Trace elements are vital for human body to maintain normal yet complex physiological functions related to body’s growth & development.

They also balance toxicity levels.

They are called trace elements because of their body concentration, which is few milligrams per kg or less.

The daily requirement of dietary trace elements is few milligrams.

They are also known as micronutrients

These are categorized as:

- Essential, probably essential or non-essential

This is based upon their biological effect, diseases that occur due to their deficiency and toxicity due to overdose.
Essential Trace Elements:
   iron, zinc, copper, cobalt, chromium, fluorine, iodine, manganese, molybdenum and selenium

Probably essential:
   nickel, tin, vanadium, silicon, boron

Non-essential:
   aluminum, arsenic, barium, bismuth, bromine, cadmium, germanium, gold, lead, lithium, mercury, rubidium, silver, strontium, titanium and zirconium is all found in plant and animal tissue.
Essential Trace Elements are divided into two sub-groups

- **Trace Elements**
  - Iron, Zinc and Copper

- **Ultra Trace-Elements**
  - Manganese, Selenium, Cobalt, Chromium, Fluorine, Iodine and Molybdenum
IRON (Fe)

- SIGNIFICANCE
- CARRIER ENZYMES
- TRANSFERRIN
- FERRITIN
- DETERMINATION OF IRON
  TYPES OF SPECIMEN
  PROCEDURES FOR SERUM IRON DETERMINATION
    Total iron estimation with protein precipitation
    Total iron estimation without protein precipitation

- TOTAL IRON BINDING CAPACITY (TIBC)
  CALCULATION OF PERCENT IRON SATURATION
  CLINICAL SIGNIFICANCE
IRON (Fe)

• **Significance of iron:**
  One of the most essential trace elements
  Body content is 4-6g and is found in the following forms;
  - Hemoglobin \( 68\% \)
  - Ferritin \( 13\% \)
  - Haemosiderin \( 12\% \)
  - Myoglobin \( 3\% \)
  - Iron enzymes \( 0.2\% \)
  - Other iron compounds \( 3.6\% \)

• **Iron dependent enzymes** *(enzymes that require iron as cofactor):*
  Cytochrome oxidase, Xanthine oxidase and peroxidase, Catalase
• Body requirement & iron conservation:
  Daily requirements:  10 mg/day (adult)
                  18 mg/day (pregnant)
  Daily excretion:   0.9 mg/day (adult)
                  1.3 mg/day (during menstruation in females)
Iron loss also increases during bleeding and hemorrhage

• Dietary Source:
  Liver, kidneys, heart and spleen, egg yolk, fish and oyster

• Body conserves iron very well. Only ~1.3 mg/day is absorbed from
digested food however the absorption increases during growth and
pregnancy. In case of iron deficiency the iron absorption from food
increases to 4 mg/day.

• Iron is absorbed in Ferrous (Fe^{2+}) form, which is measurable in
blood as free iron.
Transferrin:

- Iron binds to transferrin for transportation in plasma after re-absorption. Where as the storage occurs in hepatoparenchymal cells, reticuloendothelial cells, bone marrow, liver and spleen.

- Each transferrin molecule binds to two iron atoms

- Transferrin transports iron to various organs and tissues.

- Serum iron + amount of iron bound to transferrin = total iron in circulation.

- Determination of transferrin can provide total iron binding capacity.

- Transferrin can be measured by RIA, ELISA and Chemiluminescence
Ferritin:

- A protein having **24 subunits** of 500kDa
- It binds 4000 iron molecules which accounts for a larger iron storage
- Synthesized by cells that store iron and is later used to synthesize heme
- Ferritin in serum is derived from the breakdown of macrophages of the RES (liver, spleen and bone marrow).
- Its measurement is used to **assess iron stores in the body**
- Low Ferritin levels indicate depletion of iron stores and used as an early indication of iron deficiency
- Increased Ferritin levels are observed in liver diseases such as hepatitis, liver cirrhosis due to alcohol consumption and hepatic carcinoma
- Increased Ferritin levels are also observed in: leukemia, Hodgkin’s lymphoma and chronic inflammatory disease
- Under these conditions Ferritin is released from inflamed tissues or in case of malignancies it’s released by tumors.
- Ferritin can be measured by RIA, ELISA and Chemiluminescence
Determination of iron:

- **Type of specimen required:**
  Iron shows diurnal variation i.e. high levels in morning and low levels in the evening (decreased by 30%)
  Timing of specimen collection is important
  Fasting serum or plasma is used
  Only serum or heparinised plasma is suitable. Other anticoagulants such as EDTA, oxalate or citrate are not acceptable
  Avoid gross hemolysis

  *Remember: Iron levels decrease with age!*

- **Procedures for serum iron determination:**
  Atomic absorption spectrophotometry is widely used for determining all trace-elements however due to matrix interference and low sensitivity, calorimetric procedures for total iron estimation is most common:
A) Total iron estimation with protein precipitation:

Protein is precipitated by hot TCA, a strong acid, which also liberates iron simultaneously. The protein free filtrate is reacted with a reducing agent, usually ascorbic acid to reduce all Fe$^{3+}$ to Fe$^{2+}$

A chromogen is added to produce iron-chromogen complex, which is colored and the iron concentration is directly proportional to the color intensity.

Chromogen used are: sulfonated bathophenanthroline, ferrozine, terosite 2,4,6-tripyridyltriazine, thiocyanate (if this is used, then no need for reducing step since this chromogen also reacts with any Fe$^{3+}$).

B) Total iron estimation without protein precipitation:

Serum pH is adjusted to 6 using mild acid. This allows release of iron from protein without precipitating the proteins. Reducing agent is directly added to convert all Fe$^{3+}$ to Fe$^{2+}$

A chromogen is added to produce a colored iron-chromogen complex. Iron concentration is directly proportional to the color intensity.

Chromogen used are similar to the one used previously
Procedure (B) is more sensitive as compared to (A) where some iron is lost bound to proteins. However (A) is more specific than (B) where some protein may interfere therefore ‘blank’ samples are used to correct for interference

- **Total Iron Binding Capacity**
  
  Transferrin concentration is $\alpha$ TIBC….. representing maximum binding sites on transferrin

  Percentage iron saturation = \( \frac{\text{serum iron}}{\text{TIBC}} \)

- **Unsaturated IBC** = TIBC - serum iron

Serum iron is determined using either method (A) or (B)

Transferrin is measured using one of the immunoassays

Reference values in normal adult:

Serum Iron: 11 to 27 $\mu$mol/L

TIBC: 54 to 64 $\mu$mol/L
For complete and proper evaluation of body IRON, following sequence is recommended

- Complete hematology profile cbc, Hb, Hct, MCV, MCH, MCHC
- RBC morphology by peripheral blood smear
- Serum iron ferritin, TIBC and percentage saturation must be determined.

Determination of iron levels alone are only useful incase of iron toxicity

Iron determination is extremely important in determining iron deficiency anemia
Zinc (Zn)

Distribution and transportation

- Second most abundant trace element

- It acts in the body mainly as a cofactor for 100 diverse zinc-dependant enzymes such as DNA polymerase, alkaline phosphatase, carboxypeptidase etc.

- These enzymes mainly regulate normal growth, immune system, cell growth, collagen synthesis, wound healing, bone metabolism, reproduction, taste, smell and vision.

- Body content is 2.5g and is distributed as 60% in muscle, 30% in bone and remaining 10% in other body tissues and organs.
– Daily requirement is **3-14mg**, age and sex dependant (infants and children require as little as 3-5mg where as adult males require more than adult females however during lactation demand in females increases to 14mg/day)

– Diet rich in zinc includes: red meat, fish and sea food

– 20-30% of the dietary zinc is absorbed mainly by small intestine.

– Post absorption it’s found in the blood bound to erythrocytes (75-80%), the rest is complexed with albumin, transferrin and immunoglobulins

– Zinc is mainly excreted through GIT in the stool and to a lesser extent in urine
Zinc levels will decrease substantially in: leukemia, liver cirrhosis, hepatitis, sickle cell anemia, Infection, pernicious anemia and malnutrition

Common symptoms of zinc deficiency that must be noted include:
  - **In children**: growth retardation and skeletal abnormalities are typical symptoms in which zinc deficiency should be considered
  - **In adults**: reduced sense of taste and smell, loss of appetite, development of abnormal skin lesions and excessive hair loss

**Zn toxicity occurs due to:**

- Excessive ingestion of Zn. Some toxic compounds such as ZnCl₂ if ingested may cause generalized necrosis, burns and ulcers if applied excessively on skin. This compound is found mostly in deodorants

- Prolonged exposure to industrial compounds such as ZnO may lead to pneumonitis and sometimes even cancers
Determination of Zn

- The method of choice is atomic absorption spectrophotometer readable at a wavelength of 213.9nm, however fluorometry can be used but it’s less sensitive.

- Serum is preferred however Li-heparin may also be used to collect sample.

- Hemolyzed samples must be rejected.

- If urinary Zn needs to be determined then 24 hrs collection is preferred in a sterile plastic container (metal containers must be avoided). HCL with a pH of 2 should be added to the container to preserve Zinc from bacterial growth in such a long term collection.

**Normal Values of Zn:**

- Serum 11-23 mol/L
- Plasma 15.5-19 mol/L
- Urine 12 mol/L
- Erythrocytes 185-200 mol/L
Copper (Cu)

Source, transportation and storage

- Copper is the third most abundant trace element in the human body.

- The daily dietary requirement is between 2-6mg which is mainly obtained from red meat, cocoa, shell-fish, water pumped through copper pipes and chocolates.

- Typically 40-60% copper is absorbed by the duodenum and is transported via metalloenzymes e.g. ascorbic acid oxidase.

- In plasma 90% is bound to a conjugated metalloenzyme known as ceruloplasmin, 9% is bound to carrier proteins such as albumin and only 1% is free Cu$^{2+}$.

- Body content of copper is 80-120mg.
Body functions:

- Copper is involved in the process of erythropoiesis, erythrocyte function and regulate erythrocyte survival.

- Copper is critical for energy production in the cells. It is also involved in nerve conduction, connective tissue, the cardiovascular system and the immune system.

- Copper is closely related to estrogen metabolism, and is required for women's fertility and to maintain pregnancy.

- Copper stimulates production of the neurotransmitters epinephrine, norepinephrine and dopamine.

- Acts as a catalyst for copper-containing enzymes such as; ceruloplasmin, ascorbic acid, dopamine-β- hydroxylase, cytochrome oxidase and tyrosinase.
Clinical significance:

Hypocuperaemia is associated with:
- Anemia in infants
- Malnutrition in infants
- Menkes’ kinky-hair syndrome….genetic disorder where copper absorption leads to a brain disease which then cause characteristic wiry-steel hair.


- Nephrosis
- Hypoproteinemia.

Hypercuperaemia is associated with:
- Pregnancy
- Estrogen therapy
- Thelassemia and anemia
- Leukemia and Lymphoma particularly Hodgkin’s disease
- Rheumatoid arthritis

Copper toxicity may occur due to ingestion of excess copper or as a result of environmental exposure and this is characterize by increased tissue and serum levels
**Wilson’s disease:**

- Autosomal recessive genetic disorder with onset in the 2\textsuperscript{nd} or 3\textsuperscript{rd} decade
- Onset in children aged 4-5yrs is also reported but rarely
- Serum ceruloplasmin and serum copper is low
- Increased urine and tissue copper due to excessive deposition of copper in tissues particularly in the hepatocytes and basal ganglia of the brain leading to their steady degeneration.

**Symptoms:**

- **In the CNS:**
  Tremor, in-coordination, ataxia, rigidity, dysphagia

- **In the liver:**
  Jaundice, weakness, partial hypertension, cirrhosis, anorexia
Lab findings:
Low ceruloplasmin, low serum and fecal copper
Increased tissue, urinary and CSF copper.

Treatment of Wilson’s disease

• Lifelong treatment is recommended
• Low-copper diet - avoid shellfish, chocolate, liver, mushrooms, nuts, and various other foods.
• Vitamin B6 supplements (pyridoxine)
• Anti-copper agents
  * Zinc acetate (a chelating agent for Cu, balances Cu levels)
  * Trientine hydrochloride
  * Penicillamine and D-penicillamine
Methods of determination:
Type of specimen:
– Serum or urine, however the collection **MUST BE** in metal free containers
– Hair sample can also be used particularly from patient with Menkes’ kinky-hair syndrome. 2-5cm long hair should be washed with acetone and digested with 70% pure nitric acid.

Method of choice is atomic absorption spectrophotometer readable at the wave length of 235nm. All the glass ware used during analysis should be free of metals and to avoid contamination they should be washed with acid then dd.H₂O.

**Normal Values of Cu:**

Serum = 12-26 µmol/L
Urine = 0.05-0.55 µmol/day
SELENIUM (Se)

Ultra-trace element

– Least abundant however the most toxic of all the essential elements

– Dietary source is meat, fish and grains

– Recommended intake for adults 50-200 µg/day as in USA however intake may vary in other countries where soil Selenium levels are low.

– It’s widely distributed in the human body however highest levels are found in the liver, kidney, heart, spleen, pancreas and lungs.
Functions in human body:

- Selenium is incorporated in the enzyme Glutathione peroxidase (GTH-Px), that plays important role in normal immune system function, also plays a crucial role in the control of oxygen metabolism.

- It catalyzes the break down of $H_2O_2$ and liquid hydroperoxides in body tissues and fluids, hence protects body from oxidative damage.

- In RBCs the enzyme GTH-Px prevents peroxidation of Hemoglobin and cell membrane.

- Vitamin-E also catalyses the activity of GTH-Px

- Low GTH-Px in platelets leads to bleeding disorders

- Low GTH-Px in plasma leads to edema due to damage to capillary membranes
- In phagocytic cells (leukocytes and macrophages), there is increased activity of GTH-Px which protects these cells from destruction due to the pre-oxidase which is generated by the oxidative destruction of foreign bodies.

- GTH-Px accumulates in high concentration in liver to de-toxify hyper-oxides

- GTH-Px protects eye lens tissues from damage (i.e. inhibits cataract formation). Therefore people with cataract have 1/6 of normal Selenium levels in their eye lens tissues.

- Reduced GTH-Px due to reduced Selenium also leads to neuron damage.

- Low Selenium also leads to Keshan’s disease, which is a type of cardiac myopathy discovered in China where soil Selenium was absent.
Selenium deficiency occurs due to:
• Hemolytic anemia
• Clansman's thrombasthenia (platelet disorder)
• Carcinoma
• Gastrointestinal cancer
• Malnutrition

Increased Selenium may occur due to:
• Reticuloendothelial neoplasia
• Poisoning (toxicity)

Laboratory determination of Selenium:
• fasting whole blood, serum or plasma sample……in mental free container!
• 24hr urine sample…..in metal free container!
• Blood or urine measurement can provide good indication of body Selenium levels
• Plasma or serum is preferred over whole blood due to sensitivity of determination
• Selenium may be determined in hair sample to access degree of deficiency
- **Methods of determination:**
  1. Flameless atomic absorption spectrophotometry
  2. Spectrofluorometry
  3. Neutron activation

**Normal Values of Se:**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>whole blood</td>
<td>57-340 µg/L</td>
</tr>
<tr>
<td>RBC</td>
<td>71-340 µg/L</td>
</tr>
<tr>
<td>Serum</td>
<td>78-320 µg/L</td>
</tr>
<tr>
<td>Urine</td>
<td>5-100 µg/L</td>
</tr>
<tr>
<td>Hair</td>
<td>0.6-2.6 µg/L</td>
</tr>
</tbody>
</table>

Good correlation exists between erythrocyte GSH-Px and blood Selenium levels up to 100 µg/L and could be used as a functional analysis for the enzyme activity.
Manganese (Mn)

Ultra-trace element

• Important role in regulating metabolic processes which mainly include lipid and carbohydrate metabolism, bone and tissue formation, skeletal growth and reproduction

• Over 50 Manganese dependent enzymes have been identified and these include: catalase, peroxidase, super oxide dimutase, 5’ nucleotidase, R Nase and glucosyl- and glactosyl-transferase

• Average daily intake is 20 μg/day however daily body requirement is 2-5μg/day

• Manganese is absorbed via small intestine in similar process as iron.

• Beta-microglobulins carry Manganese to mitochondrial rich organs such as liver, pancreas, kidney and pituitary gland

• Major excretion is through bile and pancreas into GIT
• **Deficiency** of Manganese is rare however its etiology is not well known

• Several defects have yet been associated with it; such as hair color and growth alterations, loss of weight, inhibition of vit-K dependant clotting and altered cholesterol transport and utilization

• Manganese **toxicity** occurs due to inhalation of manganese dust, where minors are known to suffer from headaches, sterility, loss of appetite and speech difficulties. Excessive manganese have also been associated with psychological and neurological disturbances

• Manganese is normally **NOT** tested in routine diagnostic laboratories, however it’s known to increase in case of myocardial infarction. Reduced levels have been observed in children suffering from epilepsy and other convulsive disorders.

**Normal Values of Mn:** = 0.5 -1 µg/L
Cobalt (Co)

*Ultra-trace element*

- Biological function is limited
- Vitamin B12 is the only cobalt containing compound
- Beef, sea food and dairy products are major source of vitamin B12
- Similar to Manganese the daily intake of cobalt significantly exceeds the required dose that is $0.1 \, \mu g/L$
- Deficiency of cobalt is related to diminished vitamin B12 absorption
- >70% dietary vitamin B12 is absorbed in the presence of an intrinsic factor through the intestinal mucosa
- <5% is absorbed in the absence of I.F
• Post absorption vitamin B12 is converted to cobalamine and stored in the liver.

• Cobalt deficiency has never been reported, however vitamin B12 deficiency may occur mainly due to the absence of I.F which subsequently affects intestinal absorption followed by depletion of liver stores later on leading to pernicious anemia

• **Toxicity** may occur due to excessive inhalation or ingestion which may lead to:
  pulmonary edema, nausea, cardiomyopathy

• Laboratory testing is rarely carried out however normal plasma levels are 0.5 – 4.0 µg/L