. The reagents are stable for 4 weeks on board the analyser at 2-10°C. Protect from light and avoid contamination.

CONCENTRATIONS IN THE TEST

Copper sulfate	7 mmol/l
Sodium-potassium tartrate	20 mmol/l
Potassium iodide	6 mmol/l
Surfactant	0.05% w/v
Stabilizers	

WARNINGS AND NOTES

Product for in vitro diagnostic use only.
The standards contain 0.09% sodium azide preservative. Avoid contact with skin and mucous membranes. Reagent 1-TOTAL PROTEIN is classified as an irritant

ADDITIONAL EQUIPMENT

- Automatic analyzer or photometer able to read at 546 nm
- Thermostat at 25°C or 37°C
- General laboratory equipment

SPECIMEN

Nonhemolyzed, nonlipaemic, fresh serum is recommended. Results obtained from plasma analysis might be slightly elevated due to fibrinogen presence. Recommended anticoagulants: EDTA, heparine lithium, sodium or ammonium salt. Serum should be separated from red blood cells as soon as possible after blood collection. Serum and plasma can be stored up to 3 days at 2-8°C Nevertheless it is recommended to perform the assay with freshly collected sample.

PROCEDURE

These reagents may be used both for manual assay and in several automatic analysers. Programme Sheets are available on request.

Wavelength	546 nm
Temperature	25°C / 37°C
Cuvette	1 cm

Pipette into the cuvettes:

Reagent	Blank (B)	Standard (S)	Test (T)
R1 Total Protein Reagent	1000 µl	1000 µl	1000 µl
Bring upto the temperature of determination. Then add			
Distilled water	20 µl	-	-
R2 - Standard	-	20 µl	-
Sample	-	-	20 µl

Mix well, incubate for 10 min. at the temperature of determination. Read the absorbance of the test A (T) and standard A(S) against reagent blank (RB). The intensity of colour is stable for 30 min.

CALCULATION

Protein concentration = A (T) / A(S) x standard concentration

REFERENCE VALUES

6.6 - 8.7 g/dl

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

To Ensure adequate quality control, each run should include assayed normal and abnormal controls. If commercial controls are not available it is recommended that known value samples be aliquoted, frozen and used as controls.

For Fully Automated analyzers by using multicalibrators or total protein standard the calibration curve can plot and the same should be prepared every 8 weeks or with change of reagent lot number.

PERFORMANCE CHARACTERISTICS

Sensitivity / Limit of Quantitation : 0.17 g/dl (1.7g/l).

Linearity: up to 18 g/dl (180 g/l) using automatic analysers, up to 12 g/dl (120 g/l) for manual procedure. For higher concentration of protein dilute the sample with 0.9% NaCl and repeat the assay. Multiply the result by dilution factor.

Specificity / Interferences
Haemoglobin up to 2.5 g/dl, ascorbate up to 62 mg/l, bilirubin up to 20 mg/dl and triglycerides up to 500 mg/dl do not interfere with the test.

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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SYSTEM PARAMETERS

Method	End Point
Wavelength	546 nm
Zero Setting	Reagent Blank
Temperature Setting	37° C
Incubation Temperature	37° C
Incubation Time	10 mins
Delay Time	-----
Read Time	-----
No. of Reading	-----
Interval Time	-----
Sample Volume	0.02 ml (20 µl)
Reagent Volume	1.0 ml (1000 µl)
Standard Concentration	Refer Standard vial
Units	g/dl
Factor	-----
Reaction Slope	Increasing
Linearity	18 g/dl

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