Manual Procedure

Automated procedure on request

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12580 R 2 x 50 ml
For 100 tests

Cat. No. 12582 R 6 x 50 ml
For 300 tests

Total Protein Biuret

Colorimetric method

Liquid Reagent

Test principle

Protein + Cu^{++} —(*Alkali*)→ Colored Complex

Protein in serum forms a violet colored complex when reacted with cupric ions in an alkaline solution. The intensity of the violet color is proportional to the amount of protein present when compared to a solution with known protein concentration.

Concentrations in the test

Reagent R		
Potassium sodium tartrate	60	mmol/L
Potassium iodide	29	mmol/L
Sodium hydroxide	200	mmol/L
Copper sulfate	11.5	mmol/L
Stabilizer		
Standard : The Concentration as indicated on vial.		

Stability of reagent

Reagent R: *liquid, ready to use.*
The reagent is stable up to expiry date given on the label when stored at +2 ➔ +25°C.

Note: The reagent should be clear, pale blue, don't use in case of turbidity or presence of black precipitate.

Specimen collection and handling

1. Non-hemolyzed serum, heparinized or EDTA plasma.
2. Protein in serum is stable for 1 week at 20 - 25 °C, and 1 month at 2 - 8 °C when protected against evaporation.

Calibrator / Standard

MediCal U Cat. No.15011
Total protein STD. Cat. No.16181

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 546 nm (530 - 570 nm)
Spectrophotometer	545 nm
Cuvette	1 cm light path
Temperature	37°C / 20 - 25 °C
Measurement	against reagent blank
Reaction	end point

Assay

	Blank	Calibrator / Standard	Sample
Distilled water	20 µl	--	--
Calibrator / Standard	--	20 µl	--
Sample	--	--	20 µl
Reagent	1000 µl	1000 µl	1000 µl
Mix, incubate for 5 min. at 37 °C or 10 min. at 20 - 25 °C. Read the absorbance (A). The final color is stable for 1 hour.			

Notes:

1. When samples are pigmented (icteric or hemolyzed) or opalescent (hyperlipemic) a sample blank should be included:
   - Place 1 ml 0.9 % saline in a test tube.
   - Add 0.02 ml (20 µl) sample.
   - Zero spectrophotometer with 0.9% saline.
   - Read and record absorbance of serum blank.
   - Subtract sample blank absorbance from test absorbance.
   - Calculate as usual.
2. The Biuret method is not sensitive at low ranges (< 1 g/dl). Don't use for urine or cerebrospinal fluid specimen (CSF).

Calculation

$$\text{Conc.}_{\text{Total Protein}}\ (\text{g/dl}) = \frac{A_{\text{Sample}}}{A_{\text{Cal./STD.}}} \times \text{Conc.}_{\text{Cal./STD.}}\ (\text{g/dl})$$

Linearity

Up to 10 g/dl.
If the result exceeds 10 g/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Interferences

1. Young, et al have reviewed a number of drugs and substances that may affect protein concentration.
2. Gross hemolysis will cause elevated results.
3. Lipemic and icteric sera cause elevated results and should be run with a serum blank.
4. Sample with bromosulfophtalein (BSP) will result in falsely elevated results.

Precautions

The reagent contains sodium hydroxide which is corrosive.
Avoid contact with skin, mucous membranes and eyes.
In case of contact with skin, flush with large quantities of water.
As for eyes, flush with water then consult an ophthalmologist.

Reference range

Infants 1 d.	3.4 - 5.0	g/dl
1d. - 4 wk.	4.6 - 6.8	g/dl
2 - 12 mth.	4.8 - 7.6	g/dl
≥ 1 yr.	6.0 - 8.0	g/dl
Adults	6.6 - 8.7	g/dl

References

1. Henry, R. J., Anal. Chem. 92, 1491 (1957).
2. Peters, T. J., Clin. Chem. 14, 1147 (1968).
3. Young, DS., Effects of Drugs on Clinical Laboratory Tests, fifth editiopn 2000, AACC Press, Washington, D.C.