POINTE SCIENTIFIC, INC.
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Total Protein (Biuret) Reagent Set

Intended Use
For the quantitative determination of total protein concentration in serum. For *in vitro* diagnostic use only.

Method History
The color reaction of protein molecules with cupric ions, known as the Biuret color reaction, has been known since 1878. Since the Riegler[1] publications of 1914, several attempts have been made to stabilize the cupric ions in the alkaline reagent. Kingsley, [2,3] modified the procedure in 1939 and 1942 to include the use of sodium potassium tartrate as a complexing agent. This procedure was later modified by Weichselbaum[4] and Gornall.[5] The present method is based on these modifications.

Principle
Alkali
Protein + Cu^{++} ----------➢ Colored 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.

Reagent Content
After combining R1 and R2 the reagent contains: sodium hydroxide 750mM, copper sulfate 6mM, sodium potassium tartrate 35mM, potassium iodide 6mM, non-reactive ingredients.

Precautions
1. This reagent is for *in vitro* diagnostic use only.
2. Avoid ingestion. DO NOT PIPETTE BY MOUTH. In case of ingestion drink large amounts of water and seek medical attention quickly.
3. Avoid contact with skin and eyes. The reagent contains sodium hydroxide which is corrosive. In case of contact with skin, flush with water. For eyes, seek medical attention.

Reagent Preparation
The reagents are ready to use for analyzers capable of handling two reagents.

Reagent Storage
Store reagents at room temperature.

Reagent Deterioration
Each reagent should be a clear solution. Turbidity or the presence of a black precipitate indicates reagent deterioration and should not be used.

Specimen Collection and Storage
1. Unhemolyzed serum is the specimen of choice.
2. Gross hemolysis will cause elevated results because of the released hemoglobin as well as the increase in background color.
3. Lipemic sera cause elevated results and should be run with a serum blank.
4. Samples with bromosulfophthalein (BSP) will result in falsely elevated results.[8]
5. Protein in serum is stable for one week at room temperature (18-25°C) and for at least one month refrigerated (2-8°C) when guarded against evaporation.[6]

Interferences
Young, et al[7] has reviewed a number of drugs and substances that may affect protein concentrations.

Materials Provided
Total protein R1 and R2 reagents.

Materials Required but not Provided
1. Beckman Coulter AU™ analyzer
2. Instrument application and Operation manuals.
3. Calibrators and controls

Procedure (Beckman Coulter AU™400 application)

SPECIFIC TEST PARAMETERS

TEST NUMBER: **#** TEST NAME: **TP** ∇ TYPE: **Serum** ∇ OPERATIONAL: **Yes** ∇
SAMPLE VOL.: **2** DIL. VOL.: **0** PRE-DILUTION RATE: **1**
REAGENTS: R1 VOLUME: **150** DIL. VOL.: **0** MIN. OD MAX. OD
R2 VOLUME: **60** DIL. VOL.: **0** L H
REAGENT OD LIMIT:
WAVELENGTH: PRI. **570** ∇ SEC. **800** ∇ FIRST L: **-0.100** FIRST H: **0.900**
METHOD: **END**∇ LAST L: **-0.100** LAST H: **0.900**
REACTION SLOPE: **+** ∇ DYNAMIC RANGE:
MEASURING POINT 1: FIRST: **5** LAST: **27** L: **#** H: **#**
MEASURING POINT 2: FIRST: LAST: CORRELATION FACTOR:
LINEARITY: % A: **1.000** B:**0.000**
NO LAG TIME: ∇ ON BOARD STABILITY PERIOD: **#**

SPECIFIC TEST PARAMETERS

VALUE FLAG: **#** ∇ LEVEL L: **#** LEVEL H: **#**

NORMAL RANGES:	SEX	AGE L YEAR	AGE L MONTH	AGE H YEAR	AGE H MONTH	L	H
○ 1.	# ∇	#	#	#	#	#	#
○ 2.	# ∇	#	#	#	#	#	#
○ 3.	# ∇	#	#	#	#	#	#
○ 4.	# ∇	#	#	#	#	#	#
○ 5.	# ∇	#	#	#	#	#	#
○ 6.	# ∇	#	#	#	#	#	#
7. NONE SELECTED						#	#
8. OUT OF RANGE		L	H			#	#
PANIC VALUE:		#	#	UNIT: **g/dl**		DECIMAL PLACES: **1**	

CALIBRATION SPECIFIC PARAMETERS

CAL TYPE: **AB** ∇ FORMULA: **Y=AX+B** ∇ COUNTS: **2** PROCESS: **CONC.** ∇

	CAL. NO.	OD	CONC.	FAC/OD-L	FAC/OD-H
POINT 1.	#		#	**-9999999**	**9999999**
POINT 2.					
POINT 3.					
POINT 4.					
POINT 5.					
POINT 6.					
POINT 7.					

1-POINT CAL. POINT: ○ WITH CONC-0
MB TYPE FACTOR: CALIBRATION STABILITY PERIOD: **#**

#: User-Defined

Total Protein (Biuret)
Reagent Set

The above reagent parameters are intended to serve as a guide for use with Pointe Scientific, Inc. reagent. The parameters are based on data generated by Pointe Scientific, Inc. Please note: These parameters should be used in conjunction with your laboratory Quality Control Program for validation.

NOTE: For other instrument specific applications please contact Pointe Scientific, Inc. Technical Service Department at 1-800-445-9853

Procedure Notes

1. Serums with values above 12.0 g/dl should be diluted 1:1 with 0.9% saline, and the final answer multiplied by two.
2. ALTERNATE VOLUMES: 50ul sample to 3.0ml reagent. Calculations remain the same.

Calibration

Use an NIST-traceable serum calibrator. The procedure should be calibrated according to the instrument manufacturer's calibration instructions. If control results are found to be out of range, the procedure should be recalibrated.

Quality Control

1. The validity of the reaction should be monitored by use of the control sera with known normal and abnormal total protein values. These controls should be run at least with every working shift in which total protein assays are performed. It is recommended that each laboratory establish its own frequency of control determination.
2. Some lyophilized control sera are grossly turbid and require a serum blank. (See SPECIMEN COLLECTION AND STORAGE).

Calculation

Abs. = Absorbance

$$\frac{\text{Abs. of Unknown}}{\text{Abs. of Standard}} \times \text{Conc. of standard} = \text{Total Protein (g/dl)}$$

Example: Abs. of Unknown = 0.350, Abs. of Standard = 0.400
Concentration of Standard = 8 g/dl

Then: $\frac{0.350}{0.400} \times 8 = 7.00$ g/dl

Limitations

1. Samples with values above 12.0 g/dl should be diluted 1:1 with 0.9% saline, re-run and result multiplied by two.
2. The Biuret procedure is not sensitive at low ranges (<1 g/dl). Do not use for urine or spinal fluid.

Expected Values[8]

6.2 – 8.5 g/dl

1. The effect of posture, when blood is drawn, varies with the individual but recumbent values are usually lower than ambulatory. Differences may be as much as 1.2 g/dl.
2. It is strongly recommended that each laboratory establish its own range.

Performance

1. Linearity: 1.0 – 12.0 g/dl
2. Comparison: A comparison study performed between the Beckman Coulter AU™400 and Roche Hitachi 717 using this method resulted in a correlation coefficient of r = 0.994 with a regression equation of y=0.994x + 0.13. (n = 37, range 3.5 – 8.4 g/dl)
3. Precision:
   Within - day precision study was performed using three levels of material.
   Between - day precision study was performed using two levels of control material assayed over a 20 day period with 2 runs per day and 2 replicates per run.

Within Day (N=20)			Day to Day		
Mean	**S.D.**	**C.V.%**	**Mean**	**S.D.**	**C.V.%**
4.3	0.09	2.1	4.1	0.12	3.0
7.0	0.14	2.0	7.5	0.22	2.9
12.2	0.13	1.1			

Precision and Linearity studies were performed following modifications of CLSI Protocols EP5 and EP6[9] using a Beckman Coulter AU™400 analyzer

References

1. Riegler, E., Anal. Chem. 53:242 (1914).
2. Kingsley, G.R., J. Biol. Chem. 131:197 (1939).
3. Kingsley, G.R., J. Lab. Clin. Med. 27:840 (1942).
4. Weichselbaum, T., Amer. J. Clin. Path. 16:40 (1946).
5. Gornall, A., et al, J. Biol. Chem. 177:752 (1949).
6. Henry, R.J., et al, Clinical Chemistry: Principles and Technics, Harper & Row, New York, p. 415 (1974).
7. Young, D.S., et al, Clin. Chem. 21:1D (1975).
8. Tietz, N.W., Fundamentals of Clinical Chemistry Philadelphia, W.B. Saunders, pp. 299, (1976).
9. CLSI protocols EP5-A2, Vol. 24 No. 25, 2nd Ed, (2004) and CLSI EP6-A, Vol. 23 No. 16 (2003)

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