UDI

المصنع المتحد للكواشف الطبية
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QUALITY MANAGEMENT SYSTEM ISO 9001-2000 ASR CERTIFICATE NO. 1341 CERTIFIED

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TOTAL PROTEIN (COLOR/ENDPOINT) (BIURET METHOD)

REF 056

FOR IN VITRO DIAGNOSTIC USE

INTENDED USE
Quantitative determination of total protein in serum using a biuret reaction according Gornall.

DIAGNOSTIC SIGNIFICANCE
Through osmotic pressure, serum protein is involved in the maintenance of normal distribution of water between blood and tissues. The several fractions of serum protein vary independently and widely in disease. Low protein is primarily caused by malnutrition, impaired synthesis, loss (as by haemorrhage) or excessive protein catabolism.
Elevated protein levels are caused mainly by dehydration[1].

RANGE OF EXPECTED VALUES IN SERUM[2]
Healthy young and middle aged adults
6.0-8.0 g/dl (60-80 g/l) Recumbent
6.5-8.5 g/dl (65-85 g/l) Ambulatory.

METHOD PRINCIPLE
The biuret reaction is considered to be the method of choice for the clinical laboratory. Many modifications have been proposed to increase the stability of the copper salt required in the reaction.

The UDI procedure is based on the Gornall's[3] modification of the biuret reaction as proposed by the American Association for Clinical Chemistry (AACC)[4] and NationalCommittee for Clinical Laboratory Standards (NCCLS)[5].

The reaction sequence employed in the assay of Total Protein is as follows:

$$\text{Protein} + cu^{++} \xrightarrow[\text{pH}]{\text{Alkaline}} \text{cu -Protein Complex}$$

Protein in serum forms a blue 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.

REAGENTS
1. BIURET REAGENT: 0.3% w/v Cupric sulfate pentahydrate, 0.9% w/v Potassium sodium tartrate tetrahydrate, 0.5% w/v Potassium iodide in 0.2 N Sodium hydroxide. Keep tightly capped and protected from excessive exposure to direct sunlight. Can be used until the expiration date indicated on the bottle label.

2. TOTAL PROTEIN STANDARD (5 g/dl) : Bovine albumin fraction V with preservative. Keep tightly capped and protected from freezing. Can be used until the expiration date indicated on the bottle label.

STORAGE INSTRUCTIONS
Once opened, use the reagent within one month. Unopened kit is stable up to expiration date at *2°C to 8 °C*

CHEMICAL PRECAUTIONS
Exercise the normal precautions required for the handling of laboratory reagents. Pipetting by mouth is not recommended to any laboratory reagent

INDICATIONS OF REAGENT DETERIORATION
1. Physical appearance
The presence of a black or brown-to-red precipitate indicates reagent deterioration. The reagent must not be used if this is observed.

2. Control Assays
Failure to obtain accurate results in the assay of control materials may indicate reagent deterioration.

NOTE: UDI cannot guarantee the stability of reagents which have been:
- transferred from their original containers.
- improperly stored.
- contaminated during use.

SPECIMEN
SERUM
There is reportedly [6] no change in the total protein of clear samples kept at room temperature for one week.

MATERIAL PROVIDED
BIURET REAGENT AND TOTAL PROTEIN STANDARD (5 g/dl)

ADDITIONAL MATERIALS REQUIRED, BUT NOT PROVIDED
Reagent and sample pipettes, test vials or cuvettes, test tube rack, control serum, spectrophotometer.

PROCEDURE (AUTOMATED)
Refer the appropriate instrument application guide available from us.

1. PROCEDURE (MANUAL) MICRO METHOD
Pipette into a clean dry test tube:

	BLANK	STANDARD	TEST
Biuret Reagent	1.0 ml	1.0 ml	1.0 ml
Standard	-	0.02 ml	-
Sample	-	-	0.02 ml
Mix and let stand at room temperature for 10 minutes Read the absorbance of standard and test at 540 ± 5 nm against blank.			

2. PROCEDURE (MANUAL) MACRO METHOD

	BLANK	STANDARD	TEST
Biuret Reagent	2.5 ml	2.5 ml	2.0 ml
Standard	-	0.05 ml	-
sample	-	-	0.05 ml
Mix and let stand at room temperature for 10 minutes Read the absorbance of standard and test at 540 ± 5 nm			

STABILITY OF ENDPOINT REACTION
The final colour developed in the reaction is stable for at least 60 minutes.

CALCULATION OF RESULTS
Use the absorbance readings of STANDARD and TEST to calculate the total protein values as follows: (A = absorbance)

$$\frac{A\ (TEST)}{A\ (STANDARD)} \times \text{Conc. of STANDARD (g/dl)} = \text{Total Protein in TEST (g/dl)}$$

EXAMPLE: Assume the value of STANDARD to be 5 gm/dL and that it gave an absorbance of 0.433, while the TEST gave an absorbance of 0.55. The total protein concentration of TEST may then be calculated as follows:

$$\frac{0.55}{0.433} \times 5 \text{ g/dl} = 6.3 \text{ g/dl}$$

PROCEDURE LIMITATIONS

Moderate amounts of bilirubin, hemoglobin and lipids do not cause significant interferences with the total protein procedure[2]. However, gross hemolysis should be avoided, since the released hemoglobin, being itself a protein will cause spurious increases in serum protein levels[7].

The total protein concentration in urine and spinal fluid is too low for the biuret reagent to detect. Therefore, this procedure must not be employed for the analysis of these specimens.

For a comprehensive review of drug effects on clinical laboratory test, see reference 8.

PERFORMANCE CHARACTERISTICS

- **LINEARITY:** This method is linear up to 14.0 g/dl.
- **COMPARISON:** UDI reagent tested on MANUAL SYSTEMS (y) was compared with CAPS survey results and similar UDI reagent for other systems(x). The systematic differences between the results were within CLIA specified limits. N = 36

Correlation Coefficient	0.967
Regression Equation	y = 1x + 0.11

- **PRECISION:**

	Mean g/dL	SD	CV%
Within run	4.6	0.08	1.71
Run to run	4.4	0.15	3.46

PROCEDURE NOTES

If the total protein concentration exceeds 14.0 gm/dL (or the linear capability of the spectrophotometer), make a 1:1 dilution of the sample with 0.85% saline and re-run the test as above. Multiply the results by the dilution factor of 2.

QUALITY CONTROL

For accuracy and precision check, we recommend use of normal and abnormal UDI controls based on human serum.

ORDERING INFORMATION:

UDITROL 'N' (Normal Serum Control) REF#070N-010 2x5 ml
UDITROL 'A' (Abnormal Serum Control)REF#070A-010 2x5 ml

REFERENCES

1. Peters, T. and Biamonte, G.T., Selected Methods for the Small Clinical Chemistry Laboratory. Faulber, W.R., and Meites, S., Ed.
2. Tietz, NW, Fundamentals of Clincal Chemistry, WB Saunders, Philadelphia p. 299 (1976).
3. Gornall, AG et al, J Biol Chem, 177:751 (1949).
4. Doumas, BT. et al. Clin. Chem. 27:1642 (1981).
5. NCCLS approved standards : ACS-1, specification for standardized protein solution (Bovine Serum Albumin), 2nd ed., National Committee for Clinical Laboratory Standards, 771 E Loncaster Ave., Villanova, PA 19085, (1979).
6.Henry, RJ..: Clinical Chemistry:Principles & Techniques, Harper & Row, New York p. 173 (1964).
7. Doumas, BT., and Biggs, HG. Standard Methods of Clinical Chemistry, Vol. 7, Academic Press, New York, p. 175 (1972).
8. Young, DS et al, Clin Chem, 21:1D (1975).

EC REP
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PRODUCT AVAILABILITY

TOTAL PROTEIN (COLOR/ENDPOINT)

REF # 056-960	**4 x 240 ml**
REF # 056-480	**4 x 120 ml**
REF # 056-360	**3 x 120 ml**
REF # 056-240	**2 x 120 ml**
REF # 056-120	**1 x 120 ml**