DIAGNOSTIC TESTS

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ANANTAPUR
The Life Cycle of a Lab Test

1. Specimen taken
2. Specimen transferred to clinical laboratory
3. Tests analyze specimen & produce results
4. Clinical lab provides results to the doctor
5. Doctor interprets results
6. Doctor informs patient of results & implications
7. Patient goes to the doctor
8. Doctor orders lab test
9. Diagnosis & treatment decisions made by doctor & patient
IMPORANCE OF LABORATORY INVESTIGATIONS

- laboratory tests - important in assisting & management of the patient during treatment of disease besides diagnosis.

- Used-
  1. screen - disease in asymptomatic individual
  2. to establish or exclude presence of diseases in symptomatic patients
  3. assist the practitioner in the management of the patient.
Estimation of non protein nitrogenous substances (NPN)
**Enzymatic Method:**

Urea + H₂O $\xrightarrow{\text{Urease}}$ 2NH₃ + CO₂

1. **NESSLERIZATION:**

   \[\text{NH}_3 + \text{K}_2\text{HgI}_4 \rightarrow \text{Brown compound}\]
   (Potassium mercuric iodide)
   Measured at 450nm

2. **BERTHELOT REACTION:**

   \[\text{NH}_3 + \text{Phenol} \xrightarrow{\text{ClO}^-} \text{Indophenols} \xrightarrow{\text{Alkali Nitroprusside}} \text{Blue compound}\]
   Measured at 540nm
1. The enzyme **urease** hydrolyzes the **urea** in the patient specimen to form \( \text{CO}_2 \) & ammonium (\( \text{NH}_4 \))

\[
\text{UREA} \quad \xrightarrow{\text{urease}} \quad \text{NH}_4 + \text{CO}_2
\]

2. The ammonium is quantitated utilizing the enzyme glutamate dehydrogenase (GLDH) that reduces the coenzyme **NADH** to **NAD** with causes a decrease in absorbance that is measured at 340nm.

\[
\text{NH}_4 + \alpha\text{-ketoglutarate} + \text{NADH} \quad \xrightarrow{\text{GLDH}} \quad \text{NAD} + \text{glutamic acid} + \text{H}_2\text{O}
\]
**A**

Principle:

\[
\text{CO(NH}_2\text{)}_2 + \text{H}_2\text{O} \xrightarrow{\text{urease}} 2\text{NH}_3 + \text{CO}_2
\]

- pH indicator (phenol red)
  - 6.8
  - 8.2

**B**

Sample application and detection zone

Saliva extraction

Application of saliva sample on the strip immobilized with Urease enzyme and dye

RGB profiling using smartphone app and interpretation of response using slope method

Schematics of the biosensor showing steps for urea detection

**C**

Color change in strips with increase in urea concentration
1. The creatine is an amino acid that does not found in proteins.
2. Creatine is a nitrogenous organic acid
Formation of Creatinine

Creatine + ATP \xrightarrow{Creatine Kinase} Phosphocreatine + ADP

\[ \text{Spontaneous, non-enzymatic cyclization} \]

Creatine \xrightarrow{\text{phospho-creatine}} \text{Creatinine} \xrightarrow{\text{Pi}} \text{Spontaneous}

\[ \text{Creatin} \]
Creatine kinase

Creatine → Phosphocreatine

Creatinine

Muscle mass (body weight)

1. Waste product
2. Present in all body fluids
3. Filtered through glomeruli
4. Excreted in urine

Depends on
Creatinine:
A chemical waste molecule that is generated from muscle metabolism.

Creatinine is transported through the bloodstream to the kidneys.

The kidneys filter out most of the creatinine and dispose of it in the urine.
Automated Method Cont....

- The result are calculated as follows-

- Mg/dl, Creatinine =

\[
\frac{O.D.T[80 \text{ sec}]-O.D.T[20\text{sec}]}{O.D \text{ std}[80\text{sec}]-O.D \text{ std}[20\text{sec}]} \times \text{conc. of std}.
\]
estimation of serum creatinine by enzymatic method

Creatinine amidohydrolase

Creatinine + H\textsubscript{2}O \rightleftharpoons Creatine

Creatine amidinohydrolase

Creatine + H\textsubscript{2}O \rightarrow Sarcosine + urea

Sarcosine oxidase

Sarcosine + H\textsubscript{2}O + O\textsubscript{2} \rightarrow Glycine + formaldehyde + H\textsubscript{2}O\textsubscript{2}
Enzymatic Method

- Creatinine + H₂O $\rightarrow$ Creatine
  - Creatinine aminohydrolase

- Creatine + ATP $\rightarrow$ Creatine-P + ADP
  - Creatine Kinase

- ADP + Phosphoenolpyruvate $\rightarrow$ ATP + Pyruvate
  - Pyruvate Kinase

- Pyruvate + NADH $\rightarrow$ Lactate + NAD⁺
  - Lactate dehydrogenase

- The difference in absorbance at fixed times during conversion is proportional to the concentration of creatinine in the sample
creatine + H$_2$O $\xrightarrow{\text{creatidinase}}$ creatine

creatine + H$_2$O $\xrightarrow{\text{creatidinase}}$ sarcosine + urea

sarcosine + O$_2$ + H$_2$O $\xrightarrow{\text{sarcosine oxidase}}$ formaldehyde + glycine + H$_2$O$_2$

indicator (reduced) + H$_2$O$_2$ $\xrightarrow{\text{peroxidase}}$ indicator (oxidized) + 2H$_2$O$_2$

ABTS $\xrightarrow{H_2O_2}$ ABTS $^+$
Assay Procedure

R1: 270 µL
Sample: 8 µL

R2: 90 µL

37°C

0

5 min

550 nm

10 min

A1

A2

*Based on Hitachi 917
What Happens When the Level of Creatinine is too High?

Symptoms:
- Shortness of breath
- Nausea
- Vomiting
- Dry skin
- Headaches
- Loss of appetite
- Weight loss

Creatinine is transported through the bloodstream to the kidneys.

For More Information:
Visit: www.epainassist.com
Blood urea nitrogen : Creatinine = Normal = 12 to 20 (optimum 15)
20 : 1

- Low
  - Acute tubular necrosis
  - Low protein intake
  - Starvation
  - Severe liver disease

- High
  - Pre-renal uremia
  - High protein intake
  - After GI bleeding

- High with raised Creatinine
  - Post renal obstruction
  - Pre-renal uremia with Renal disease
Ingested Nucleic acid  Tissue destruction

Breakdown

Liver

Purines $\rightarrow$ Adenosine + Guanine

Plasma

Uric acid

Kidney $\rightarrow$ GIT

In urine  Uric acid + Bacterial enzyme
Uric acid metabolism

- Dietary purine intake
- Tissue nucleic acids
- Endogenous purine synthesis

Urate pool

- Gut excretion
- Renal excretion

Overproduction

Underexcretion

Hyperuricemia

- Asymptomatic
- Acute gout attack
- Chronic tophaceous gout
- Renal manifestations
Hyperuricemia may lead to

Formation of kidney stones: Most of uric acid is removed by the kidneys and disposed of in the urine.
In hyperuricemia Uric acid crystals precipitate in the kidney and may block filtering tubules leading to renal failure.

Uric acid crystals in a urine sample

Sodium urate/kidney stones
Swollen and inflamed joint

Uric acid crystals
Estimation of serum calcium

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Calcium homeostasis

Storage bone:
- Calcium deposition
- Calcium resorption

Intake:
- Calcium in the diet

Blood:
- Ca++ absorbed into blood

Excretion:
- Ca++ lost in urine
- Calcium lost in feces

Small intestine:
Calcitonin, calcitriol & PTH act together

Thyroid releases CALCITONIN

Increase Ca\(^{2+}\) deposition in bones
Decrease Ca\(^{2+}\) uptake in intestines
Decrease Ca\(^{2+}\) reabsorption from urine

If Ca\(^{2+}\) levels too high

Calcium levels fall

If Ca\(^{2+}\) levels too low

Calcium levels rise

Parathyroid releases PTH

Increase Ca\(^{2+}\) release from bones
Increase Ca\(^{2+}\) uptake in intestines
Increase Ca\(^{2+}\) reabsorption from urine

Homeostasis (normal calcium levels in blood)
Hypercalcemia related to hyperparathyroidism

LOW BLOOD CALCIUM DETECTED AT PARATHYROIDS

PARATHYROIDS

PTH

KIDNEY

PTH stimulates:
1) reabsorption of calcium from urine (and excretion of phosphates)
2) activation of Vitamin D

SMALL INTESTINE

Vitamin D causes increased absorption of calcium from the diet

BONE

PTH stimulates osteoclast activity. Bone is resorbed which releases calcium into the bloodstream

Active Vitamin D

INCREASED SERUM CALCIUM

By- Professor Namrata Chhabra (MD Biochemistry)
Hypocalcemia

The bones become fragile due to decreased level of calcium (Hypocalcemia) and often show signs of osteoporosis...

For More Information,
Visit: www.epainassist.com
Cats of Hypocalcemia

C: Convulsion
A: Arrhythmias
T: Tetany
S: Spasm and Stridor