**Intended Use**
For the quantitative determination of total protein concentration in serum using the Mindray BS-200 analyzer.

**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\(^4\) 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\(^6\) and Gornall.\(^5\) The present method is based on these modifications.

**Principle**
Alkali
Protein + Cu\(^{++}\) \[\text{Colored Complex}\]

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\(15\text{-}30^\circ \text{C}\). The reagent is stable until the expiration date appearing on the label when stored as directed.

**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. A serum Blank should be performed.
4. Samples with bromosulfophthalein (BSP) will result in falsely elevated results.\(^6\)
5. Protein in serum is stable for one week at room temperature \(18\text{-}25^\circ \text{C}\) and for at least one month refrigerated \(2\text{-}8^\circ \text{C}\) 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. Mindray BS-200 Analyzer
2. BS-200 operation manual
3. Chemistry Calibrator, catalog number C7506-50
4. Chemistry control, catalog number C7592-100

**BS-200 Test Parameters**

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<tr>
<th>Test</th>
<th>TPRO</th>
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<th>R2:</th>
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**Calibration Parameters**

<table>
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<tr>
<td>Coefficient:</td>
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**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 re-calibrated.

**Quality Control**
1. Use control sera with known total protein concentrations to monitor the integrity of the reaction.
2. Quality control requirements should be performed in conformance with local, state, and/or Federal regulations or accreditation requirements.
Total Protein
(Biuret)
Reagent Set

Calculation (Example)
Abs. = Absorbance

Abs. of Unknown x Conc. of = Total Protein (g/dl)
Abs. of Standard standard

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

Then: \( \frac{0.350 \times 8}{0.400} = 7.00 \text{ g/dl} \)

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
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 study was performed between the Mindray BS-200 and a similar analyzer and method, resulting in a correlation coefficient of 0.996 with a regression equation of \( y=0.919x + 0.36 \).
3. Precision: Precision studies were performed using the Mindray BS-200 analyzer following a modification of the guidelines which are contained in NCCLS document EP5-T2.9

<table>
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<tr>
<th>Within Run</th>
<th>Day to Day</th>
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<td>7.46</td>
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References