LABORATORY MEASURMENT OF COMMOM ELEMENTS

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CALCIUM IN PLASMA

- *40% As **Bound** to Proteins Including Albumin (80%) and Glubolins (20%)*
- *10% As **Complex** with Anions Such as Bicarbonate, Citrate, Lactate, Phosphate, Pyruvate, Beta-Hydroxybutyrate, Sulfate, Heparin*
- *50% As **Free***

FACTORS AFFECTING BOUND-CALCIUM

- *pH*
- *Protein Concenteration*
- *Abnormal Proteins*
- *Competition for Binding*

FACTORS AFFECTING COMPLEXED-CALCIUM

Concentration of Anions

- ***Citrate***
- ***Heparin***
- ***Lactate***
- ***Ketone Bodies***

CALCIUM DETERMINATION

INDIRECT METHODS

Precipitation by Organic or Inorganic Anions

- ***Direct Determination by Gravimetry***
- ***Indirect Determination by Measuring Anion***

CALCIUM DETERMINATION

DIRECT METHODS

Photometry (Colorimetry)

- ***O-Cresolphthaleine Complexone (CPC)***
- ***Arsenaso III***
- ***Other Dyes***

Atomic Absorption Spectrophtometry (AAS)

CALCIUM DETERMINATION

FREE CALCIUM DETERMINATION

Ion Selective Electrode (ISE)

CALCIUM DETERMINATION

SAMPLE FOR TOTAL CALCIUM

- *Calcium Binds to Alb*
- *Using Heparinized Plasma or Serum (Avoiding Citrate, Oxalate & EDTA)*
- *Acid Wash*
- *Hemolysis Has Two Different Effects*
- *Not Using Lipemic & Icteric Samples*
- *Stable at 4^{0C} for a Few Days and a Few months at Freeze*

CALCIUM DETERMINATION

SAMPLE FOR FREE CALCIUM

- *Whole Blood with Heparin or Serum Calcium Binds to Alb*
- *pH Effect*
- *Effect of Heparin*
- *Assay without Delay*

MAGNESIUM DETERMINATION

Total Magnesium

1) Colorimetric Methods

- *Calmagit*
- *Formazan Dye*
- *Methyl Thymol Blue*
- *Titan Yellow*

2) Atomic Absorption Spectrophotometry (AAS)

3) Enzymatic with Hexokinse

Free Magnesium

Ion Selective Electrode (ISE)

MAGNESIUM DETERMINATION

SAMPLE

- *Magnesium Binds to Proteins*
- *Increasing During Activity*
- *Using Heparinized Plasma or Serum (Avoiding Citrate, Oxalate & EDTA)*
- *Higher Concentration in Cells*
- *Not Using Lipemic & Icteric Samples*
- *Urine Collection with Acid*

PHOSPHATE DETERMINATION

- *Phosphate not Phosphrous*
- *Phosphates Exist as Organic & Inorganic*
- *Phosphates Are Intra- and Extracellular*

PHOSPHATE DETERMINATION

Direct (Nonenzymatic) Methods

1) Ammonium Molybdate

Phosphate Ions + Ammonium Molybdate

Phosphomolibdate + Ammonium Ions

- *UV Spectrophotometry (Phosphomolybdate Has Absorption at 340nm)*
- *Colorimetry (Reduction to Molybdenum)*

2) Vanadate Molybdate

PHOSPHATE DETERMINATION

Indirect (Enzymatic) Methods

1) *Glycogen Phosphorylase , Phosphoglucomutase and G6PD*
2) *Sucrose Phosphorylase , Phosphoglucomutase and G6PD*
3) *Nucleotide Phosphorylase , Xanthine Oxidase and Peroxidase*

PHOSPHATE DETERMINATION

SAMPLE

1) *Effects of age, Diet , Activity, Diurnal variation*
2) *Serum or Heparinized Plasma*
3) *Hemolysis*
4) *Effect of Detergents*
5) *Large Amount in RBC*
6) *Storage at 4^{0C} for few Days and few Months at Freeze*
7) *Collection Urine With Acid*

IRON METABOLISM ASSESSMENT

- *Hct, Hb, RBC, Erythrocytic Indexes*
- *SI, TIBC, UIBC, Tsat*
- *Transferin, TrfR, Ferittin,*

VARIATION OF SERUM IRON

DECREASED

- *Iron Deficiency*
- *Malnutrition*
- *Chronic Disesese*
- *Response to hematinic therapy*
- *Recent blood lose*

INCREASED

- *Iron Overload*
- *Iron Therapy*
- *Aplastic Anemia*
- *Hepatic Disease*

SERUM IRON DETERMINATION

- *In Three Stages*
- *Releasing Iron by Reducing pH*
- *Reduction of Fe^{3+} to Fe^{2+} by Reducing Agent*
- *Iron Chelating by Chromogens such as **Bathophenantroline** and **Ferrozine***

IRON DETERMINATION

SAMPLE

- *Changing During Day*
- *Protein Binding*
- *Serum or Heparinized Plasma*

  Avoid Using EDTA, Citrate & Oxalate
- *Hemolysis*
- *Iron Contamination*
- *3 Days at RT and 7 Days at 4^{0C}*

SERUM IRON DETERMINATION

- *Decreased In Iron deficiency Anemia & Anemias of Chronic Diseases*
- *Increased In Sideroblastic Anemia*
- *It Is Normal or High In β-Thalassemia Trait*

Serum Iron Is Not Recommended for Detection of Iron Deficiency Anemia , Because of:

- *Technical Problems*
- *Diurnal Variation*
- *Weak Corroleation*

MEASURMENT OF TRANSFERIN

- *It Is*

  Increased In Iron Deficiency

  Normal or Decreased In ACD

  Decreased In Sideroblastic Anemia

  Normal In Beta-Thalassemia Trait

- *It Is Measured Directly by Immunoassays*

  Or Calculated from TIBC

  TRF (mg/dL) = 0.7 x TIBC (μg/dL)

IRON BINDING CAPACITY (IBC)

TIBC Measurment

- *Adding Excess Fe^{3+}*
- *Saturation of TRF*
- *Removing Free Fe^{3+} by MgCO3*
- *Iron Measuring in Supernatant*

UIBC Measurment

- *Adding Fe^{3+} In Known Amount*
- *Saturation of TRF*
- *Measuring Free Fe^{3+}*
- *UIBC = Added Fe - Free Fe*
- *TIBC = UIBC + SI*

MEASURMENT OF FERRITIN

- *Selective Method for Assessing Iron Body Storage*
- *It Is Sensitive Marker for Iron Deficiency*
- *It Is A Positive Acute Phase Reactants*
- *Increases During Hepatocyte Damage*
- *Is Not a Sensitive Marker for Iron overload*

CONDITIONS AFFECTING ON SI, Tf, and TIBC

- *Diurnal Variation*
- *Menstural Cycle*
- *Pregnancy*
- *Oral Contraceptive*
- *Iron Contamination*

CONDITIONS AFFECTING ON SI, Fer, Tsat, Tf, and TIBC

CONDITION	SI	Fer	Tsat	Tf	TIBC
Iron deficeincy	Decreased	Decreased	Decreased	Increased	Increased
Malnutrition	Decreased	Decreased	Decreased	Decreased	Decreased
Chronic infection	Decreased	Increased	Decreased	Decreased	Decreased
Malignancy	Decreased	Increased	Decreased	Decreased	Decreased

CONDITIONS AFFECTING ON SI, Fer, Tsat, Tf, and TIBC

CONDITION	SI	Fer	Tsat	Tf	TIBC
Iron Overdose	Increased	Increased	Increased	Decreased	Decreased
Hemochro-Matose	Increased	Increased	Increased	Slightly Decreased	Slightly Decreased
Sideroblastic anemia	Increased	Increased	Increased	Normal/ Decreased	Normal/ Decreased
Acute liver disease	Increased	Increased	Increased	Variable/ Increased	Variable/ Increased
Viral hepatitis	Increased	Increased	Normal/ Increased	Increased	Increased