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

Intended Use
For the quantitative determination of total protein concentration in serum.

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
$$\text{Protein} + Cu^{++} \xrightarrow{\text{Alkali}} \text{Colored Complex}$$

Protein in serum forms a violet 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
Sodium Hydroxide 600mM, Copper Sulfate 12mM, Sodium Potassium Tartrate 32mM, Potassium Iodide 30mM, 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
Reagent comes in a ready to use form.

Reagent Storage
Store reagent at room temperature.

Reagent Deterioration
The reagent should be a clear, pale blue 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.
    a. Place 1.0ml 0.9% saline in test tube.
    b. Add 0.02ml (20ul) sample.
    c. Zero spectrophotometer with 0.9% saline.
    d. Read and record absorbance of serum blank.
    e. Subtract blank absorbance from test absorbance.
    f. Calculate as usual.
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 reagent.

Materials Required but not Provided
1. Accurate Pipetting devices.
2. Timer.
3. Test Tubes and rack.
4. Spectrophotometer.

Procedure (Automated)
Refer to specific instrument application instructions.

Procedure (Manual)
1. Label test tubes “Blank”, “Standard”, “Control”, “Patient”, etc.
2. Pipette 1.0ml of working reagent to each tube.
3. Add 0.02ml (20ul) of standard and patients to appropriate tubes and mix by inversion.
4. Let the tubes stand at room temperature (18-25°C) for 5 minutes.
5. Set the spectrophotometer at 540nm and zero instrument with the reagent blank.
6. Read and record absorbance readings of each tube.

Procedure Notes
1. Final color is stable for 60 minutes at room temperature.
2. Serums with values above 15.0 g/dl should be diluted 1:1 with 0.9% saline, and the final answer multiplied by two.
3. ALTERNATE VOLUMES: 50ul sample to 3.0ml reagent. Calculations remain the same.

Calibration
Use an aqueous Protein standard (8 g/dl) or serum calibrator.

Quality Control
1. Use control sera with known total protein concentrations to monitor the integrity of the reaction.
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 15.0 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 – 15.0 g/dl
2. Comparison: A comparison study when performed between this procedure and another procedure based on the same principle resulted in a correlation coefficient of 0.999 with a regression equation of y=1.00 x + 0.00.
3. Precision:

Within Run			Run to Run		
Mean	S.D.	C.V.%	Mean	S.D.	C.V.%
4.4	0.04	0.9	4.5	0.05	1.1
5.5	0.04	0.7	5.6	0.10	1.8

References

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