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Total Protein Examination Using the Biuret Method

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ABSTRACT

Protein is the main cellular component that makes up half of the weight of cells and has an important role in the structure and function of organisms.1Proteins are formed from one or more polypeptides which play a role in forming specific similarities (Figure 1).2Protein has a special function, namely building and maintaining body cells and tissues.2Total protein is all types of protein found in serum or plasma consisting of albumin, globulin and fibrinogen.3Protein analysis can be done in two ways, namely qualitatively and quantitatively. Qualitative protein analysis is by Xanthoprotein reaction, Hopkins-Cole reaction, Millon reaction, Nitropusicide reaction, and Sakaguchi reaction, while quantitative protein analysis is done using the Kjedahl method, formol titration method, Lowry method, visible spectrophotometry (Biuret) method, and UV spectrophotometry method.3 Examination of total protein levels in the blood is carried out using the biuret method which is seen from the color reaction between the alkaline copper reagent and the CO-NH peptide chain which produces a purple color. 4 This method is based on the number of fixed CO-NH chains per unit mass of protein. The working principle of this method is the color equation with copper ions, where the protein in the serum reacts with copper ions in an alkaline (basic) environment, thus forming a purple complex compound whose color intensity is proportional to the total protein content in the blood. 4,5 The intensity of the purple color indicates the total protein content in the sample so that it can be assessed photometrically by measuring its absorbance using a spectrophotometer at a light wavelength of 546 nm. The advantages of the biuret method compared to other methods in measuring total protein levels are that the examination is not affected by temperature, produces different colors in different proteins, has good examination repeatability, and high accuracy, while the disadvantages of the biuret method are low sensitivity and many interfering factors, EDTA samples can interfere with the interpretation of the results.

Keywords: total protein; biuret method; spectrophotometer.

INTRODUCTION

Protein is the main cellular component that makes up half of the weight of cells and has an important role in the structure and function of organisms.[1]Proteins are formed from one or more polypeptides which play a role in forming specific similarities (Figure 1).[2] Protein has a specialfunction, namely building and maintaining body cells and tissues.[2] Total protein is all types of protein found in serum or plasma consisting of albumin, globulin, and fibrinogen.3Proteins in the body that are globularin shape are called globular proteins.

Globular proteins are classified based on their chemical properties, namely albumin and globulin.

Albumin is the main protein with an amount of 55% of the total protein, globulin with an amount of 38% of the total protein, and fibrinogen with an amount of 7% of the total protein[4]. All total proteins are synthesized in the liver except gamma globulin. Albumin is a single-chain polypeptide with a molecular weight of 65 kD and contains 584 amino acids and functions to maintain oncotic pressure so that blood cells remain in the

blood vessels, which help transport fat, are important for growth and tissue healing. Globulin is a single-chain polypeptide with a molecular weight of 66 kD and contains 585 amino acids that function totransport and bind to metals such as iron in the blood and function in the immune system. Globulin consists of different types of proteins, namely alpha, beta, and gamma types.4Fibrinogen is a single chain polypeptide with a molecular weight of 66 kD and contains 585 amino acids and has a function in the coagulation system.[4]

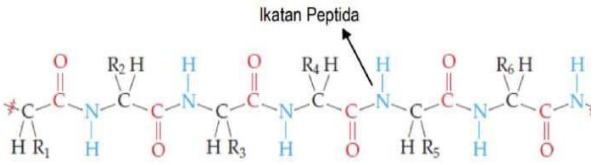


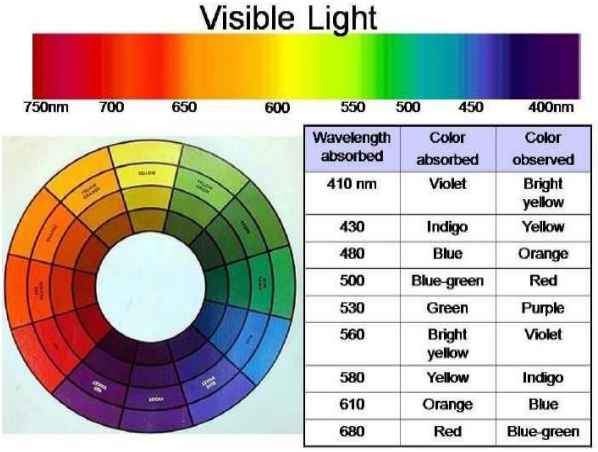
FIGURE 1: Primary structure of protein[4].

Total protein examination can use venous blood made into plasma or serum. Making plasma from venous blood will give an osmotic effect because the addition of anticoagulants causes water to leave the cells and enter the plasma, thus thinning the plasma and reducing the concentration. The effect of this effect depends on the type and concentration of anticoagulant, so the use of serum is more recommended because the serum concentration of lipoprotein will get more accurate results according to the patient's condition when taking the blood specimen.[5] Serum is the fluid that remains after blood is allowed to clot in a tube. Serum is similar to plasma except that fibrinogen and other coagulation factors are reduced by the clotting process.[5]

Total protein is affected by factors that result in increased ordecreased test results. These factors are patient preparation and sample preparation during total protein level testing.[5] Total protein levels are affected by patient preparation, namely diet. Excess protein in the body due to frequent consumption of foods containing animal and vegetable protein can increase total protein levels, while protein deficiency in the body due to insufficient consumption of foods containing protein can also decrease total protein levels.[6]Sample preparation factors affect total protein levels, such as examination using plasma samples can cause total protein levels to be 3–5% higher due to the influence of fibrinogen in plasma.The use of a tourniquet increases total protein levels in the blood because prolonged holding with hard pressure when taking venous blood samples causes hemoconcentration and blood infiltration into the tissue. Other factors that affect total protein levels include body weight, age, growth, hormones, gender, pregnancy, lactation, nutrition, stress, and fluid loss.[6]

Protein analysis can be done in two ways, namely qualitatively and quantitatively. Qualitative protein analysis is by Xanthoprotein reaction, *Hopkins-Cole* reaction, *Millon* reaction, *Nitropusicide* reaction, and *Sakaguchi* reaction, while quantitative protein analysis is done using *Kjedahl* method, formol titration method, *Lowry* method, *visible spectrophotometry* (Biuret) method, and *UV spectrophotometry* method.[3] In this tutorial we will discuss the examination of total protein in serum/plasma using the biuret method (*visible spectrophotometry).* The advantages of thebiuret method compared to other methods in measuring total protein levels are that the examination is not affected by temperature, produces different colors in different proteins, has good examination repeatability, and high accuracy, while the disadvantages of the biuret method are low sensitivity and many interfering factors, EDTA samples can interfere with the interpretation of the results.

Examination of total protein levels in the blood is carried out using the biuret method which is seen from the color reaction between the alkaline copper reagent and the CO-NH peptide chain which produces a purple color.[7] This method is based on the number of fixed CO-NH chains per unit mass of protein. The working principle of this method is the color equation with copper ions, where the protein in the serum reacts with copper ions in an alkaline (basic) environment, thus forming a purple complex compound whose color intensity is proportional to the total protein content in the blood.[7,8] The intensity of the purple color indicates the total protein content in the sample so that it can be assessed photometrically by measuring its absorbance using a spectrophotometer at a light wavelength of 546 nm (Figure 2).



Wavelength absorbed	Color absorbed	Color observed
410 nm	Violet	Bright yellow
430	Indigo	Yellow
480	Blue	Orange
500	Blue-green	Red
530	Green	Purple
560	Bright yellow	Violet
580	Yellow	Indigo
610	Orange	Blue
680	Red	Blue-green

FIGURE 2: Wavelength of colored light.

Total protein examination using the biuret method uses a spectrophotometer to read the wavelength of light. Spectrophotometry as the name implies is a tool consisting of a spectrometer and a photometer.

The spectrophotometer produces light from a spectrum with a certain wavelength and the photometer is a tool for measuring the intensity of light transmitted or absorbed. So, the spectrophotometer is used to measure the relative energy if the energy is transmitted, reflected, or emitted as a function of wavelength. The working principle of the spectrophotometer is the electromagnetic spectrum covers a wide wavelength region from high-energy short-wave gamma rays to microwaves. The absorption spectrum in the ultraviolet and visible regions generally consists of one or more wide absorption bands, all molecules can absorb radiation in the UV-visible region. The wavelength at the time of absorption depends on how tightly the electrons are bound in the molecule. Electrons in a single covalent bond are tightly bound and radiation with high energy, or short wavelengths, is required for excitation. Simply put, a spectrophotometer instrument called a spectrophotometer consists of a Light source – monochromatic – sample cell – detector – readout (Figure 3).[9]

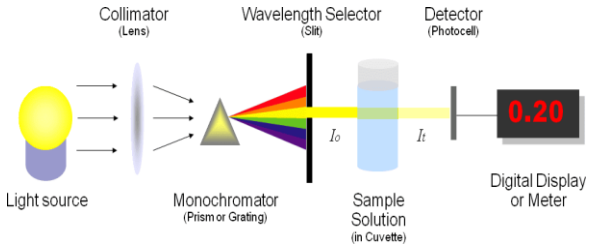


FIGURE 3: How a spectrophotometer works[9].

A decrease in total protein levels can occur in patients who experience prolonged malnutrition, starvation, low-protein diets, malabsorption syndrome, gastrointestinal cancer, ulcerative colitis, Hodgkin's disease, severe liver disease (*terminal liver failure)*, chronic renal failure, severe burns, and water intoxication. Increased total protein levels can occur in patients who experience dehydration (hemoconcentration), vomiting, diarrhea, multiple myeloma, respiratory distress syndrome, *chronic liver disease,* and sarcoidosis.[10]

AIM

This tutorial aims to explain the method and principles of quantitative examination of total protein levels using the biuret method.

METHOD

(A) Pre-analytic[10]

1. Patient preparation

No special preparation of the patient is required before sampling

2. Sample preparation

a. The sample used is serum or plasma EDTA or heparin
b. Hemolysis, lipemic, and icteric samples are not recommended
c. Storage of stable serum/plasmas can be stored in the refrigerator for 3 days at a temperature of 2-8°C and 6 months at a temperature of 20°C

3. Reagent Preparation

Reagent storage will be stable up to the specified expiration date, if stored at a temperature of 2-8oC. Keep the reagent away from directsunlight and also do not freeze it. Avoid shaking the reagent vigorously. The reagent is ready to use.

4. Instruments and materials

a. Test tube
b. Micropipette
c. Blue and yellow tip
d. LISS ID rack/ coombs test card
e. Reagent 1 :*Biuret Reagent*(consist of :*cupric sulphate, potassium iodide, Na+/K+tartarate, sodium hydroxide, stabilizers, inactive ingredients and surface-active agents)*
f. Reagent 2 :*Total Protein Standard*(6mg/dL)
g. Serum sample
h. *Incubation chamber*
i. Photometer
j. Tissue and timer

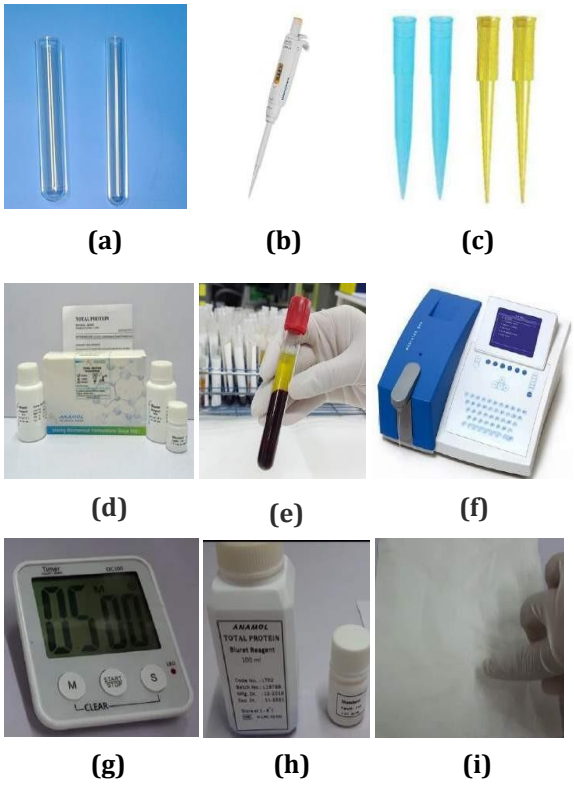


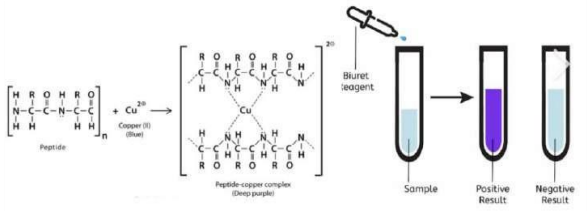
FIGURE 1: Tools and materials; (a) Test tube, (b) Micropipette, (c) Blue and yellow tip, (d) Kit components, (e) Serum sample, (f) Photometer, (g) Timer, (h) Biuret reagent and, Total Protein Standard (i) Tissue (Source: Instructions Total Protein Method-Biuret, 2016 Anamol Laboratories)[10] (Source: Personal documentation).

(B) Analytic

1. Test principle[10]

Proteins bind to copper forming a purple complex in an alkaline solution. *Cupric sulphate*on is the reagent that will complex the peptide bond in a basic environment. The intensity of the purple color indicates the total protein content in the sample so that it can be assessed photometrically by assessing its absorbance using a spectrophotometer at a light wavelength of 546 nm.

The absorbance of this complex is proportional to the protein concentration in the sample (Figure



2).

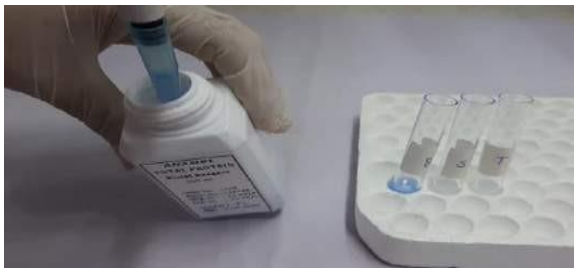
FIGURE 2: Biuret Method Test Principle[10].

2. The procedure[10]

a. Prepare the tools and materials to be used.
b. Prepare three test tubes labeled B (*Blank),* S (*Standard), and* T (*Test).*
c. Mix the reagents and samples/standard above according to the specified and homogenize.

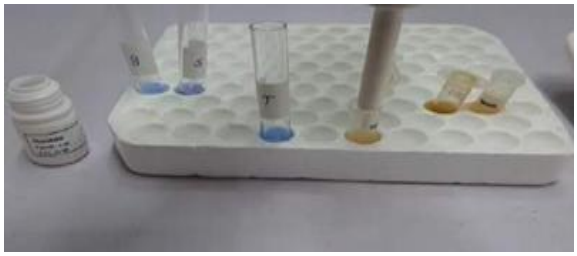
	Blank	*Standard*	*Test*
Reagent	1000 µl	1000 µl	1000 µl
Standard	NA	10 µl	NA
Sample	NA	NA	10 µl

d. Add 1000 µl of biuret reagent to test tube B (*Blank),* S (*Standard),* T (*Test).*



e. Add 10 µl of standard total protein reagent into test tube S (Standard) and homogenized.

f. Add 10 µl of serum sample into test tube T (*Test*) and homogenized.



g. Incubate three tubes B (*Blank*), S (*Standard*), T (*Test*) for 5 minutes at 37^{0}C.

h. Aspirate the reaction mixture in test tubes B (*Blank*), S (*standard*), and T (*Test*) into *the flow cell* and measure the absorbance of the sample at a wavelength of 546 nm.

h. The results of the total protein examination will be calculated based on the calculation formula and the results will be recorded.

$$Total\ Protein\ (mg/dL) = \frac{Abs.of\ sample\ x\ 6}{Abs.of\ standard}$$

(C) Post-analytic[10]

1. Reference value

Adults = 6.2 – 8.5 mg/dL

2. Interpretation

a. Hyperproteinemia

The total protein level is above the normal value. Total protein levels increase in dehydration, multiple myeloma, *and chronic liver disease.*

b. Hypoproteinemia

The total protein level is above the normal value. Total protein levels decrease in chronic kidney failure and liver failure (*terminal liver failure)*, severe burns, malnutrition, low protein diet, gastrointestinal cancer, ulcerative colitis, and Hodgkin's disease.

3. Linearity

a. Lowest linearity: 0.35 mg/dL
b. Highest linearity: 18 mg/dL

4. Troubleshooting

If the total protein value exceeds the linearity limit, the valueabove 18 g/dL is diluted with normal saline at a ratio of 1:1, homogenized, and re-examined. In this case, the test value must be multiplied by the dilution factor, which is multiplied by 2 to obtain the correct total protein value. For example, the total protein result obtained is 20 mg/dL, then multiplied by 2, the result becomes 40 mg/dL. The diluent solution used can use 0.9% NaCl or sterile aquabidest. However, the most commonly used is 0.9% NaCl, but if there is interference in the examination, especially electrolyte disturbances, it is better to use sterile aquabidest.

5. Limitations

a. There is significant interference in samples containing bilirubin up to 20 mg/dL and hemoglobin up to 500 mg/dL
b. This kit is intended for in vitro diagnostic use only.
c. Discard the reagent if the absorbance of the reagent exceeds 0.300 ODagainst D/W at 546 nm.
d. Avoid direct contact with light when assessing total protein values, the final color value results are stable for 8 hours if notexposed to direct light.

Conflict of Interest Statement :

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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