

TOTAL PROTEIN

BIURET METHOD

Principle:

Colorimetric determination of the total protein based on the principle of Biuret reaction (copper salts in an alkaline medium). Protein in plasma or serum forms a blue coloured complex when treated with cupric ions in alkaline solution. The intensity of the blue colour is proportional to the protein concentration.

Reagent Concentration:

Biuret Reagent:
Sodium iodide 100 mmol/l
Potassium sodium tartrate 15 mmol/l
Copper sulphate 5 mmol/l
Potassium Iodide 15 mmol/l

Standard:
Albumin Bovine Fraktion V 7 g/dl

Preparation and Stability

Reagent and Standard are ready to use.
The reagents are stable up to the stated expiry date when stored at +2 to +8 °C.

Samples:

Serum, heparinised or EDTA-plasma.
Stability in serum at +2 to +8 °C is up to 1 month and at +15 to +25°C is up to 1 week.

Procedure:

Wavelength: Hg 540nm (530-570nm)
Temperature: +25°C
Cuvette: 1cm light path
Zero adjustment: Reagent blank
Each series needs one reagent blank only

	Blank	Standard	Sample
Reagent/R1	1000µl	1000µl	1000µl
Standard/R4	---	25µl	---
Serum or Plasma	---	---	25µl

Mix. Incubate for 15 minutes at 30-37C. Wait for 5 minutes at room temperature. Measure absorbance of sample and standard against reagent blank.

Calculation:

By Standard:
? A sample
----- x standard conc (g/dl. = Protein in g/dl
? A standard

Standard concentration: 7g/dl

Linearity:

The method is linear up to 15g/dl or 150g/l.
If the protein concentration is greater than 15 g/dl in the serum or plasma, dilute sample 1:2 with saline solution and repeat test. Multiply result by 2.

Normal Values:

Serum, Adults: 6.7-8.7 g/dl (67-87 g/l)
Newborn: 5.2-9.1 g/dl (52-91g/l)
Children: 5.4-8.7 g/dl (54-87 g/l)

Notes:

The sample blank for clear, colourless sera is equivalent to 0.2 g/dl and is therefore negligible. A sample blank must be determined for haemolytic and liperimic sera by pipetting 20µl serum to 1000µl physiological saline and measurement against distilled water. The absorbance of the sample blank has to be subtracted from the absorbance of the sample.

Patients who have received intravenous amounts of polydextrans yield values which are too high to be measured by the Biuret method. In such cases the protein concentration should be measured using the Kjeldal method.

Quality Control:

For accuracy and reproducibility control:-
Assayed Multi-Sera Normal and Elevated.
For reproducibility control:-
Multi-Sera Loaw, Normal and Elevated.

Presentation:

TOP0350 2 x 125ml, 250 tests
Biuret Reagent 2x125ml
Standard 1x5ml

Safety precautions:

For in vitro diagnostic use only. Do not pipette by mouth. Exercise the normal precautions required for handling laboratory reagents.

Health and Safety data sheets are available on request.

Literature:

Weichselbaum, T. E., Amer. Clin. Path., 16, 40 – 48 (1946)
Josephson, B. and Gyllenswärd, A. Scand. J. Clin. Lab. Invest., 9, 29 (1975).
Henry R.J et al. Anal. Chem.92 1491 (1957)
Peters T.J. Clin. Chem. 14 1147 (1968)