College of pharmacy

Clinical laboratory training

Fifth stage

Dr. Maytham Ahmed

Lecture: 5

Blood Proteins and Bilirubin

Blood Proteins and Bilirubin

Total Protein Test

The total protein test is a simple test. It looks for normal or abnormal protein levels in the body. It may be used to aid in the diagnosis of nutritional problems, kidney diseases or liver diseases.

Principle: The serum proteins are a diverse group consisting of transport proteins, enzymes, immunoglobulins, inhibitors, and others. In spite of functional differences between the various serum proteins, they have certain common biophysical and biochemical properties. These include (1) a basic composition of carbon, hydrogen, nitrogen, and oxygen; (2) a backbone of peptide bonds which join the amino acid units together; and (3) absorption maxima in the ultraviolet and far ultraviolet regions. Based on these properties, laboratory methods have been developed to determine the concentration of proteins in serum, often with the assumption that each of the several hundred individual proteins present in serum reacts similarly in chemical reactions.

The major measured serum proteins are divided into two groups albumin and globulin. There are four major types of globulin (alpha-1, alpha-2, beta, and gamma globulins) each with specific properties and actions.

Gamma globulins are a class of globulins, identified by their position after serum protein electrophoresis. The most significant gamma globulins are immunoglobulins (antibodies), although some immunoglobulins are not gamma globulins, and some gamma globulins are not immunoglobulins.

The five primary classes of immunoglobulins (antibodies) are IgG, IgM, IgA, IgD, and IgE. These are distinguished by the type of heavy chain found in the molecule.

The **Biuret method** is commonly used to estimate total serum protein in the given sample. Proteins give violet-blue complex with copper salts in an alkaline medium. The intensity of the color formed is proportional to the total protein concentration in the sample (Biuret reaction).

Specimen: Serum and plasma may be used and yield comparable results, although because of the presence of **fibrinogen**, plasma concentration of total protein is 2 to 4 g/L higher than serum concentration. A fasting specimen is not required but may be desirable to decrease lipemia. Lipemia causes clinically significant interferences for phosphorus,

creatinine, total protein and calcium measurement. Turbid samples (e.g., due to lipemia) must be treated before analysis. Total protein is stable in serum and plasma for 1 week at room temperature , for at least 1 month when refrigerated, and for at least 2 months at −20°C. Heat treatment (56°C, 30 minutes) of serum does not cause any clinically significant change in the concentration of total protein.

Interpretation

The **two major causes** for alterations of serum total protein concentration are (1) changes in the volume of plasma water and (2) changes in the concentration of one or more of the specific proteins in the plasma. Decrease in the volume of plasma water (hemoconcentration) is reflected as relative hyperproteinemia, with the concentration of all the individual proteins increased to the same degree. This is seen in dehydration due to inadequate water intake or excessive water loss, as in severe vomiting, diarrhea, Addison's disease, or diabetic acidosis. On the other hand, increase in the volume of plasma water (hemodilution) is reflected as relative hypoproteinemia, with the concentration of all the individual proteins decreased to the same degree. This occurs with water intoxication or salt-retention syndromes and during excessive intravenous infusion. Specimens collected in gel and non-gel separator tubes give comparable results. Only disorders affecting the concentration of albumin and/or the immunoglobulins will give rise to abnormal total protein levels.

Low total protein: You could have a liver or kidney, or a digestive disorder like celiac disease (your body can't absorb protein the way it should).

High total protein: Too much protein in your blood can be a sign of chronic infection or inflammation (like HIV/AIDS or viral hepatitis). It can also be an early sign of a bone marrow disorder.

Albumin Test

The concentration of albumin in the serum is important in maintaining its volume since it accounts for approximately 80% of serum colloid osmotic pressure. A reduction in serum albumin concentration often results in oedema. Albumin is quantitatively the most important protein synthesized in the liver, with 10–15 g/day being produced in a healthy man. The most widely used **methods** for the analysis of serum albumin are **dye-binding procedures**. Dye-binding techniques are based on a shift in the absorption maximum of the dye when bound to albumin. The shift in the absorption maximum allows the resulting color to be measured in the presence of excess dye, which in concert with the high-binding affinity of

albumin allows all of the albumin molecules to take part in the reaction. A wide variety of dyes has been employed for the measurement of albumin, including methyl orange, 2-(4′-hydroxyazobenzene) benzoic acid (HABA), bromcresol green (3,3′,5′-tetrabromo-m-cresolsulfonphthalein, or BCG) and bromcresol purple (5,5′-dibromo-ocresolsulfonphthalein, or BCP). BCG and BCP are most commonly used nowadays.

Specimen

Serum is the specimen of choice, but heparinized plasma can also be used if precautions are taken to prevent heparin interferences. Fasting is not required, although it may be desirable, since marked lipemia interferes in the BCG assay. Venostasis should be avoided when collecting samples; hemoconcentration increases the apparent concentrations of albumin and other plasma proteins.

Interpretation

Plasma albumin level can be affected by many conditions. **Hyperalbuminemia** is usually attributable to dehydration or hemoconcentration.

Hypoalbuminemia is usually the result of

(1) Hemodilution.

(2) A rate of synthesis less than the rate of albumin loss. Decreased synthesis may be because of malnutrition, malabsorption, or an inability of the liver to synthesize albumin because of diseases such as acute or chronic hepatitis. Plasma albumin concentration below the lower reference limit may imply hepatic disease chronicity. In chronic liver disease, albumin is a good indicator of prognosis.

(3) Diseases that cause a large albumin loss from urine, skin, or intestine. Low plasma albumin concentration may be a result of large losses in the case of diseases such as nephrotic syndrome, protein-losing enteropathy, exudative skin lesions, or burns. Burns in particular may be associated with severe albumin loss.

(4) Increased catabolism observed in fevers, untreated diabetes mellitus, and hyperthyroidism.

The albumin/globulin (A/G) ratio

This ratio provides information about the amount of albumin you have compared with globulin.

Low A/G ratio: could indicate your albumin levels are too low (hypoalbuminemia), or your globulin levels are too high. High globulin indicates inflammation and immune system activity. This might be the sign an autoimmune disorder. It can also point to kidney disease or cirrhosis (which is inflammation and scarring of the liver). In some cases, a low A/G ratio can be a sign of a tumor in bone marrow.

High A/G ratio: High albumin concentration can be caused either by overproduction of albumin by the liver or severe dehydration. A high A/G result might also indicate low levels of globulin, which are found in people with antibody deficiencies. Low globulin levels can also occur in in malnutrition.

If your doctor feels any of your levels are too high or low, you may need to have more precise blood or urine tests. For instance, your doctor may give you a serum protein electrophoresis (SPEP) if your total serum protein is high or if you have otherwise unexplained signs and symptoms that might suggest you could have a plasma cell disorder, like multiple myeloma. Your doctor will give you more details about your results and let you know what, if any, other tests you need.

Normal values:

Total proteins	(6-8 g/dL)	(60-80 g/L SI units)
Albumin	(3.4 - 5.4 g/dL)	(34 to 54 g/L SI units)
Globulin	(2 - 3.9 g/dL)	(20 to 39 g/L SI units)

The Albumin-globulin ratio between 1.1 and 2.5

Bilirubin Test

Bilirubin

Bilirubin is a yellow compound. It is obtained mainly from destruction of hemoglobin, then conjugated in the liver to bilirubin diglucuronide and then excreted in the bile.

Jaundice

Increased plasma bilirubin results in condition called jaundice (yellow pigmented skin & sclera). Jaundice categorized as: (1) pre-hepatic (hemolytic jaundice), (2) hepatic (hepatocellular jaundice) and (3) post-hepatic (obstructive jaundice). Bilirubin can accumulates in plasma for many reasons.

Jaundice is common in the newborn and is most commonly due to elevation in unconjugated bilirubin as a result of immaturity of the liver (physiological jaundice) or due to hemolytic disease of the newborn. Serum unconjugated bilirubin may reach more than 400 μmol/L in hemolytic disease. In the adult, increased bilirubin and jaundice can arise due to increased production (prehepatic), diseases of the liver (hepatic), or obstruction to bile flow (posthepatic). In prehepatic jaundice, the bilirubin is predominantly unconjugated, and the concentration rarely exceeds 100 μmol/L. In obstructive jaundice, the bilirubin is predominantly conjugated bilirubin, and concentrations in excess of 700 μmol/L can be seen.

Jaundice usually becomes clinically apparent when the plasma bilirubin concentration reaches about 50 μmol/L (hyperbilirubinaemia).

Van den Bergh reaction

This reaction is highly useful in understanding the nature of jaundice. It helps to identify the type of jaundice. It is a chemical reaction used to measure bilirubin levels in blood. More specifically, it determines the amount of conjugated bilirubin in the blood.

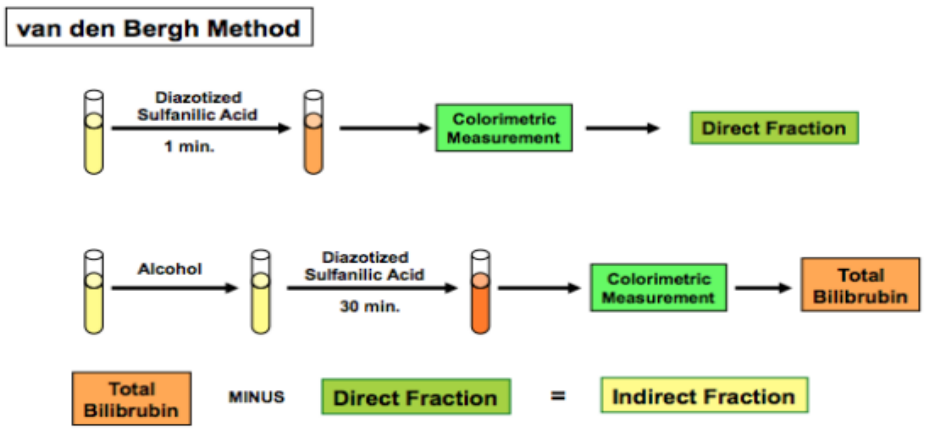
Van den Bergh reaction principle

Bilirubin reacts with diazotised sulphanilic acid to produce purple coloured azobilirubin.

The serum of the patient is mixed with diazo reagent. If a red colour develops immediately it is called a direct positive. It happens if conjugated bilirubin is present. In an indirect positive test, the patient's serum is first treated with alcohol and later mixed with diazo reagent. This causes development of a red colour. It is seen if unconjugated bilirubin is present. If both conjugated and unconjugated bilirubin are present the reaction is termed a biphasic reaction.

Conjugated bilirubin also is called (direct bilirubin) because it reacts directly with the reagent, and unconjugated bilirubin is called (indirect bilirubin) because it has to be solubilized first. When alcohol is added to the test system, however, both the direct and indirect forms react.

The pigment that reacted in the absence of alcohol was termed “direct.” The pigment that required the presence of alcohol was termed the “indirect” bilirubin fraction. The response of serum to Van den Bergh’s test, with or without the presence of alcohol, has been the basis for several classifications of jaundice.



Laboratory Analysis of Bilirubin

1- Total bilirubin: measures both forms of bilirubin (unconjugated and conjugated)

2- Direct bilirubin: measures only conjugated form

3- Indirect bilirubin calculated by : Total bilirubin – direct bilirubin = (indirect bilirubin)

Normal values

• Total bilirubin: 0.3-1 mg/dL (5-17 µmol/L SI units)

• Conjugated bilirubin: 0 - 0.4 mg/dL (0-7 µmol/L SI units)

• Unconjugated bilirubin: 0.1-1 mg/dL (1-17 µmol/L SI units)

• Urine bilirubin: negative

Conversion formula for converting µmol/L to mg/dL: **Multiply µmol/L by the number 0.058**

Normally the unconjugated bilirubin makes up 70-85% of the total bilirubin.

• Critical condition occur when total bilirubin >15 mg/dL

Specimen

• Serum is usually used for analysis obtained from venous blood. Total bilirubin determinations using a diazo method require either serum or plasma.

• Fasting for 4 to 8 hours is required before the test. Water is permitted.

• Avoid hemolysis during sample collection.

• Use heel puncture for blood collection in infants.

Interfering factors

1- Blood hemolysis and lipemia can produce false results.

2- Avoid the exposure of the specimen to sun light or high intensity artificial light at room temperature because this will decrease bilirubin content because it is very sensitive to and destroyed by light and heat, and specimens should be protected from ambient light prior to and during analysis. Concentrations may decrease by 30% to 50% per hour if exposed to direct sunlight.

3- Air bubbles and shaking of specimen may cause decreased bilirubin levels.

4- Certain food (carrots, yams) may increase the yellow hue in serum thus cause increase bilirubin level by spectrophotometer method

5- Delayed transportation of blood sample before the sample was processed increases bilirubin level could be due to haemolysis.

6- Prolonged fasting raises bilirubin level as does anorexia.

7- Examples of drugs that may increase total bilirubin: allopurinol, anabolic steroids, antimalarials, ascorbic acid, chlorpropamide, diuritics, methyldopa, monoamino oxidase inhibitors, morphine, oral contraceptives, rifampicin, theophylline and vitamin A.

8- Examples of drugs that may decrease total bilirubin: barbiturates, caffeine, penicillin, corticosteroids and salicylates.