# Cihan University - Sulaimaniya

# Clinical Biochemistry

## Lab. 4

## Measurement of Serum Urea

Prepared by :

**Darya Shorsh Hamad**

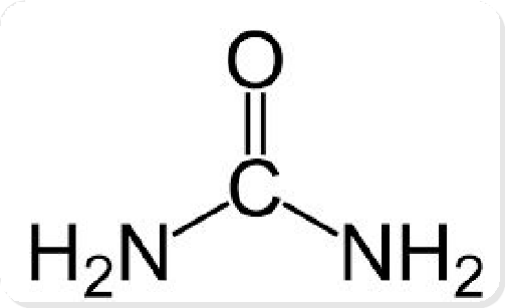
**Mcs.** in Clinical Biochemistry

E-mail: darya.shorsh@sulicihan.edu.krd

**Academic Year 2022-2023**

# Introduction

- Urea is the highest **non-protein nitrogen** compound in the blood.
- Urea is the major **excretory product of protein metabolism**.
- It is formed by **urea cycle in the liver** from **free ammonia** generated during **protein catabolism**.
- Since historic assays for urea were based on measurement of nitrogen, the **term blood urea nitrogen (BUN)** has been used to refer to urea determination.




**Formula: BUN (mg/dl) = Urea (mg/dl) / 2.1428**

# Clinical significance of urea measurement

- Measurement of Blood Urea Nitrogen (BUN) alone is **less useful in diagnosing kidney diseases** because it's blood level is influenced by **dietary protein and hepatic function.**

| | Type | Cause | Note |
|---|---|---|---|
| **High urea** (High urea concentration in plasma is called **azotemia**) | Pre-renal | • Congestive heart failure.<br>• Dehydration.<br>• High protein diet.<br>• Increased protein catabolism. | Caused by reduced renal blood flow, less blood is delivered to kidney, then less urea is filtered. |
| | Renal | • Renal failure . | |
| | Post-renal | • Urinary tract obstruction. | |
| **Low urea** | | • Low protein intake.<br>• Liver disease.<br>• Pregnancy. | |

# Measurement of serum Urea by Colorimetric Method

**Aim:** Determine the concentration of **Urea in the serum**

**Principle:**Urea is hydrolyzed in the presence of water and urease to produce **ammonia and carbon dioxide**. Then, Ammonium ions react with **hypochlorite** and **salicylate** to give **a green dye.** The intensity of the color formed **at 600 nm** is proportional to the **urea concentration** in the sample.

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

$$NH_3 + \text{Salicylate} + \text{Hypochlorite} \longrightarrow \text{2,2- Dicarboxyindophenol}$$

Green Dye

# Reagents

**R1** **UREA** Salicylate

| | | |
|---|---|---|
| Salicylate | 31 | mmol/L |
| Nitroprussiate | 1.67 | mmol/L |

**R2** **UREA** Urease

| | | |
|---|---|---|
| Urease | ≥ 15 | KUI/L |

Working reagent (R1+R2) is not classified as dangerous.

**R3** **UREA** Base

| | | |
|---|---|---|
| Sodium hypochlorite | 7 | mmol/L |
| Sodium hydroxide | 62 | mmol/L |

**R4** **UREA** Standard Urea 40 mg/dL (6.66 mmol/L)

# Reagents Preparation

**Working reagent:** Add content of vial R2 into Vial R1. Mix gently by inversion.

**Base (Vial R3):**

Standard (Vial**R4):** ready for use

# Specimen Collection and Handling

Unhemolysed serum or heparinised plasma. Avoid fluoride or ammonium as anticoagulant which interfere with the assay.

Urea is stable in serum or plasma for:

- 24 h at room temperature.
- several days at 2-8°C.
- at least 2-3 months frozen.

24h Urine: diluted (1+19) with demineralised water before assay.

Urea is stable in urines for:4 days at 2-8°C.
Add antibacterial agent as Thymol to improve the stability.

# Procedure

Manual procedure

Let stand reagents and specimens at room temperature.

| Pipette into test tubes | Blank | Standard | Assay |
|---|---|---|---|
| Working reagent (R1+R2) | 1 mL | 1 mL | 1 mL |
| Demineralised water | 5 µL | | |
| Standard | | 5 µL | |
| Specimen (Note 1) | | | 5 µL |
| Mix and wait for 4 minutes at room temperature or 2 minutes at 37°C | | | |
| Base (vial R3) diluted ¼ | 1 mL | 1 mL | 1 mL |
| Mix. Let stands for 8 minutes at room temperature or 5 minutes at 37°C. Read absorbance at 600 nm (590-610) against blank (Note 3). Reaction coloration is stable for 2 hours. | | | |

# Calculation

Manual procedure:

Serum and plasma:

$$\text{Result} = \frac{\text{Abs (Assay)}}{\text{Abs (Standard)}} \times \text{Standard concentration}$$

Urines diluted (1+19): Multiply the result by 20 (dilution factor).

To calculate blood urea nitrogen (BUN): multiply the value of urea (mg/dL) by 0.467.

Converting of Urea concentration to the BUN:

**Formulas: BUN (mg/dl) = Urea (mg/dl) / 2.1428**

# Reference Interval

| In serum and plasma | mg/dL | [mmol/L] |
|---|---|---|
| In cord | 45-86 | [7.5-14.3] |
| Premature | 6-54 | [1.1-8.9] |
| < 1 year | 9-41 | [1.4-6.8] |
| Children | 11-39 | [1.8-6.4] |
| 18-60 years | 13-43 | [2.1-7.1] |
| 60-90 years | 17-49 | [2.9-8.2] |
| > 90 years | 21-66 | [3.6-11.1] |
| In urines | 26-43 g/24 h | [0.43-0.71 mol/24 h] |