TOTAL PROTEIN

INTENDED USE

Vitro total protein reagent is intended for the in vitro quantitative determination of total protein in serum or plasma on both automated and manual systems.

METHOD

Biuret colorimetric endpoint method.

BACKGROUND

Plasma proteins are synthesized predominantly in the liver plasma cells, lymph nodes, spleen and in bone marrow. In the course of disease the total protein concentration and also percentage represented by individual fractions can significantly deviate from normal values. Hypoproteinemia can be caused by diseases and disorders such as loss of blood, sprue, nephrotic syndrome, severe burns, salt retention syndrome and Kwashiorkor (acute protein deficiency). Hyperproteinemia can be observed in cases of severe dehydration and illnesses such as multiple myeloma. Changes in the relative percentage of plasma protein fraction can be due to a change in the percentage of one plasma protein fraction. Often in such cases the amount of total protein does not change. The A/G-ratio is commonly used as an index of the distribution of albumin and globulin fractions. Marked changes in this ratio can be observed in cirrhosis of the liver, glomerulonephritis, nephrotic syndrome, acute hepatitis, and lupus erythematosus as well as in certain acute and chronic inflammations. Total protein measurements are used in the diagnosis and treatment of a variety of diseases involving the liver, kidney, or bone marrow as well as other metabolic or nutritional disorders[2].

ASSAY PRINCIPLE

One of the earliest methods of protein measurement is based on the conversion of nitrogen in proteins and other compounds to ammonia by digestion followed by titration. The original Kjeldahl procedure has undergone numerous modifications. The most widely used being that described by Hiller et al.[3] These methods are generally time-consuming and complex. Methods using the biuret reaction[1] are more suitable for routine analysis. These methods are based on the formation of colored complexes between peptide bonds and cupric ions in alkaline medium. Vitro total protein reagent is based on the biuret reaction where divalent copper reacts with the peptide bonds of protein under alkaline conditions to form the characteristic pink to purple biuret complex.

$$\text{Protein} + Cu^{2+} \xrightarrow{\text{Alk. pH}} \text{Cu-Protein complex}$$

The intensity of color is directly proportional to the total protein concentration in the specimen. It is determined by measuring the increase in absorbance at 530 - 570 nm.

EXPECTED VALUES

Adults[4]	
Standing	6.3 – 8.3 g/dl
Recumbent	6.0 – 7.8 g/dl
Children[5]	
> one year	6.0 – 8.0 g/dl
< one year	4.6 –7.6 g/dl
Newborns[5]	

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range. For diagnostic purposes, the protein results should always be assessed in conjunction with the patient's medical history, clinical examination, and other findings.

REAGENTS

R_1	Protein standard	6.0	g/dl
	Sodium hydroxide	0.2	N
R_2	$EDTA_2$	18	mmol/l
	Cupric sulfate	12	mmol/l

- **Reagent Preparation & Stability**

All reagents are ready for use and stable up to the expiry date given on label when stored at 2–8^{0}C.

SPECIMEN

- Serum or plasma.
- The only acceptable anticoagulants are heparin and EDTA.

Specimen Preparation & Stability

Patients should be following their usual diet and be in their usual state of health. Specimen may be stored at 2-8^{0}C prior to analysis. Serum total protein is reported to be stable for at least 3 days at 2-8^{0}C and 6 months at –20^{0}C.[6]

In theory, the total protein level in plasma should be about 3 g/l greater than in serum due to the presence of fibrinogen and other clotting proteins. In actuality, however, the plasma protein level is usually no higher due to diffusion of water from the erythrocytes into the plasma to compensate for the increased salt concentration from the anticoagulant. Total protein levels are reported to be 4–8 g/l lower when measured in serum collected from subjects in the supine position rather than when standing erect[6].

PROCEDURE

- **Manual Procedure**

Wavelength	530 - 570 nm
Cuvette	1 cm light path
Temperature	20-25 or 37 ^{0}C
Zero adjustment	against reagent blank
Specimen	Serum or plasma

	Blank	Standard	Specimen
R_2	1.0 ml	1.0 ml	1.0 ml
Standard	.....	20 µl	.....
Specimen	.....	.....	20 µl

Mix, incubate for 5 minute at 20-25^{0}C. Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.
The color is stable for 60 minutes.

Automated Procedure

User defined parameters for different auto analyzers are available upon request.

CALCULATION

Calculate the total protein concentration by using the following formulae:

Total protein Concentration=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times \text{Standard value}$$

- **Unit conversion**

mg/dl x 1.45 = mmol/l

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or

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- At least once a shift, or
- When a new bottle of reagent is used, or
- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established total protein values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Anticoagulants:**
  Heparin and EDTA are the only accepted anticoagulants.
- **Bilirubin:**
  No significant interference from free or conjugated bilirubin up to a level of 25 mg/dl.
- **Drugs:**
  Young[7] in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.
- **Haemoglobin:**
  Haemoglobin levels higher than 7.5 g/l increase the apparent total protein concentration significantly.
- **Lipemia:**
  No significant interference.

WARNING & PRECAUTION

- Vitro total protein reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- The reagents should be brought to room temperature before use.
- The reagent and sample volumes may be altered proportionally to accommodate different spectrophotometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- Don't use the reagent if it is turbid.
- Reagent 2 contains sodium hydroxide which is can cause caustic burns. In the event of contact, flush affected area with copious amount of water. Get immediate medical attention after contact with eyes.

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run	
Control	Level I	Level II
Number of samples	40	40
Mean (g/dl)	5.8	4.9
SD (g/dl)	0.08	0.06
CV (%)	1.4	1.3

	Between Day	
Control	Level I	Level II
Number of samples	40	40
Mean (g/dl)	5.8	4.9
SD (g/dl)	0.19	0.16
CV (%)	3.2	3.4

Method Comparison

Comparison studies were carried out using another commercially available Total Protein reagent as a reference. Normal and abnormal human serum samples were assayed in parallel and the results compared by least squares regression. The following statistics were obtained.

Number of sample pairs	60
Range of sample results	2.1-9.2 g/dl
Mean of reference method results	7.18 g/dl
Mean of Total Protein reagent results	7.15 g/dl
Slope	0.955
Intercept	0.29 g/dl
Correlation coefficient	0.9844

Sensitivity

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a pathlength of 1 cm.

When run as recommended the sensitivity of this assay is 0.2 g/dl.

LINEARITY

When run as recommended, the assay is linear up to 15 g/dl.

If result exceeds 13 mg/dl, specimen should be diluted with 0.9% NaCl solution and reassayed. Multiply the result by the dilution factor.

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SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
13501	2 X 100 ml
13502	2 X 125 ml
13503	4 X 125 ml
13504	1 X 500 ml
13505	2 X 500 ml

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