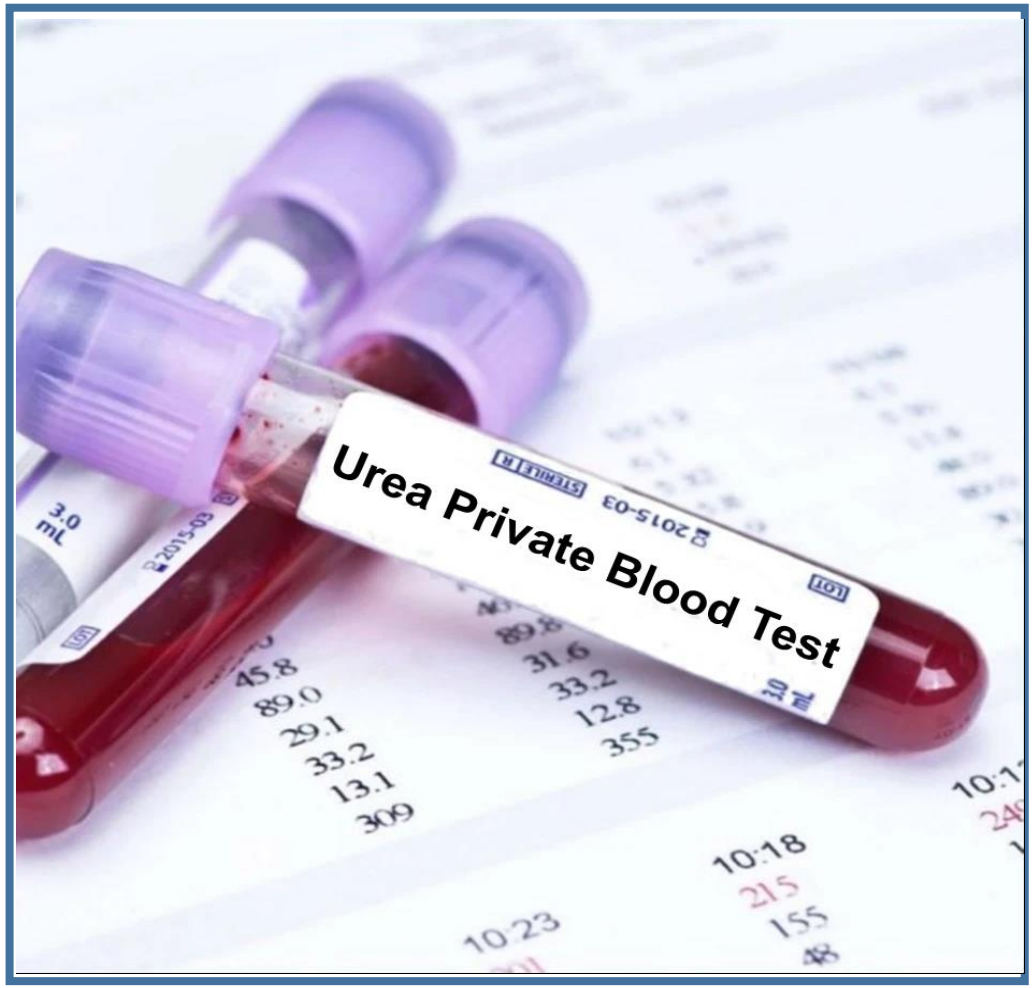

Quantitative Determination of Blood Urea

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Quantitative Determination of Blood Urea

Introduction

Quantitative Determination of Blood Urea is considered one of the most commonly performed procedures in clinical biochemistry laboratory. Urea is the main non-protein nitrogen compound in the blood. Urea is synthesized in the liver. Urea is produced as a by-product of the deamination reactions of amino acids after proteins break down. Urea elimination in the urine is the major route for nitrogen excretion, it is filtered from the blood at the glomerulus, but passive tubular reabsorption occurs in low rate of urine flow.

Quantitative Determination of Blood Urea

Clinical Significance

Urea is the main end product pf protein metabolism in the body. The importance of the urea concentration in blood lies in its value as an indicator of kidney function. Azotemia (an abnormal increase in blood urea level) is seen mainly in renal disorders, dehydration, increase protein catabolism, high-protein diets, and gastrointestinal hemorrhage. There are two types of Azotemia:

1- Prerenal Azotemia: It is caused by impaired perfusion of the kidneys due to decreased cardiac output or for any of the former causes.

2- Postrenal Azotemia: It is caused by an obstruction in the urine outflow such as nephrolithiasis, prostatism, and tumors of the genitourinary tract.

From the other hand, urea production decreased in acute dehydration, malnutrition, pregnancy, low-protein intake and liver disease.

Sample Handling

Samples for urea analysis should be delivered to the laboratory as soon as possible after being drawn from the patient to separate the blood serum or heparinized plasma for analysis because urea can be lost via bacterial action. Fluoride tubes were not used in blood collection in this assay because they interfere with this assay by inhibiting urease reaction. Urea in the blood serum is stable for 7 days at 2-8 °C, we can freeze it for longer storage but avoiding repeated freezing and thawing.

Quantitative Determination of Blood Urea

Principle

Urea is hydrolyzed by urease into ammonia and carbon dioxide. The ammonia generated reacts with alkaline hypochlorite and sodium salicylate in presence of sodium nitroprusside as coupling agent to yield a green chromophore. The intensity of the color formed is proportional to the concentration of urea in the sample.

$$\text{Urea} + H_2O \xrightarrow{\text{Urease}} 2NH_3 + CO_2$$

$$NH_4^+ + \text{Sakicylate} + NaClO \xrightarrow[OH^-]{\text{Nitroprusside}} \text{Indophenol} + NaCl$$

Reagents

1- Enzyme Reagent (Vial R1): Urease (> 500 U/mL).

2- Buffered Chromogen (Vial R2): Phosphate Buffer (20 mmol/L, PH=6.9), EDTA (2 mmol/L), Sodium Salicylate (60 mmol/L) and Sodium Nitroprusside (3.4 mmol/L).

3- Alkaline Hypochlorite (Vial R3): Sodium hypochlorite (10 mmol/L) and Sodium hydroxide (150 mmol/L).

4- Standard Concentration Solution of Urea (50 mg/dL).

Reagent Preparation

Working Reagent Solution was prepared by mixing and dissolving the content of Vial R1 in the content of Vial R2 and store it at 2-8 °C away from light.

Quantitative Determination of Blood Urea

Procedure

1- Collect blood specimen from patients in serum blood tubes and leave it to coagulate for 20 minutes.
2- Separate serum sample from the blood by centrifuging at 3000-4000 RPM for 10-20 minutes.
3- Collect the serum in labeled tubes.
4- Three sets of tubes were prepared as below:

Solutions / Tubes	Blank	Standard	Serum
Working Reagent	**1 mL**	**1 mL**	**1 mL**
Standard	**-**	**10 µL**	**-**
Sample (Serum)	**-**	**-**	**10 µL**

5- All tubes were mixed well by vortex and incubated them for 5 minutes at 37ºC or 10 minutes at (16-25˚C).
6- Add 1 mL of R3 solution to all tubes.
7- All tubes were mixed well again by vortex and incubated them for 5 minutes at 37ºC or 10 minutes at (16-25˚C).
8- The absorbance (A) of the serum samples and standard were measured against the blank at wave length 600 nm by using cuvette of 1 cm light path of Sepctrophotometer.

**** Advantage of Blank Tube is to zero the instrument and eliminate all the effects of light (like refraction, diffraction, ..etc), measurement tube material and all other compounds in the solution except the target compound.**

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Calculations

$$\text{Level of Urea (mg/dL)} = \frac{\text{A (Sample)}}{\text{A (Standard)}} \times N$$

Where: N = Concentration of urea in standard solution = 50 mg/dL.

Reference Range

Normal Urea Level in Serum (Newborns < 10 days) = 6.4 – 53.5 mg/dL.

Normal Urea Level in Serum (Adults 12-60 years) = 15 – 40 mg/dL.

Normal Urea Level in Serum (Adults over 60 years) = 17 – 50 mg/dL.

** Urea levels tend to be slightly higher in males than in females due to high muscle mass in males than in females.