Cihan University - Sulaimaniya

Clinical Biochemistry

Lab. 5

Measurement of Serum Creatinine

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Introduction

Clinical Significance

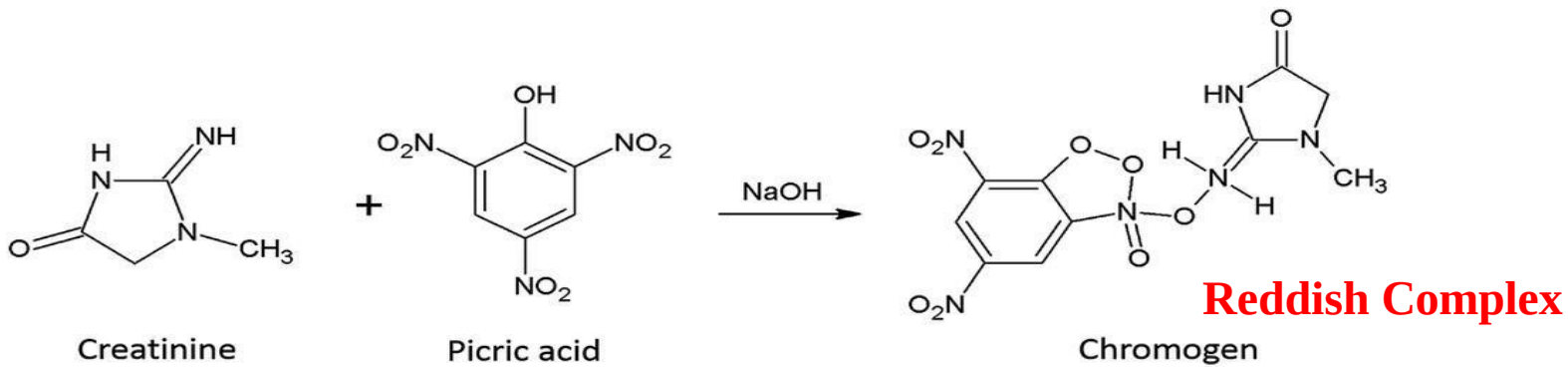
- **Creatinine** is synthesized in the body at a **fairly constant rate** from **creatine.**
- Creatinine in the blood is then removed by filtration trough the **glomeruli of the kidney** for excretion in the urine.
- **the *creatinine clearance (CC)* *test*** is one of the **most sensitive tests** to **diagnose renal function** especially the **glomerular filtration rate (GFR).**
- **Elevated levels** of **creatinine** in serum are usually associated with **renal diseases**, especially those related to **GFR such as glomerular nephritis**.

Quantitative measurement of Creatinine

Aim: Quantitative measurement of Creatinine in the serum

Principle

This procedure is based upon a modification of the original picrate reaction (Jaffe). Creatinine under alkaline conditions reacts with picrate ions forming a **reddish complex**. The formation rate of the complex measured through the increase of absorbance in a prefixed interval of time is proportional to the concentration of creatinine in the sample.



Reagent Composition

R1: Picric acid

R2: Alkaline Reagent: NaOH

R3: Standard: 2mg/dL

REAGENT PREPARATION

Working reagent. Mix 1 volume of **R1** + 1 volume of **R2**. Stable for 1 week at room temperature, stored **tightly closed and protected from light.**

Samples

- ✓ Serum or heparinized plasma, and urine.
- ✓ Creatinine in serum or plasma is stable up to 24 hours at 2-8ºC. Freeze for longer storage.
- ✓ Creatinine from random samples of urine is stable for 4 days at 2-8ºC. Freeze for longer storage.
- ✓ The 24-hour urine samples for the Clearance Test should be collected on a **preservative (fluoride thymol)** and **immediately refrigerated.**

Procedure

1. Adjust the instrument to zero with distilled water

	Blank	Standard	Sample
Working Reagent	**1ml**	**1ml**	**1ml**
Standard Solution		**100µl**	
Distilled Water	**100µl**		
Serum			**100µl**

1. Mix and start stop watch
2. Read the absorbance for each tubes at 592 nm **(A1) after 30 seconds** and **after 90 seconds (A2)** of the sample addition
3. Calculate **ΔA (A2-A1)**

Calculation

$$\text{Creatinine (mg/dL)} = \frac{(\Delta A)\ \text{Sample} - (\Delta A)\ \text{Blank}}{(\Delta A)\ \text{Standard} - (\Delta A)\ \text{Blank}} \times \text{Standard Concentration}$$

Standard Concentration: 2mg/dl

Reference Intervals:

Male: 0.7 - 1.4 mg/dL

Female: 0.6 - 1.1mg/dL

Calculation of Creatinine Clearance

$$\text{mL/min} = \frac{\text{mg creatinine/ dL URINE x mL 24-h}}{\text{mg creatinine/ dL SERUM x 1440 min}}$$

Cockcroft-Gault Formula for Estimating Creatinine Clearance

$$\textbf{CrCl (mL/min)} = \frac{\text{(140-age) x Lean Body Weight (kg)}}{\text{Serum Creatinine (mg/dL) x 72}} \text{ (x 0.85 if female)}$$

Reference Intervals:

Clearance Test

Men	97 - 137 mL/min
Women	88 - 128 mL/min