Estimation of Serum Creatinine, Urine Creatinine and Creatinine Clearance
Kidney functions:

- The kidneys serve three essential functions:

1. They function as filters, removing metabolic products and toxins from the blood and excreting them through the urine.

2. They regulate the body’s fluid status, electrolyte balance, and acid-base balance.

3. The kidneys produce or activate hormones that are involved in erythrogenesis, Ca2+ metabolism, and the regulation of blood pressure and blood flow.
-Renal function tests (RFT):

• Are used to detect the presence of renal diseases and assess their progress.

• The most widely used test is to measure the glomerular filtration rate (GFR), that is, the rate of filtrate formation by the kidneys.
- **Glomerular Filtration Rate:**

  • Under normal conditions, approximately **625 mL of plasma** flow through the kidneys each minute and the volume of plasma filtered is **125 ml/ min** which is called the glomerular filtration rate.

  • **Glomerular filtration rate (GFR):** is the volume of plasma filtered by the kidneys per unit of time.

  • **GFR** is an important and the best overall measurement in the evaluation of kidney function.
-Measuring the GFR:

• Accurate measurement of the GFR by clearance tests requires determination of the concentration, in plasma and urine, of a substance is known to be completely filtered from the plasma at the glomerulus.

• This substance must not be reabsorbed nor secreted by renal tubules, broken down, or accumulated by the tubules and must remain at a constant concentration in the plasma throughout the period of urine collection.

• It's clearance is given by: clearance = U.V/ P , where:

  ➔ U= concentration of any substance in urine.
  ➔ P= concentration of the same substance in plasma .
  ➔ V= volume of urine( ml/min).
### Substances clearance used for Measuring GFR

<table>
<thead>
<tr>
<th>Source</th>
<th>Inulin Clearance</th>
<th>Creatinine Clearance</th>
<th>Urea Clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td>Not reabsorbed or secreted.</td>
<td>An endogenous product of muscle metabolism; near constant production.</td>
<td>An endogenous product of protein.</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>Not made by body; must be injected (exogenous).</td>
<td>Small amount is secreted.</td>
<td>-Partially reabsorbed. -synthesis varies with diet.</td>
</tr>
</tbody>
</table>
- Creatinine:

- Creatinine is derived from “creatine” which is synthesized in the liver, kidney and pancreas. It moves through the circulation and is taken up entirely by muscles.

- Creatinine is a substance that, in health, is easily excreted by the kidney.

- It is the byproduct of muscle energy metabolism and is produced at a constant rate according to the muscle mass of the individual.

- Endogenous creatinine production is constant as long as the muscle mass remains constant.
- Serum Creatinine:

- High plasma creatinine:

  - Plasma creatinine tends to be higher in subjects with a large muscle mass.

  - Other non-renal causes of increased plasma creatinine include the following:

    1. A high meat intake can cause a temporary increase.

    2. Transient, small increases may occur after vigorous exercise.
- Urine Creatinine:

• Decreased urine creatinine is found in:
  - Advanced renal disease.
  - Renal stenosis.
  - Hyperthyroidism.

Increased urine creatinine is found in:
  - Diabetes mellitus.
  - Hypothyroidism.
- Creatinine clearance:

- A measure of the amount of creatinine eliminated (filtered) from the blood by the kidneys.

- Creatinine is cleared from the body fluids almost entirely by glomerular filtration (small amount is secreted by kidney tubules).

- Therefore, the clearance of creatinine can be used to assess GFR.

- Because measurement of creatinine clearance does not require intravenous infusion into the patient, this method is much more widely used than inulin clearance for estimating GFR clinically.
- Clinical Implications:

1. Decreased creatinine clearance is found in any condition that decreases renal blood flow:
   
a. Impaired kidney function.
   
b. Shock, dehydration.
   
c. Hemorrhage.

2. Increased creatinine clearance is found in:
   
a. Pregnancy.
Reference Values:

- Urine creatinine: 1-2 g/24h
- (Serum) creatinine: 0.6–1.2 mg/dL
- Normal creatinine clearance = 100-130 ml/min/1.73m²

- Note:
  - What 1.73 m² means?

  - Kidney function is proportional to kidney size, which is proportional to body surface area. A of 1.73 m² is the normal mean value for young adults.

  - Adjustment for body surface area is necessary when comparing a patient’s estimated GFR to normal values or to the levels defining the stages of Chronic kidney disease (CKD).
## Chart 2 - Chronic kidney disease staging

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
<th>GF (ml/ min/1.73m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Kidney lesion with normal or increased GF</td>
<td>≥ 90</td>
</tr>
<tr>
<td>II</td>
<td>Kidney lesion with mild GF decrease</td>
<td>60-89</td>
</tr>
<tr>
<td>III</td>
<td>Kidney lesion with moderate GF decrease</td>
<td>30-59</td>
</tr>
<tr>
<td>IV</td>
<td>Kidney lesion with marked GF decrease</td>
<td>15-29</td>
</tr>
<tr>
<td>V</td>
<td>Functional kidney failure or undergoing SRT</td>
<td>&lt; 15</td>
</tr>
</tbody>
</table>

Practical Part
- **Objective:**

1- To estimate creatinine in serum and urine.

2- To calculate creatinine clearance value.

- **Principle:**

  *(Jaffe’s method):*

  - Colorimetric estimation of creatinine using the **alkaline picrate method**:

    Creatinine + picric acid → **Creatinine picrate (orange)**

  - Absorbance at **520 nm.**
**Method:**

1- Set up a series of 8 test tube as follows:

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Standard (3mg/dl) (serum)</th>
<th>Test (serum)</th>
<th>Standard (0.75mg/dl) (Urine)</th>
<th>Test (urine)</th>
<th>Blank</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(A)</td>
<td>(B)</td>
<td>(C)</td>
<td>(D)</td>
<td>(E)</td>
</tr>
<tr>
<td>Water</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
<td>1.5 ml</td>
</tr>
<tr>
<td>Standard (serum)</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Serum Sample</td>
<td>-</td>
<td>-</td>
<td>0.5 ml</td>
<td>0.5 ml</td>
<td>-</td>
</tr>
<tr>
<td>Standard (Urine)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.5 ml</td>
</tr>
<tr>
<td>Urine Sample</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Picric acid</td>
<td>6 ml</td>
<td>6 ml</td>
<td>6 ml</td>
<td>6 ml</td>
<td>6 ml</td>
</tr>
</tbody>
</table>


2- Immerse the Tubes carefully in the boiling water bath for 40 seconds.

4- Pipette 0.6 ml of NaOH to all tube.

5- Let the tubes stand for 20 min.

6- Read the absorbance at 520 nm.

-Results:

<table>
<thead>
<tr>
<th>Tube</th>
<th>Standard (serum)</th>
<th>Test (serum)</th>
<th>Standard (urine)</th>
<th>Test(Urine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(A)</td>
<td>(B)</td>
<td>(C )</td>
<td>(D)</td>
<td></td>
</tr>
<tr>
<td>(A)</td>
<td>(B)</td>
<td>(C )</td>
<td>(D)</td>
<td></td>
</tr>
</tbody>
</table>

Absorbance at 520 nm

Average(Mean of Absorbance)
- **Calculation:**

**Patient information:** 24h urine volume = 100ml, gender: women, body surface: 1.6m², DF=100.

1- **Serum creatinine =**

(Mean Absorbance of serum test ÷ Mean Absorbance of Standard) X concentration of Serum standard = ........ mg / dl

2- **Urine creatinine =**

(Mean Absorbance of Urine test ÷ Mean Absorbance of Standard) X concentration of Urine standard X DF= ...... mg / dl

(To compare with normal range, convert from mg/dl to g/24 h)

3- **Creatinine Clearance :**

=U.V/ P

= [ (Urinary creatinine (mg/dl)) / (plasmac creatinine (mg/dl)) ] x Urine volume(ml/min) = B

B -------------------------------> 1.6 m² (person surface area )

? ---------------------------------> 1.73 m²

-Corrected for surface area= ....... ml/min/1.73 m²
-Example:

- Find the Creatinine Clearance if you know that the Urine creatinine $U = 488 \text{ mg/dl}$, Serum creatinine $P= 2.32 \text{ mg/dl}$, Volume of urine in 24 h $=100 \text{ ml}$ and A ”surface area” $=1.6 \text{ m}^2$ ?

$\Rightarrow$ Creatinine Clearance: $= \frac{U \times V}{P}$

$= \left( \frac{488 \text{ mg/dl}}{2.32 \text{ mg/dl}} \right) \times \left( \frac{100}{1440} \right) = 14.6 \text{ ml/min}$

14.6 ml/min in 1.6 m2, find the creatinine clearance for 1.73 m2 surface area :

$= \left( 14.6 \times 1.73 \right) / 1.6 = 15.8 \text{ ml/min/1.73m}^2$

OR

$\Rightarrow$ Creatinine Clearance: $= \frac{( U \times V \times 1.73)}{(P \times 1440 \times A)}$

$= \left( \frac{488 \text{ mg/dl} \times 100 \times 1.73}{2.32 \times 1440 \times 1.6} \right)$

$= 15.8 \text{ ml/min /1.73m}^2$

* To convert 24 hour to min $(24 \times 60 = 1440)$
Discussion:

• Comment on the concentration of creatinine in serum.
• Comment on the concentration of creatinine in urine.
• Comment on the value of Creatinine Clearance.