Baghdad University   **Practical Clinical analysis**
College of Sciences for women   **Lab-10 / 4st Class**
Biology Department   **Dr. Rasha Majid Abd-ulameer**

Clinical Biochemistry

These tests are very important for physician to detect the diseases so we must to be careful during the procedure and this depend on: -

- Fluids types.

- Procedures.

- The staff (professional, beginner)

The Purpose of these examinations is for qualitative and quantitative testing of body fluids, such as blood (whole, serum, plasma), urine, cerebrospinal fluid (CSF), etc………

The most important things that the technical should be know:

-The sample that we need (blood, serum, plasma).
- Normal values of tests.
-Principle of chemical reaction.

1-Blood urea:

Urea is the final result of protein catabolism in liver (urea represent 50% of the non- proteiniceous nitrogen substances).

Normal values:

Serum (urea): 10-50 mg / dl or 1.7 - 8.3 µmol /L.
Urine (urea): 20-35 g / 24h   or   333- 583 µmol / 24h.

The urea level rise in the cases:

- Renal failure.
- Renal diseases…. more than 100 mg / dl.
- In dehydration accompanied with renal failure….. 500 mg / dl.

Urea level decrease in very rare cases such as: liver diseases.

Specimens: serum, urine, heparinized plasma.

Procedure:

1-Assay condations:
Wavelength………………….…500 nm.
Temperature…………………………..37 C.

2- Reagents:

Standard (STD) = R1.
Working solution = R2 + R3.
Alkaline Reagent = R4.

3- Set up the following tubes:

	Reagent (BLANK)	Standard (STD)	sample
Standard (STD)	--------------	10 µl	---------
sample	--------------	----------	10 µl
Working solution	(1 mL)	(1 mL)	(1 mL)

4- Mix carefully & incubation (5 min at 20-25 C).

5- Add:

	Reagent (BLANK)	Standard (STD)	sample
Alkaline Reagent = R4	200 µl	200 µl	200 µl

6- Mix carefully & incubation (10 min at 20-25 C or 5 min at 37 C) & measure the absorbance at 500 nm within 60 min.

Calculation:

C = 100 x A sample / A STD =   mg /dl.
C = 5.55 x A sample / A STD =   µmol /L.

2- Serum Creatinine:

Creatinine is the anhydride creatine and is from the non-proteinicous nitrogen substances, it represent as waste product so it excreted by kidney.
Formed in liver….. stored in muscle… librated as creatinine in urine.

Normal values:

Serum:
In men: 0.7 -1.4 mg/ dl or   61.8 - 123.7 µmol /L.
In women: 0.5 – 1.1 mg/ dl or   53.0 – 97.2 µmol /L.

In urine:

In men: 10-20 mg/ kg/24h   or   88 - 177 µmol/ kg/24h .
In women: 8-18 mg/ kg/24h   or   71- 177 µmol/ kg/24h .

Rising of creatinine more than this levels may be as a result of kidney infection or obstructive of urinary tracts.

Specimens: serum, urine, heparinized plasma.

Procedure:

1-Assay condations:

Wavelength…………………..…492 nm.
Temperature…………………………..37 C.

2- Reagents:

Standard = (STD).
R1 =Picric acid.
R2 = Alkaline Reagent.
Working solution = R1+ R2 .

3- Set up the following tubes:

	Reagent (BLANK)	Standard (STD)	sample
Standard (STD)	--------------	100 µl	----------
sample	-------------	----------	100 µl
Working solution	(1 mL)	(1 mL)	(1 mL)

4- Mix carefully .

5- Read the absorbance (A1) after 30 seconds and after 90 seconds (A2) of the sample addition.

6- Calculate: A sample =A2-A1.   : A (STD) =A2-A1.

Calculation:

A2-A1= A sample (or STD).

Specimen: Serum.

C = 2 x A sample / A STD =   mg /dl.
C = 88.4 x A sample / A STD =   μmol /L.

Specimen: urine.
C= 100 x A sample / A STD =   mg /dl.

3- Uric acid test:

Uric acid is the final product of purins metabolism, it represent the nucleic acid which found in nucleoprotein.

Specimens:
Serum, urine, heparinized & EDTA plasma.

Normal values:

Serum:
In men: 3.4-7 mg/ dl or 200-420 μmol /L.
In women: 24-5.7 mg/ dl or 140-340 μmol /L.

In urine:
In men & women : 250- 750 mg/dl or 1.5-4.5 mmol/24h.

Uric acid level rise in: gout disease 6.5-12 mg/dl (high level of uric acid…….precipitate in and around joints).

Procedure:

1-Assay condations:

Wavelength………………….…520 nm.

Temperature………………………..37 C.

2- Reagents:

Standard = (STD).

R1 =Buffer.

R2 = Enzymes.

Working solution = R1+ R2 .

3- Set up the following tubes:

	Reagent (BLANK)	Standard (STD)	sample
Standard (STD)	--------------	25 µl	---------
sample	-------------	----------	25 µl
Working solution	(1 mL)	(1 mL)	(1 mL)

4- Mix carefully & incubation(10 min at 20-25 C or 5 min at 37 C).

5- Read the absorbance at 520 nm (A) of the sample and the Standard, against the blank.

Calculation:

Specimen: Serum, EDTA plasma.

C = 6 x A sample / A STD =   mg /dl.

C = 59.5 x A sample / A STD =   µmol /L.

Specimen: urine.

C= 6 x A sample / A STD x Vol (dl) urine 24 h =   mg /24 h.