

PROTEINS (TOTAL)

TP 0500 CH

4 x 125 ml

SUMMARY OF TEST

The two general causes of alterations of serum total proteins are a change in the volume of plasma water and a change in the concentration of one or more of the specific proteins in the plasma. Decrease in the volume of plasma water (hemoconcentration) is reflected as relative hyperproteinemia; concentration of all the individual plasma proteins are increased to the same degree. Hyperproteinemia is noted in dehydration due to inadequate water intake or to excessive water loss as in severe vomiting, diarrhea, Addison's disease, or diabetic acidosis. Hemodilution (increase in plasma water volume) is reflected as relative hypoproteinemia; concentration of all the individual plasma proteins are decreased to the same degree. Hemodilution occurs with water intoxication or salt retention syndromes, during massive intravenous infusions, and physiologically when a recumbent position is assumed. A recumbent position decreases total protein concentration by 0.3 to 0.5 g/dl

Among the individual serum proteins, albumin is present in such high concentration that low levels of this protein alone may cause hypoproteinemia. Such hypoproteinemia is common and has many causes. Mild hyperproteinemia may be caused by an increase in the concentration of specific proteins normally present in relatively low concentration, as for example, increases in APRs and polyclonal immunoglobulins, as a result of infection. Marked hyperproteinemia may be caused by high levels of the monoclonal immunoglobulins produced in multiple myeloma and other malignant paraproteinemias.

Historically, total protein was first determined by Kjeldahl's method, in which acid digestion was used to convert nitrogen in the protein to ammonium ion. The method was well defined and reproducible but so time consuming and inconvenient that it was impractical for widespread routine use. Kjeldahl's determination, however, still remains a means of defining reference materials for the biuret method in use today. The biuret method depends on the presence of peptide bonds in all proteins. When a solution of protein is treated with Cu(II) ions in a moderately alkaline medium, a colored chelate is formed between the Cu(II) ion, the carbonyl oxygen and amide nitrogen atoms of the peptide bond. An analogous reaction occurs between cupric ion and the organic compound biuret, hence the name. Amino acids and dipeptides do not react, but tri-, oligo-, and polypeptides do react to give pink to reddish-violet products. The intensity of the color produced is proportional to the number of peptide bonds that are reacting and therefore to the number of protein molecules present in the reaction system. Thus, the biuret reaction with protein is suitable for quantitative determination of total protein by spectrophotometry.

PRINCIPLE OF THE METHOD

Proteins peptidic bonds react with Cu(II) in alkaline solution to form blue-purple complex, the absorbance of which is measured at 520-560 nm. Each Cu(II) can complex up to 6 peptidic bonds. Tartrate salt is a stabilizer and iodide ions are added to prevent self-reduction of alkaline cupric complex. For automatic analyzers, set the reference wavelength to 600-700 nm.

KIT COMPONENTS

For in vitro diagnostic use only.

The components of the kit are stable until expiration date on the label.

Keep away from direct light sources.

Reagent A: 4 x 125 ml (liquid) blue cap

Composition: cupric sulphate 6 mM, sodium-potassium tartrate 21 mM, potassium iodide 6 mM, NaOH 0.75 M.

Standard: proteins solution 6 g/dl - 5 ml

Store all components at 15-25°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

REAGENT PREPARATION

Use reagent ready to use.

Stability: up to expiration date on labels at 15-25°C.

Stability since first opening of vials: ≥ 60 days at 15-25°C.

PRECAUTIONS

Labelling: R36/38 Irritating to eyes and skin.
Contains potassium iodide. May produce an allergic reaction.
S20/21 When using do not eat, drink or smoke.
S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
S36/37/39 Wear suitable protective clothing, gloves and eye/face protection.

S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

S46 If swallowed, seek medical advice immediately and show this container or label.

SPECIMEN

Either serum or plasma may be used, but serum is preferred. A fasting specimen is not required but may be desirable to decrease lipemia. Hemolysis should be avoided. Tightly stoppered samples of serum are stable for 1 week at room temperature or 1 month at 2-8°C.

Specimens that have been frozen and thawed should be thoroughly mixed before assay.

TEST PROCEDURE

Wavelength: 540 nm (allowed 520 ÷ 560 nm)
Lightpath: 1 cm
Temperature: 25, 30 or 37°C

dispense:	blank	standard	sample
reagent	2 ml	2 ml	2 ml
water	20 µl	-	-
standard	-	20 µl	-
sample	-	-	20 µl

Mix, incubate at 25, 30 or 37°C for 10 minutes.
Read absorbances of standard (As) and samples (Ax) against reagent blank.

RESULTS CALCULATION

Serum, plasma:

proteins g/dl = Ax/As x 6 (standard value)

EXPECTED VALUES

Ambulatory adult: 6.3 - 8.3 g/dl
Recumbent adult: 6.0 - 7.8 g/dl
(after age of 60 years, levels are approximately 0.2 g/dl lower)

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:

QN 0050 CH QUANTINORM CHEMA 10 x 5 ml
with normal or close to normal control values

QP 0050 CH QUANTIPATH CHEMA 10 x 5 ml
with pathological control values.

If required, a multiparametric, human based calibrator is available:

AT 0030 CH AUTOCAL H 10 x 3 ml

Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity

the method is linear up to 12 g/dl.

If the limit value is exceeded, it is suggested to dilute sample 1+9 with saline and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)

the limit of detection is 0.1 g/dl.

Interferences

no interference was observed by the presence of:

hemoglobin ≤ 350 mg/dl
bilirubin ≤ 20 mg/dl
lipids ≤ 200 mg/dl

Precision

intra-assay (n=10)	mean (g/dl)	SD (g/dl)	CV%
sample 1	5.03	0.10	2.00
sample 2	5.54	0.10	1.80

inter-assay (n=20)	mean (g/dl)	SD (g/dl)	CV%
sample 1	5.12	0.11	2.20
sample 2	5.31	0.17	3.20

Methods comparison

a comparison between Chema and a commercially available product gave the following results:

Proteins total Chema = x
Proteins total competitor = y
n = 97

y = 1.02x - 0.11 g/dl r = 0.97

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.








REFERENCES

Flack C.P. and Woollen J.W. - Clin.Chem. 30, 559 (1984).
Tietz Textbook of Clinical Chemistry, Second Edition, Bur-
tis-Ashwood (1994).

MANUFACTURER

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SYMBOLS

	for in vitro diagnostic use only
	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions