Clinical Chemistry

QUALITY CONTROL

TERMS TO REMEMBER:

- **Quality:** a feature/characteristic of a product which meets the expected criteria of a consumer (customer).
- **Control:** a solution that resembles a human sample that is used for QC purposes only
- **Standard:** a colorless solution with **known** concentration of substances used for calibration
- **Specificity:** defined as the ability of a method to measure the analyte of interest ONLY.
- **Sensitivity:** defined as the ability of a method to measure analytes even at its **lowest concentration**
- **Accuracy:** nearness of measured value to that of the target value
- **Precision:** nearness of measured values to each other
- **Diagnostic specificity:** defined as the ability of a method to detect a population of individuals **absent of a disease process**
- **Diagnostic sensitivity:** defined as the ability of a method to detect a population of individuals having the **presence of disease**
- **Intralab QC (internal QC):** control samples are run simultaneously with a patient to ensure reliability of methods and result. Used **for daily monitoring** of accuracy and precision of method used. Detects **random and systematic errors.**
- **Interlab QC (external QC):** laboratories are given samples with unknown concentrations for them to test and results are compared with other laboratories thus maintaining "long-term accuracy" to methods utilized.
    - Results difference of **greater than 2SD** indicates disagreement with other lab included**.**
- **Mean:** average of a set of values (**mean = Σx/n**). Measures central tendency.
- **Median:** midpoint of a set of values
- **Mode:** the most frequent among all values/data
- **Range:** Simplest expression of spread or distribution
- **Standard Deviation:** it is defined as the measure of dispersion of values to that of the mean. Most frequent used measure of variation.
    - $SD = \sqrt{\frac{\Sigma(x-mean)^2}{n-1}}$
- **Coefficient of variation:** mean expression in percentile. Index of precision
    - $CV = \frac{SD}{mean} x\ 100$
- **Variance:** square of SD. $V=SD^2$
- **T-test:** this is used to assess if there is a statistical difference between the **means** of 2 groups of data
- **F-test:** this is used to assess if there is a statistical difference between the **SD** of 2 groups of data
- **Shewhart Levey-Jennings Chart:** most widely used QC chart
- **Trend:** six or more consecutive values that either increase or decrease gradually **(will cross the mean) – main cause:** reagent deterioration
- **Shift:** six or more consecutive values that are distributed on one side or other side of the mean **(does NOT cross the mean) – main cause:** improper instrument calibration

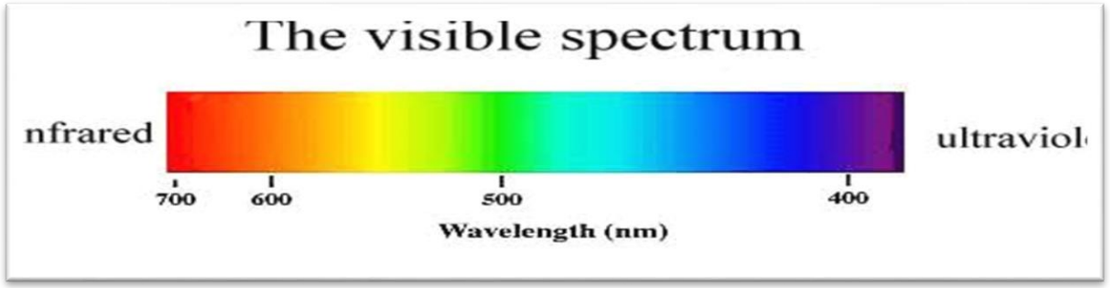
WESTGARD RULES		
TYPE OF ERROR	**RULES**	**SOURCES OF ERROR**
RANDOM - **Tests for** imprecision	1_{2s} (warning rule), 1_{3s} and R_{4s}	**By chance errors:** mislabeling, pipetting error, fluctuations in temperature & voltage
SYSTEMATIC - **Tests for** inaccuracy	2_{2s}, 4_{1s} and 10x	Improper calibration, reagent deterioration, contaminated solutions, instability of both samples and solutions

- **Delta check:** used to check if there are significant differences between present set of values to that of past values on the sample of same individual.
- **Six Sigma:** a way of improving product processing to **eliminate defects**

INSTRUMENTATION

DEFINITION OF TERMS:

- **Energy:** entity that this transmitted by electromagnetic waves
- **Wavelength:** defined as the distance between two successive peaks
- **Nanometer:** unit expression of wavelength
- **Frequency:** number of waves that passes a point of observation per one unit of time



SPECTROPHOTOMETRY

- Measures **transmitted light** in a colored solution
- Measurement is based upon **Beer-Lambert-Bouguer Law (Beer's Law/Beer-Lambert's Law)**

BEER-LAMBERT LAW

- States that concentration of an unknown analyte is **directly proportional to the light absorbed and inversely proportional to light transmitted.**
  - ✓ Absorbance is proportional to the inverse log of transmittance

$$A = -logT = \frac{\log 1}{T}$$

SINGLE-BEAM SPECTROPHOTOMETER

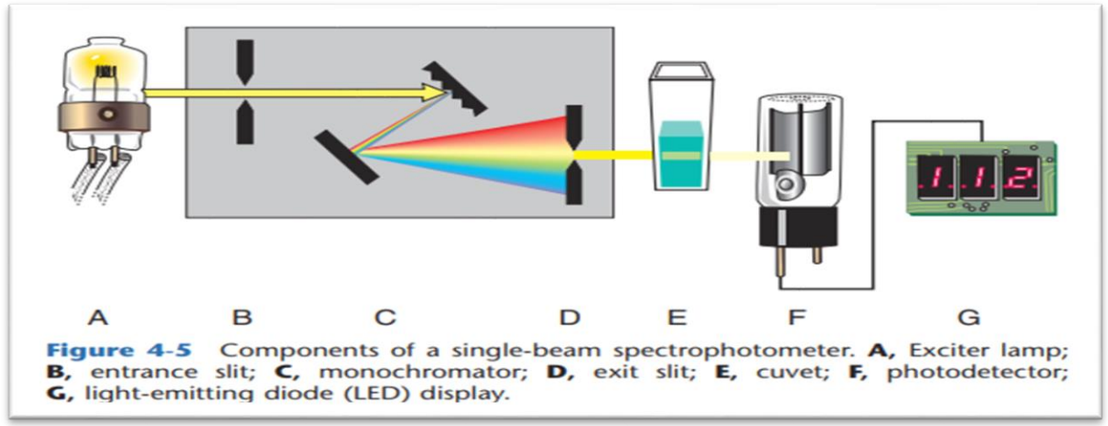


Figure 4-5 Components of a single-beam spectrophotometer. **A,** Exciter lamp; **B,** entrance slit; **C,** monochromator; **D,** exit slit; **E,** cuvet; **F,** photodetector; **G,** light-emitting diode (LED) display.

Photo reference: Henry's Clinical Diagnosis and Management by Laboratory Methods, 22nd edition

DOUBLE-BEAM SPECTROPHOTOMETER

- Double- beam in time – 1 photodetector
- Double-beam in space – 2 photodetectors (1- sample beam, 2- reference beam)

PARTS OF SPECTROPHOTOMETER

1. **LIGHT SOURCE**
   - ✓ Tungsten: for visible and near infrared region
   - ✓ Deuterium: for UV region
   - ✓ Xenon discharge lamp: for UV and Visible region
2. **ENTRANCE SLIT** – minimizes the entry of stray light to the monochromator
3. **MONOCHROMATOR** – isolates specific wavelength
   - ✓ Prisms: light is **refracted**

- ✓ Diffraction gratings: light is **bent; most commonly used**
- ✓ Filters: light enters one side and is **reflected** on the other side.

4. **EXIT SLIT** – controls bandpass (total range to which wavelengths are transmitted. The narrower the bandpass, the grater the resolution)
5. **CUVETTE –** contains the solution (known as absorption cell/analytical cell/sample cell)
6. **PHOTODETECTOR –** aids in the conversion of light transmitted to photoelectric energy
   - ✓ Barrier layer cell: simplest. Temperature sensitive. Radiation and visible region.
   - ✓ Photodiode: has excellent linearity.
   - ✓ Photomultiplier tube: most commonly used. Chemiluminiscence and Fluometry. Measures visible and UV region.
   - ✓ Phototube: cathode and anode enclosed in glass case. Fluometry.
7. **READ-OUT DEVICE** – a monitor that displays the output

ATOMIC ABSORPTION SPECTROPHOTOMETRY

- Measures the amount of light that have been absorbed by a ground state atom
- For measurement of unexcitable metals like calcium and magnesium
- Hollow-cathode lamp: light source
- Atomizer: used for the conversion of ions to atoms
- Chopper: used to modulate amount of light from the hollow-cathode lamp

FLAME EMISSION PHOTOMETRY

- Flame permits the excitation of the electrons; after which, electrons return to a ground state thus radiation is emitted.
- **Flame serves as both light source and cuvette.**
- Internal standards used: Cesium and Lithium (preferred)
- For measurement of excited ions such as sodium **(yellow)** and potassium **(violet)**.
- Calcium also shows a colored **(brick red)** flame

FLUOROMETRY

- Light is absorbed by atoms at a specific wavelength and is emitted at a longer wavelength (with lower energy)
- Light source: xenon lamp or mercury arc
- There are two monochromators
  - ✓ Primary monochromator: selects wavelength that is best absorbed by solution that is to be measured
  - ✓ Secondary monochromator: this prevents the incident light from striking the detector
- Disadvantage: **Quenching**

TURBIDIMETRY

- Measures **light blocked** by molecules
- Used for immunoglobulins, immune complexes and complement

NEPHELOMETRY

- Measures **light scattered** by molecules
- Used for measuring amount of antigen-antibody complexes

CHROMATOGRAPHY

- Separation is based upon differences in characteristics (both physical and chemical) of substances
- Used for amino acid determination, drugs and sugars

LIST DOWN ALL TYPES OF CHROMATOGRAPHY AND RESEARCH ON ITS PURPOSE/PRINCIPLE

POTENTIOMETRY

- Measures electric potential
- pH electrode – glass electrode
- pCO_2 electrode
- ion – selective electrode
  - ✓ Sodium: glass electrode
  - ✓ Potassium: Valinomycin gel
  - ✓ Chloride: Tri-N-octyl propyl ammonium chloride decanol

ELECTROPHORESIS

- Separation of proteins is aided by an electric current

	IONS		POLE
POSITIVE	CATIONS	⟷	CATHODE
NEGATIVE	ANIONS	⟷	ANODE

- pH of buffer: **8.6**
- support materials:
  - ✓ Agarose gel - separation by electric charges
  - ✓ Cellulose acetate – separation by molecular size
  - ✓ Polyacrylamide gel – separation by charge and molecular size

ELECTROPHORETIC PATTERN OF CERTAIN CONDITIONS	
Alpha$_1$-globulin flat curve	Juvenile cirrhosis
Alpha$_2$-globulin band spike	Nephrotic syndrome
Beta-gamma bridging	Hepatic cirrhosis
Monoclonal gammopathy (gamma spike)	Multiple myeloma
Polyclonal gammopathy	Rheumatoid arthritis and malignancy
Small spike in Beta-region	Iron deficiency anemia

CARBOHYDRATES

- Composed of carbon, hydrogen and oxygen
- Are water soluble
- Are important source of energy for the body's mechanisms
- Classifications:
  - ✓ Monosaccharides: Glucose, fructose and galactose
  - ✓ Disaccharides: **maltose** (glucose + glucose), **lactose** (galactose + glucose) and **sucrose** (fructose + glucose; most common non reducing sugar)
  - ✓ Polysaccharides: starch and glycogen

GLUCOSE

- Primary sugar found circulating in the body
- Carbohydrate metabolism:
  - ✓ **Glycolysis**: glucose → lactate or pyruvate → energy **(↑ glucose)**
  - ✓ **Glycogenolysis**: breakdown of glycogen to glucose **(↑ glucose)**
  - ✓ **Glycogenesis:** formation of glycogen from sugars for storage **(↓glucose)**
  - ✓ **Gluconeogenesis:** formation of glucose from non-carbohydrate sources **(↓ glucose)**
- Hormones for glucose regulation
  - ✓ **Hypoglycemic**
    - Insulin – released by β cells of islet of Langerhans
      - Entry of glucose in the cell
      - Falsely low measurement of serum insulin is seen in the presence of **hemolysis.**
  - ✓ **Hyperglycemic**
    - Glucagon – released by α cells of islet of Langerhans
      - Primary hormone that increases glucose concentration.
      - NV in fasting plasma: 25-50pg/mL
    - Somatostatin – released by delta cells of islet of Langerhans

- Inhibits the action on inulin, GH and glucagon.
- Cortisol
- Epinephrine
- Growth hormone
- Thyroxine
- ACTH

- MUST KNOW FOR SPECIMEN FOR GLUCOSE DETERMINATION
  - ✓ FBS should be obtained from an 8-10 hours fasting sample
  - ✓ In terms of glucose levels: **capillary > venous but < arterial**
  - ✓ Glucose is metabolized at:
    - Room temperature: 7 mg/dL/hr
    - 4°C: 2 mg/dL/hr
  - ✓ Tube of choice: Gray top **(anticoagulant: _____; anti-glycolytic agent: _____)**

GLUCOSE DETERMINATION

METHOD	PRINCIPLE	REAGENTS	END PRODUCT/ COLOR REACTION
i. CHEMICAL METHOD			
A. OXIDATION REDUCTION METHOD			
1. ALKALINE COPPER REDUCTION METHOD			
Folin-Wu - **Modification: Benedict's Test**	Copper Reduction	Alk. Copper reagent Phosphomolybdic Acid	Molybdenum – **BLUE**
Nelson- Somogyi	Copper Reduction	Alk. Copper reagent Arsenomolybdic acid	Molybdenum – **BLUE**
Neocuproine	Copper Reduction	Cuprous ions Neocuproine	Cuprous-Neocuproine Complex – **YELLOW/ YELLOW ORANGE**
2. ALKALINE FERRIC REDUCTION METHOD			
Autoanalyzer (Hagedorn-Jensen)	Ferricyanide reduction (Inverse Colorimetry)	$K_3Fe(CN)_6$	$K_3Fe(CN)_6^{-4}$
B. Condensation Method			
Ortho-Toluidine	Dubowski reaction; Condensation Method	O-toluidine Glacial Acetic Acid	Glycosylamine – **BLUE GREEN**
II. ENZYMATIC METHODS			
Glucose Oxidase - **Saifer Gernstenfield** - **Clarke electrode**	Enzymatic - Colorimetric - Polarographic	Glucose Oxidase Peroxidase O-dianisidine	Oxidized o-dianisidine – **ORANGE BROWN**
Hexokinase **(REFERENCE METHOD)**	Enzymatic	Hexokinase G6PD	$NADPH^+$

LABORATORY TESTS

- Screening Tests
  - ✓ **Fasting Blood Sugar** – 8-10 hours fasting

- Normal: <100 mg/dL
- Impaired fasting glucose: 100-125 mg/dL
- **Diabetic:** ≥126 mg/dL

✓ **2-hours post-prandial** – a fasting blood samples is extracted, after which, patient is given glucose load (75g). After 2 hours, blood glucose is measured.

- Normal: <140 mg/dL
- Impaired: 140-199 mg/dL
- Diabetic: ≥ 200 mg/dL

- Confirmatory Tests
  - ✓ **Oral Glucose Tolerance Test** – series of glucose testing
    - Patient is instructed to consume a **normal to high CHO diet per day for 3 days prior to procedure**
    - Patient should be ambulatory
    - The patient should be finished **within 5 minutes**
    - Glucose loads: **adult (75g), pregnant (100g) and children (1.75g/kg)**
    - Normal: <140 mg/dL
    - Impaired: 140-199 mg/dL
    - Diabetic: ≥ 200 mg/dL
- Monitoring Test
  - ✓ **HbA1c** – long term monitoring (2-3 months)
    - Dependent upon the patients' RBCs lifespan
    - Sample: EDTA whole blood, non-fasting
    - **For every 1% increase in HbA1c = 35mg/dL change in plasma glucose!**
- **Fructosamine** – short term monitoring (2-3 weeks)
  - ✓ Levels of albumin affects results

CLINICAL SIGNIFICANCE

HYPERGLYCEMIA	**Increased glucose levels**
	DIABETES MELLITUS / **DIABETES INSIPIDUS**
	Involvement of insulin / Involvement of ADH
	Polyuria / Polyuria (with no hyperglycemia)
	High specific gravity urine / Low specific gravity urine
	DIABETES MELLITUS
	TYPE 1 / **TYPE 2**
	Autoimmune process / Resistance to insulin
	Insulin-dependent DM / Non-insulin dependent DM
	Juvenile-onset DM / Adult-onset DM
HYPOGLYCEMIA	Decreased glucose levels
	Whipple's triad: ✓ Low blood glucose level (<60 mg/dL) ✓ Presence of signs and symptoms ✓ Reversal of symptoms (if glucose is administered)
GESTATIONAL DM	Due to hormonal imbalance; occurs in pregnant women

GLYCOGEN STORAGE DISEASES	
TYPE	**DEFECTS**
Ia – Von Gierke	Glucose-6-phosphatase
II – Pompe	Lysosomal acid alpha glucosidase (GAA) acid maltase
III – Cori-Forbes	Glycogen debranching enzyme
IV – Andersen	Glycogen branching enzyme
V – McArdle	Muscle phosphorylase
VI – Hers	Glycogen phosphorylase
VII – Tarui	Phosphofructokinase
XI – Fanconi-Bickel	Glycogen transporter 2
0	Glycogen synthetase

LIPIDS AND LIPOPROTEINS

- Lipids are more commonly referred to as **fats**
- **Insoluble in water** but soluble in organic solvents
- Major forms of lipids:
  - ✓ **FATTY ACIDS**
    - Simplest
    - Building blocks of lipids
    - Saturated (no double bonds) or unsaturated (with double bonds)
  - ✓ **TRIGLYCERIDES**
    - Tri – three molecules of fatty acids + one molecule of glycerol
    - Breakdown is facilitated by **lipoprotein lipase**
    - Primary cause of **turbid** serum
    - **Main storage form of lipid**
    - Requires a fasting specimen (12-14 hours)
    - > 500mg/dL highg risk for CAD
    - RV: <500 mg/dL - normal
      150-199 mg/dL - borderline high
      200-499 mg/dL - high TAG
      >500 mg/Dl - very high TAG (acute / recurrent pancreatitis)
  - ✓ **CHOLESTEROL**
    - Not readily catabolized = **not a source of fuel**
    - No fasting is required
    - Four ringed structure made by hepatocytes
    - Constituent of cell membranes and precursor of some hormones (steroids: progestin, glucocorticoids, mineralocorticoids, androgen and estrogen).
    - Estrogen promotes transport and excretion of CHOLE
    - Should be measured in adults ≥ 20 y/o at least once every 5years.
    - RV: <200 mg/dL = desirable
      200 – 239 mg/dL = borderline high
      ≥240 = high cholesterol
    - Two forms: esterified (60-70%) and free cholesterol (30-40%)
    - TAG and Chole most important lipids in management of CAD
  - ✓ **PHOSPHOLIPIDS**
    - Structure: 2 fatty acids + phospholipid attached to glycerol
    - Most abundant lipid
    - Can also be found as **surfactants** in lungs. Def in neonates: RDS
    - Forms: Lecithin/phosphatidylcholine (major, 70-75%), sphingomyelin (18-20%), phosphatidylserine and phosphatidylethanolamine (3-6%) and lysophosphatidylcholine (4-9%)
    - RV: 150 – 380 mg/dL (serum)
    - Sphingomyelin
      - Component of cell membranes (RBC and nerve sheath)
      - Niemann-pick dxs: accumulation in the liver and spleen. (lipid storage disorder)
  - ✓ **LIPOPROTEINS**
    - Carrier proteins for lipids
    - Major lipoproteins
      A. **Chylomicrons**: largest and least dense.
         - Contains mostly TAG.
         - Produced in the intestines.
      B. **VLDL/Pre-beta lipoprotein.** Made in the liver.
      C. **HDL/ Alpha Lipoprotein:** smallest but most dense lipoprotein.
         - Removes excess cholesterol from cells.
         - Produced by liver and intestine.
         - Maintains balance of cholesterol.

- CDC Reference method for determination: ultracentrifugation, precipitation with heparin-$MnCl_2$ and Abell-Kendal assay.

D. LDL/Beta Lipoprotein: Marker of CHD risk.

- most cholesterol-rich and most atherogenic.
- major end-product of VLDL catabolism.

	HDL	LDL	VLDL	Chylomicrons
	Good cholesterol	Bad cholesterol	Carrier of endogenous TAG	Carrier of exogenous TAG
Migration	Alpha	Beta	Pre-beta	Origin
Size	70-100	100-300	2000	> 2000
Density	1.063-1.125 (bottom layer)	1.019-1.063	0.95-1.006	< 0.95 (top layer)
Protein	50%	20%	4-8%	1-2%
LIPID CONTENT (%)				
Free cholesterol	3-5	6-8	4-8	1-3
Esterified	15-20	45-50	16-22	2-4
TAG	2-7	4-8	45-65	80-95
Phospholipid	26-32	18-24	15-20	3-6
Lipid: protein ratio	50:50	80:20	90:10	99:1
Apolipoproteins	A-1, A-II, C	B-100, E	B-100, A-1, C, E	A1, B-48, C, E

- Minor lipoproteins:
  - A. IDL – Subclass
    - Migrates either in the pre-beta or beta region
    - Major apolipoprotein: Apo B-100
  - B. **Lp(a)** aka **sinking pre-beta,** linked to atherosclerosis
- Abnormal lipoproteins: LpX – linked to **obstructive jaundice,** β-VLDL aka **floating β lipoprotein**
  - Indicator of cholestasis**.**
- Beta-VLDL: floating beta lipoprotein
  - Migrates with LDL in beta region found in type 3 hyperlipoproteinemia or dysbetalipoproteinemia.
  - VLDL rich in cholesterol

APOLIPOPROTEINS

- **Apo A –** major protein component of HDL
  - ✓ **Apo A-I:** LCAT activator
  - ✓ **Apo A-II:** may inhibit hepatic & lipoprotein lipases; increases plasma TAG
- **Apo B** – major protein component of LDL
  - ✓ **Apo B-48:** found in **chylomicron**
  - ✓ **Apo B-100:** synthesized in liver; found in **VLDL & LDL**
- **Apo C** – major protein component of VLDL**;** minor in HDL and LDL
  - ✓ **Apo C-I:** may inhibit the hepatic uptake of VLDL and cholesterol ester transfer protein
  - ✓ **Apo C-II:** if deficient – there would be reduced clearance of TAG-rich lipoproteins
  - ✓ **Apo C-III:** main form found in HDL. Lipolysis of TAG-rich lipoproteins is inhibited by this form
- **Minor apolipoproteins**
  - ✓ Apo D: aids in the activation of LCAT
  - ✓ Apo E: Arginine rich
    - Apo E-I
    - Apo E-II: associated with type III hyperlipoproteinemia
    - Apo E-III: most common isoform
    - Apo E-IV: associated with high levels of LDL, increased risk for Alzheimer's and CHD

✓ Apo F, Apo H and Apo J

LIPID QUANTITATION

1. **TRIGLYCERIDES**
   - **A.** CHEMICAL METHOD **(Van Handel and Zilversmit method** and **Modified Van Handel Zilversmit method)**
     STEP 1: EXTRACTION BY ORGANIC SOLVENT
     ✓ This is for the removal of lipids from proteins
     ✓ There is an additional adsorption step to remove non-triglycerides
     STEP 2: SAPONIFICATION OR HYDROLYSIS BY KOH IN ETOH
     ✓ TAG → fatty acids + glycerol
     STEP 3: OXIDATION
     ✓ Oxidizes glycerol to measurable compounds
     STEP 4: COLORIMETRY
     ✓ 500-600nm
   - **B.** ENZYMATIC METHOD – lipase and glycerokinase serve in the initial enzymatic reaction

2. **TOTAL CHOLESTEROL**
   - **A.** COLOR REACTION
     - **Liebermann Burchardt Reaction**
       ✓ **Principle:** Dehydration and Oxidation of cholesterol to form a colored compound
       ✓ **Reagents:** Acetic anhydride-sulfuric acid
       ✓ **End product:** Cholestadienyl monosulfonic acid – **GREEN**
     - **Salkowski Reaction**
       ✓ Methods:
         ○ **Bloor's method** – extraction of cholesterol by Bloor's, L-B reaction
         ○ **Abell-Kendall method** – extraction of cholesterol by Zeolite, L-B reaction
   - B. Enzymatic Method
     - Cholesterol oxidase reaction measures amount of hydrogen peroxide produced.
     - Interference: (+) hemoglobin, (-) Bilirubin and ascorbic acid.

   CDC reference method: Abell, Levy and Brodie method (3 step method: Saponification, extraction, and colorimetry)

3. **HDL**
   Methods: Electrophoresis & Modified Bloor's
4. **Ultracentrifugation**: density gradient
   a. Reference method for quantitation of lipoprotein.
   b. Svedverg (s) units
   c. Reagent: potassioum bromide solution with 1.063 density.
5. **Electrophoresis**
6. **Chemical precipitation** (HDL and LDL)

Put here your own mnemonic for the classification

Formula for LDL-Cholesterol (LDL-C) = total cholesterol – HDL- VLDL

WRITE THE FRIEDEWALD AND DELONG'S FORMULA

FREDERICKSON AND LEVY'S CLASSIFICATION OF HYPERLIPOPROTEINEMIA		
TYPES	**STANDING PLASMA TEST***	**GEL ELECTROPHORESIS**
TYPE I	Creamy layer – Clear plasma	Normal
TYPE IIa	Negative – Clear plasma	Increased β band
TYPE IIb	Negative – Cloudy plasma	Increased β and pre- β band
TYPE III	Occasional – Cloudy plasma	Increased pre- β band (broad β band)
TYPE IV	Negative – Cloudy plasma	Increased α2 band
TYPE V	Creamy layer – Cloudy plasma	Increased α2 band

**plasma is placed in a test tube and stored at 4°C overnight. Presence of "cream" floating and turbidity of plasma is observed for presence of chylomicron and VLDL respectively*

LIPID STORAGE DISEASES	
Fabry's disease	alpha galactosidase deficiency
Gaucher	beta galactosidase deficiency
Krabbe	cereboside beta galactosidase deficiency
Metachromatic Leukodystrophy	arylsufatase A deficiency
Niemann Pick	sphingomyelinase deficiency
Sandhoff	hexosaminidase A and B deficiency
Tay Sach	hexosaminidase A deficiency

LIPID PROFILE			
	Desirable	**Borderline High**	**High**
Triglycerides	<150 mg/dL	150-199 mg/dL	200-499 mg/dL
HDL-C	40 mg/dL	n/a	n/a
LDL-C	<130 mg/dL	130-159 mg/dL	160-189 mg/dL
Total Cholesterol	<200 mg/dL	200-239 mg/dL	≥240 mg/dL

STRATIFIED RISK FACTORS FOR CHD		
Age (in years)	**Moderate Risk (mg/dL)**	**High Risk (mg/dL)**
2-19	>170	>185
20-29	>200	>220
30-39	>220	>240
40- above	>240	>260

PROTEINS

- Composed of carbon, hydrogen, oxygen and **nitrogen**
- **Most abundant macromolecule in the body**
- Amphoteric in nature
- Synthesized in the liver except for immunoglobulins (which are synthesized by plasma cells)
- In alkaline Ph = proteins are negatively charged
- In acidic pH = proteins are positively charged
- Structures:
  - ✓ Primary: amino acid sequence
  - ✓ Secondary: conformations could either be alpha-helix, beta-pleated, sheath and bend form
  - ✓ Tertiary: actual 3D configuration
  - ✓ Quaternary: protein already consists of 2 or more polypeptide chains

PLASMA PROTEINS

FRACTIONS	SPECIFIC PROTEINS
Prealbumin	Aka transthyretin **Marker for malnutrition** 2nd most predominant protein in the CSF Transfer T4 and retinol (Vitamin A) ↑ Alcoholism, Chronic renal failure, steroid txm. ↓ poor nutrition RV: 18 – 45 mg/ dL
Albumin	Most abundant protein Acts as a transport protein **Negative acute phase reactant** Maintains osmotic pressure Elevated in Cystic fibrosis Negative acute phase reactant Low level: nephrotic syndrome Analbuminemia: albumin absence Bisalbuminemia: there are 2 bands seen in the albumin region Hypoalbuminemia: low levels of albumin RV: 3.5 – 5.0 g/dL
GLOBULIN	Measurement: TP – A = G ↑ Early cirrhosis RV: 2.3 – 3.5 g/dL
Alpha$_1$ globulin	**Alpha$_1$ antitrypsin (AAT)** Acute phase reactant. Released from WBC to combat inf Protease inhibitor NV 2.3-3.5 mg/dL **AFP** Tumor marker for hepatocellular carcinoma (hepatic and gonodal cancer). Increased in presence of twins and neural tube defect. Decreased in down syndrome. Screening for maternal AFP for NTD and DS: 15 and 20 weeks of gestational age. **RV: 5 ng/ml both in adults and children** **Alpha-1-acid-glycoprotein (orosomucoid)** Carrier proteins for steroid hormones (Progesterone). Increased in neonatal bacterial inf. **RV: 55-140 mg/dL** **Alpha$_1$-antichymotrypsin** Inhibits serine Proteinases Acute phase reactant. Binds and inactivates PSA Associated with Alzheimer's dxs, ↓ in liver dxs **RV: 30 – 60 mg/dL** **Gc-globulin**

	Affinity with vit D and actin. **Alpha-1-lipoprotein** Transports lipids
Alpha$_2$ globulin	**Ceruloplasmin** Transports copper ↓ **Wilson's Disease (kayser-fleisher rings: deposition in cornea)** Menkes' kinky-hair syndrome Method: copper oxidase activity. RV: 18-45 mg/dL **Haptoglobin** transports free hemoglobin Acute phase reactant **Alpha$_2$ macroglobulin** Inhibits protease 10x elevation is seen in nephrosis RV: 150-420 mg/dL
Beta globulin	**Pre-beta-lipoprotein** Transports lipids (VLDL, TAG) **Beta-lipoprotein** Transports lipids (LDL, CHOLE) **Beta$_2$ microglobulin** Light chain component of HLA Elevated in RA and SLE, MM, HIV and Renal Failure. RV: 0.2-2.8 ug/dL **Complement System** Immune response ↑ in inflammation ↓ DIC, hemolytic anemia and malnutrition **CRP** Acute phase reactants Promotes phagocytosis Cardiac marker RV: <1.0 mg/dL
	Fibrinogen Protein present in plasma but not in serum Largest protein in the blood Precursor of fibrin clot Method for measurement: **Parfentjev method** RV: <1.0 **Hemopexin** Acute phase reactant Binds heme Indicates early hemolysis RV: 50-115 mg/dL **Transferrin/SIderophilin** Transports iron ↑ hemochromatosis, ↓ liver dxs, malnutrition, nephrotic syn.
Gamma globulin	**Immunoglobulins**: synthesized in plasma cells **IgG** most abundant **IgA** found in mucous secretions **IgM** first to appear **IgE** allergy and anaphylactic reactions **IgD** present in surface of B cells **CRP** (in other references)

OTHERS PROTEINS

- **Myoglobin**
  - ✓ Carries oxygen in muscles
  - ✓ Nephrotoxin
  - ✓ Marker of chest pain (angina) and early det. Of AMI
  - ✓ ↑ **2-3 hours of onset, peak at 8-10 hours**
  - ✓ ↑ **AMI, angina, rhabdomyolysis**, muscle trauma, acute renal failure
- **Troponin (cTnI)**

- ✓ Regulates actin and myosin
- ✓ Marker for **acute coronary syndrome**
- ✓ Most important marker for AMI
- ✓ RV: <0.1 ng/mL

- **BNP**
  - ✓ ↑ ventricular systolic and diastolic dysfunction
  - ✓ Congestive heart failure
- **Cystatin C**
  - ✓ Marker for kidney function (GFR)
  - ✓ Endogenous renal marker
- Beta-trace protein
  - ✓ Marker for CSF leakage
- Amyloid
  - ✓ Fibrous protein aggregates
- Bence-Jones protein: protein found in patients with Multiple Myeloma
  - ✓ **Unique feature:** Coagulates at 40-60°C and dissolves at 100°C
  - ✓ **Method for measurement:** Immunofixation
  - ✓ **Electrophoretic pattern:** "tall spike" or "monoclonal peak"

METHODS FOR ALBUMIN QUANTITATION

- Electrophoresis
- Biuret Method
  - ✓ Principle: measurement of at least 2 peptide bonds and formation of a **violet colored chelate.**
  - ✓ Measured at 540nm
  - ✓ Reagents: Rochelle salt (NaK tartrate), Alkaline $CuSO_4$, NaOH and KI
- Kjeldahl Method
  - ✓ Reference method
  - ✓ Based upon the digestion of protein and measurement of nitrogen content of proteins
  - ✓ Albumin nitrogen x 6.25 = albumin
- Lowry (Folin-Ciocalteu) method
  - ✓ Reagent: Phosphotungstomolybdic acid
- Dye-binding method
  - ✓ BCG: most commonly used
  - ✓ BCP: most sensitive, specific and precise
  - ✓ H-ABA: with salicylates and bilirubin interferences

CSF OLIGOCLONAL BANDING

- **Multiple sclerosis:** 2 or more IgG bands in the gamma region
- Other dxs with two more bands in the CSF: **Encephalitis, neurosyphilis, Gullain-Barre syndrome, neoplastic dxs**
- Serum **banding** in CSF: **Leukemia, lymphoma and viral inf**.

AMINOACIDOPATHIES

- **Alkaptonuria**
  - ✓ Absence of **homogentisate oxidase** in tyrosine pathway
  - ✓ Ochronosis: tissue pigmentation
  - ✓ Darkening of urine upon standing
- **Homocystinuria**
  - ✓ Impaired activity of **cystathionine B-synthetase**
  - ✓ Elevated **homocysteine** and **methionine** in blood and urine
  - ✓ Screening test: Modified Guthrie Test (L-methionine sulfoximine)
- **MSUD**
  - ✓ Reduced or absence of **a-ketoacid decarboxylase**
  - ✓ Accumulation of leucine, isoleucine and valine.
  - ✓ Screening test: Modified Guthrie Test (4-azaleucine)

- **Phenylketonuria**
  - ✓ Def of **phenylalanie hydrolase**
  - ✓ Phenylpyruvic acid in both blood and urine
  - ✓ Musty odor urine
  - ✓ Screening: Guthrie Bacterial Inhibition Assay (*Bacillus subtillis)*
- Tyrosinemia
  - ✓ Def. of either of these enzymes **tyrosine aminotransferase, 4-hydroxyphenylpyruvic acid oxidase, fumarylacetoacetate**
  - ✓ Increased levels of **methionine** and **p-hydroxyphenolpyruvic acid** in blood.
  - ✓ Results to liver damage or cirrhosis

NON – PROTEIN NITROGEN

- Monitor and asses renal function.
- Result from the breakdown of protein and nucleic acids.

UREA

- Most abundant (45-50%) NPN
- **Major end product of protein metabolism**
- First metabolite to increase in kidney dxs
- BUN:Crea Ratio 10:1-20:1
- Urea is decreased in severe hepatic dxs
- Methods:
  - ✓ Micro-Kjeldahl Nesslerization method
    - Indirect method
    - Nitrogen x 2.14 = urea x 0.467 = BUN
  - ✓ Rosenthal method
    - Direct method
    - **Diacetyl monoxime method**
  - ✓ Enzymatic method
    - Urease
  - ✓ IDMS
    - Reference method

CREATININE

- **Major end product of muscle catabolism**
- Produced by three AA: methionine, arginine and lysine
- Index of overall renal function
- Evauluate fetal kidney maturity
- 100% is excreted

- Creatine: 100% is reabsorbed by kidney
- RV: Male = 0.9 – 1.3 mg/dL (80 – 115 umol/L)
  Female = 0.6 – 1.1 mg/dL (53 – 97 umol/L)
- Methods:
  - ✓ Jaffe reaction
  - ✓ Note: falsely elevated in px taking caphalosporin
    - **Color reagent:** Alkaline picrate
    - Lloyd's reagent: sodium aluminum silicate
    - Fuller's Earth: aluminum magnesium silicate
    - Enzymatic method
      - Creatinine aminohydrolase CK Method
      - Creatinase-hydrogen peroxide method

AMINO ACIDS

- Building blocks of proteins

AMMONIUM

- Used to monitor **hepatic coma**
- Important indicator of Reye's syndrome

URIC ACID

- **Major product of purine metabolism**
- Forms crystals in joints **(tophi)**
- Methods:
  - ✓ Folin method
  - ✓ Henry's method
  - ✓ Enzymatic method

LIVER FUNCTION TEST

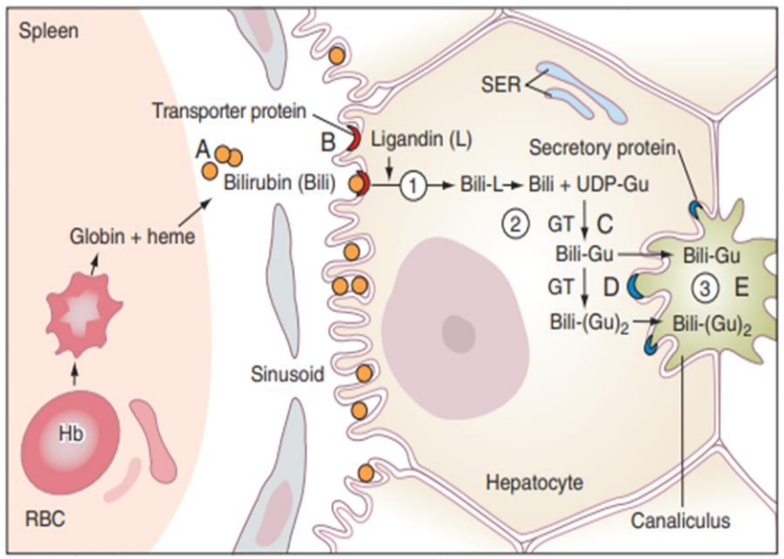


Figure 21-2 Schematic summary of the pathway of bilirubin *(Bili, in brown circles)* transport and metabolism. Bilirubin is produced from metabolism of heme, pri in the spleen, and is transported to the liver bound to albumin. It enters the hepatocyte by binding to a transporter protein *(red crescents)* and crosses the cell mem *(circled 1)*, thus entering the cell. It binds to Y and Z proteins *(not shown)* and then to ligandin for transport to the smooth endoplasmic reticulum (SER). In the SER, bi is conjugated to glucuronic acid by UDP-glucuronyl transferase 1 *(circled 2 and labeled GT)*, producing monoglucuronides and diglucuronides of bilirubin—Bili-Gu ar $(Gu)_2$. Conjugated bilirubin is then secreted into the canaliculi *(circled 3)* by the adenosine triphosphate-binding cassette transporter protein MRP2/cMOAT/ABCC2 *(as blue crescents)*. In overproduction disease **(A)**, such as hemolytic anemia, unconjugated bilirubin is produced at rates that exceed the ability of the liver to clear it, l to a usually transient increase in unconjugated bilirubin in serum. In both Gilbert's and Crigler-Najjar syndromes, mutations in the gene encoding UDP glucuronyl trans *(UDPGT1A1)*, shown at **C** in the figure, result in buildup of unconjugated bilirubin in hepatocytes and ultimately in serum. In Gilbert's syndrome, there may also be a in the bilirubin transporter protein, shown at **B** in the figure. Mutations in the *MRP2/cMOAT/ABCC2* gene result in defective secretory proteins, causing buildup of conj bilirubin in hepatocytes and, ultimately, in serum, resulting in the Dubin-Johnson syndrome **(D)**, an autosomal recessive disease. Conjugated hyperbilirubinemia found in the Rotor syndrome, possibly virus induced. In adults, blockade of any of the major bile ducts, especially the common bile duct, by stones or space-occu lesions such as tumors **(E)**, is the most common cause of conjugated hyperbilirubinemia.
Hb, Hemoglobin; *RBC*, red blood cell.

Photo reference: Henry's Clinical Diagnosis and Management by Laboratory Methods, 22nd edition

METHODS

- Van den Bergh: color reaction for bilirubin
  - ✓ Color reagent: Diazo reagent
  - ✓ Product: Azobilirubin
  - ✓ Evelyn-Malloy
    - **Medium: ACID**
    - **Dissociating agent: 50% methanol**
    - **End color: red/reddish purple**
  - ✓ Jendrassik-Grof
    - **Medium: ALKALINE**
    - **Dissociating agent: Caffeine sodium benzoate**
    - **End color: blue**
- Icterus index
  - ✓ Applicable to newborn and neonates
- Bromsulfonpthalein Dye Excretion test
  - ✓ **Rosenthal White**
    - Double collection method
    - Collection is done after 5 mins and 30 mins
    - Reference values: 50**%** dye retention (5mins) 0% (30mins)
  - ✓ **Mac Donald**
    - Single collection method
    - Collection: done after 45 mins (± 5% dye retention)

DISEASES

- Gilbert syndrome: defect in transport protein in liver
- Crigler-Najjar syndrome: defective conjugation due to deficiency of UDP-GTase
- Dubin-Johnson syndrome: defective excretion due to blockage by stones

ENZYMES

- Catalyzes reaction

DEFINITION OF TERMS

- Apoenzyme: protein portion of enzyme without cofactor
- Holoenzyme: complete active enzyme
- Active site: site where enzymatic reaction occurs
- Allosteric site: site other than the active site
- Isoenzyme: forms of enzyme that are different from each other but still catalyzes same reaction

CATEGORIES

1. **Oxidoreductase**
   - ✓ For oxidation/reduction reactions
   - ✓ Ex: LDH, G6PD and Malate dehydrogenase
2. **Transferase**
   - ✓ Catalyzes transfer of groups from one substrate unto another
   - ✓ Ex: AST, ALT, CK, GGT
3. **Hydrolase**
   - ✓ Hydrolysis
   - ✓ Ex: ACP, ALP, 5'NT, AMS, LPS, CHS
4. **Lyase**
   - ✓ Removal of groups but with no hydrolysis
   - ✓ Ex: Aldolase
5. **Isomerase**
   - ✓ Interconversion of isomers
6. **Ligase**
   - ✓ Joins to 2 substrate molecules
   - ✓ Ex: synthases

ENZYME	METHODS	SUBSTRATES	FACTS
HEPATIC ENZYME PROFILE			
ALP **Liver Kidney Bone Placenta Intestine WBC**	Bodansky Shenowara Jones King-Armstrong Bessy Lowry-Brock	B-glyceroPO_4 B-glyceroPO_4 p-nitrophenylPO_4 p-nitrophenylPO_4	Optimum pH: 10 Greatly elevated in Paget's disease Avoid using EDTA-Citrate-Oxalate
ALT (SGPT) **Liver RBCs**	Reitman-Frankel (DNPH)	Alanine α-keto	Liver-specific Marked elevation with viral hepatitis De ritis ratio: >1 = viral; <1 = non-viral
LD **All tissues**	Wacker Method (forward) Wrobleuski La Due (reverse) Wrobleuski Cabaud Berger Broida		NAD+ (cofactor) LD4 and LD5 Storage: 25°C up to 24 hours
GGT **Canaliculi of hepatic cells, Kidney, Prostate and Pancreas**	SZAZ	Gammaglutamyl p-nitroanilide	Most sensitive marker for alcoholic hepatitis
ChE	Pseudo- Michael; Ellman	Acetylcholine	ChE: CNS, RBC, Lungs, Spleen Pseudo: Liver – Succinylcholine (relaxant);

			anesthetic poisoning
CARDIAC ENZYME PROFILE			
CK **Cardiac, skeletal and brain tissues**	Tanzer-Gilvarg (forward) Oliver-Rosalki (reverse)		CK-BB (fastest migrating; most anodal), CK-MB, CK-MM (slowest; least anodal) Sensitive indicator of AMI & Duchenne disorder Highest elevation of total CK: Duchenne's muscular dystrophy Light and pH sensitive
AST (SGOT) **Liver, heart, skeletal muscle**	Karmen Method (Ph 7.5; 340 nm)	Aspartate α-keto	Most sensitive enzyme for skeletal muscle disease Inhibited by all anticoagulants except heparin (but ammonium heparin should not be used)
LD **All tissues**	Wacker Method (forward) – pH 8.8 Wrobleuski La Due (reverse) – pH 7.2 Wrobleuski Cabaud Berger Broida		LD1 (anodic & heat stable) LD2 (heat stable & major isoenzyme in the sera of healthy persons) LD5 (cathodic & cold labile) Flipped ratio: LD1>LD2
			LD/HBD(LD1) ratio: 1.2-1.6; if 0.8-1.2 suspect for MI
Myoglobin			Responsible for O_2 supply of striated muscle
Troponin			3 subunits: I (inhibitory), T (tropomyosin-binding) & C (calcium-binding)
ACUTE PANCREATITIS PROFILE			
AMS **Salivary glands, Pancreas**	Saccharogenic Iodometric/Amyloclastic Chromogenic Kinetic Method	Pancreatic AMS: diastase Salivary AMS: ptyalin	MicroAMS: unbound (free) MacroAMS: bound to IgG and IgA Earliest pancreatic marker Smallest enzyme in size Salivary AMS: inhibited by wheat germ lectin
LPS **Pancreas**	Cherry-Crandall Sigma-Tietz Titration	Olive Oil/Triolein (pure form of TAG)	End product: Fatty Acids Most specific pancreatic marker
PROSTATIC CANCER PROFILE			
ACP **RBC Prostate**	Chemical Inhibition Test RBC-ACP: inactivated by Cu++, unaffected by Tartrate P-ACP: unaffected by Cu++, inactivated by	Organophosphates	Very labile (add 5M acetate buffer/citrate tablet to preserve)

	Tartrate		
PSA			Most useful for tumor marker for prostate cancer RR: 0-4ng/mL

ACUTE MYOCARDIAL INFARCTION MARKERS (Bishop, Rodriguez, Coderes)			
Marker	**Onset (hours)**	**Peak (hours)**	**Duration (hours)**
Myoglobin	1-3	5-12	18-30
Trop I	3-4	10-24	7days up to 10-14days
Trop T	3-6	12-18	5-10 days
CK-MB	4-6	12-24	48-72
AST	6-8	24	5 days
LDH	12-24	48-72	10-14 days

ELECTROLYTES

ELECTRONEUTRALITY

$$Na^+ + K^+ + 7 = Cl^- + HCO_3^- + 25$$

ANION GAP: difference between unmeasured anions and unmeasured cations

AG = Na^+ - (Cl^- + HCO_3^-) **Ref. range: 7-16 mmol/L**

AG = Na^+ + K^+) - (Cl^- + HCO_3^-) **Ref. range: 10-20 mmol/L**

ELECTROLYTES	INFORMATION	
Sodium	Most abundant **cation** in the **ECF** Has the greatest influence in serum osmolality **Aldosterone**: responsible for the reabsorption in tubules **Atrial natriuretic factor:** blocks secretion of both aldosterone & renin **Hyponatremia is the most common electrolyte disorder** ~for every 100mg/dL increase in blood glucose, there is a decrease by 1.6 mmol/L of serum sodium	
	Hypernatremia	**Hyponatremia**
	Excessive water loss	Increase water retention
	Water intake is decreased	Water imbalance
	Increase Na+ intake/retention	Sodium loss
	Methods: Flame Emission Photometry (FEP) - yellow ISE – **glass aluminum silicate** AAS Colorimetry - **Albanese Lein**	
Potassium	Major intracellular cation Regulates ICF volume regulation and H+ concentration, contraction of the heart and excitability of mucles	
	Hyperkalemia	**Hypokalemia**
	Extracellular shift	Renal loss
	Increased intake	GI loss
	Renal excretion is decreaed	Intracellular shift
	Artifactual (eg. Hemolysis, thrombocytosis)	Intake is decreased
	Methods: FEP – **violet** ISE – **valinomycin gel** AAS Colorimetry **– Lockhead and Purcell**	
Chloride	Major extracellular anion Only anion that serves as an enzyme activator Sweat chloride: diagnosis for cystic fibrosis	
	Hyperchloremia	**Hypochloremia**
	GI loss	Hyperparathyroidism
	Diabetic ketoacidosis	Low reabsorption of HCO_3
	Low Na+ levels	
	Mineralocorticoid excess & deficiency	
	Methods: Mercurimetric method: Schales and Schales (indicator: diphenylcarbazone) Coulometric amperometric titration: Cotlove chloridometer Colorimetry ISE – electrodes with AgCl membranes	
Calcium	Ion that is the most abundant in the body 3rd most abundant in blood 99% (bone) and 1% (blood) PTH: promotes bone resorption Calcitonin: promotes bone deposition Vitamin D3: promotes intestinal absorption of calcium Methods:	

	Clark & Collip Precipitation (titration with $KMnO_4$) FEP AAS – reference method ISE – **liquid membrane**
Magnesium	2nd major cation in ICF 4th most abundant ion in the body 2nd mostly affected by hemolysis (after potassium) Methods: AAS – reference method Colorimetry – **Calmagite (reddish-violet)** Dye method – **Titan yellow**
Bicarbonate	2nd most abundant ECF anion Acts as buffer Diffuses out of the cell in exchange for chloride Increased levels: alkalosis, vomiting, hypokalemia Decreased levels: acidosis Methods: ISE – Clark electrode Enzymatic method: Phosphoenolpyruvate carboxylase & dehydrogenase
Phosphorus	Unstable ion Inversely proportional to calcium and PTH Best preserved by acidic filtrate

SUMMARY (memorize this ☺)

ELECTROLYTES	FUNCTION
HCO_3, K, Cl	Acid-Base Balance
Ca, Mg	Blood coagulation
Mg, Ca, Zn	Cofactors in enzyme activation
K, Mg, Ca	Myocardial rhythm and contractility
K, Ca, Mg	Neuromuscular excitability
Mg, PO_4	Production and use of ATP from glucose
Mg	Regulation of ATPase pumps
Na, K, Cl	Volume and osmotic regulation

BLOOD GAS

DEFINITION OF TERMS:

- **Acid**: a compound that could **donate** a H+ ion
- **Base**: a compound that could **accept** a H+ ion
- **Acid-Base Balance:** a mechanism by which the pH of blood is maintained at 7.35-7.45 for homeostasis
- **Buffer:** a weak acid/base with its conjugate salt that resists changes in Ph

ACID BASE BALANCE

HENDERSON-HASSELBACH EQUATION

- Implicates the relationship between pH, and the two involved organs - lungs and kidneys

$$pH = 6.1 + log\frac{HCO_3}{PCO2\ x\ 0.0307}$$

EXPANDED FORM: $pH = 6.1 + \log\frac{[TCO2-(PCO2\ x\ 0.03)]}{PCO2\ x\ 0.03}$

FOUR BASIC ABNORMAL STATES						
Imbalance	**pH**	**pCO_2**	**H_2CO_3**	**HCO_3**	**Primary compensation**	**Seen in:**
Respiratory Acidosis	↓	↑	↑	**N**	Kidneys retain bicarbonate & excrete hydrogen	Pneumonia, emphysema
Respiratory Alkalosis	↑	↓	↓	**N**	Reverse of respiratory acidosis	Hyperventilation, early salicylate poisoning
Metabolic Acidosis	↓	**N**	**N**	↓	Hyperventilate (CO_2 blew off)	Diabetic ketoacidosis, renal disease and prolonged diarrhea
Metabolic Alkalosis	↑	**N**	**N**	↑	Hypoventilation (CO_2 retention)	Vomiting, antacids, $NaHCO_3$ infusion

EVALUATING ACID-BASE DISORDERS

1. Determine if the pH is high (alkalosis) or low (acidosis)
2. Compare pCO_2 and HCO_3 to normal values
   - ✓ **If pCO_2 is opposite to pH =** respiratory
   - ✓ **If HCO_3 is in the same direction with pH =** metabolic
3. If pH is within normal range, full compensation has occurred
4. if main compensatory mechanism has already occurred yet the pH is still out of range, **partial compensation** happened.

NORMAL VALUES

- pH: **7.35 – 7.45**
- pCO_2: **35-45 mmHg**
- pO_2: **81-100 mmHg**
- HCO_3: **21-28 mEq/L**
- TCO_2: arterial (19-24 mmol/L); venous (**22-26 mmol/L**)
- H_2CO_3: **1.05-1.035 mmol/L**
- O_2 saturation: **94-100%**

COMMON SOURCES OF ERROR

Error	pCO2	pH	pO2	Effect
Sample sitting at room temperature for more than 30 mins	↑	↓	↓	**Acidosis**
Bubbles in syringe, uncapped specimen	↓	↑	↑	**Alkalosis**
Hyperventilation				**Alkalosis**
Specimen exposed to air				**Alkalosis**

SAMPLE:

1. **pH = 7.25, pCO_2 = 42 and HCO_3 = 16**
   - ✓ determine acid-base status

ENDOCRINOLOGY

- study of endocrine glands and the hormones they secrete

HORMONES

- are chemical signals that are secreted by cells into the blood stream that travels to its target tissues

POSITIVE FEEDBACK

An increase in the hormone product results to an elevated activity (another hormone production) of the system

NEGATIVE FEEDBACK

A decrease in the hormone product results to a decreased activity (another hormone production) of the system

CLASSIFICATION OF HORMONES

CLASSIFICATION	EXAMPLE
PEPTIDES/POLYPEPTIDES • **Water soluble**	
A. **GLYCOPROTEIN**	HCG, TSH, EPO, FSH
B. **POLYPEPTIDES**	ADH, GH, ACTH, Prolactin
STEROIDS • **Synthesized from cholesterol** • **Insoluble**	Aldosterone, Estrogen, Cortisol, Progesterone, Testosterone, Vitamin D
AMINES	Epinephrine, norepinephrine, T3, T4, melatonin

GLAND	HORMONES	INFORMATION
Hypothalamus	**Releasing Hormones**	TRH – regulates production of TSH and prolactin GnRH – regulates production of LH and FSH GHRH – regulates production of GH CRH – regulates production of ACTH
	Somatostatin	Inhibitor of GH and TSH production
	Dopamine	Prolactin release inhibitor
Anterior pituitary	**GH**	Most abundant pituitary hormone **Gigantism**: increase (excess) in GH **before** the closure of epiphyseal plate **Acromegaly**: increase (excess) in GH **after** the closure of epiphyseal plate **Dwarfism**: a deficiency of GH
	Prolactin	Initiates and maintains lactation Highest levels at 4am, 8am, 8pm and 10pm **Prolactinoma:** most common type of functional pituitary tumor
	TSH	Stimulation for the production of T3 and T4
	LH	For secretion of progesterone; for ovulation
	ACTH	Stimulation for the production of adrenocortical steroid formation and secretion
	FSH	For secretion of estrogen For development of seminiferous tubules; spermatogenesis
Posterior pituitary ~ only releases hormones (doesn't	**Oxytocin**	Stimulates contraction of the uterine "Fergusson reflex" Also acts in parturition and in transport of

produce)		sperm Also, for milk ejection (suckling asstimulator)
	ADH	Aka vasopressin For water balance and blood pressure elevation Deficiency: diabetes insipidus
Thyroid gland	**T3 and T4:** produced by follicular cells	For metabolism There are more T4 than T3 T3 is more biologically active **Primary hyperthyroidism (Graves' disease)** ✓ increased T3 and T4 but decreased TSH ✓ presence of anti-TSH receptor antibody Secondary hyperthyroidism: both FT4 and TSH are increased **Primary hypothyroidism (Hashimoto's thyroiditis)** ✓ increased TSH but decreased T3 and T4 ✓ presence of anti-TPO antibody **Myxedema:** manifestation of Hashimoto's disease
	Calcitonin: produced by parafollicular cells	A calcium and phosphate regulator
Parathyroid gland: smallest gland in the body	**PTH**	Produced and secreted by chief cells of parathyroid gland For **bone resorption**
		Primary hyperparathyroidism: increased ionized calcium **Secondary hyperparathyroidism:** decreased ionized calcium
Adrenal gland	**Cortisol**	Secreted by zona fasciculate Highest levels in: 6am-9am Lowest levels: 11pm-3am **Cushing's syndrome**: increased levels of cortisol and ACTH but decreased levels of aldosterone and renin are notable ✓ screening test: 24hr urine free cortisol test ✓ Confirmatory: low dose dexamethasone suppression test and CRH stimulation test **Cushing's disease:** increased levels of ACTH due to tumor on the pituitary gland Methods: **Porter-Silber reaction** (corticosteroids); + reaction = yellow pigment **Zimmerman reaction** (ketosteroids) + reaction = reddish purple color
	Aldosterone	Secreted by zona glomerulosa Most important mineralocorticoid Responsible to Na+ and K+ retention **Barterr's syndrome:** there is a defect in the kidney's ability to reabsorb sodium **Conn's syndrome** (1' hyperaldosteronism): there is hypokalemia and hypernatremia **Liddle's syndrome:** there is an excess sodium reabsorption and excretion of potassium due to defect in the DCT

	Catecholamines	Secreted in medulla 80% epinephrine, 20% norepinephrine **Pheochromocytoma**: tumor that results to overproduction of catecholamines
Reproductive glands	**Testosterone**	Principal androgen in the blood Most potent male androgen **Synthesized by the Leydig cells**
	Estrogen	**Estrone:** most abundant in **menopausal women** **Estradiol:** most potent; most abundant in pre-menopausal women **Estriol:** major estrogen detected during pregnancy; produced by placenta; marker for down syndrome **Kober reaction:** used to analyze **estrogens**
	Progesterone	Single best hormone to evaluate if ovulation has occurred
Pancreas	**Insulin**	Hypoglycemic agent
	Glucagon	Hyperglycemic agent

METABOLITES OF HORMONES!

- Dopamine: Homovanilic acid
- Serotonin: 5-HIAA
- Epinephrine: Vanillyl mandelic acid and metanephrine
- Norepinephrine:
  - ✓ Urine: 3-methoxy-4-hydroxyphenylglycol
  - ✓ Blood: vanillyl mandelic acid

TOXICOLOGY

TOXIC AGENTS

- Alcohol
  - ✓ Ethanol (grain alcohol): most commonly abused chemical substance

STAGES OF IMPAIRMENT	
BLOOD ALCOHOL (% w/v)	**SIGNS AND SYMPTOMS**
0.01 – 0. 05	No obvious impairment, some changes observable on performance testing
0.03 – 0.12	Mild euphoria, decreased inhibitions, some impairment of motor skills
0.09 – 0.25	Decreased inhibitions, loss of critical judgment, memory impairment, diminished reaction time
0.18 – 0.30	Mental confusion, dizziness, strongly impaired motor skills (staggering, slurred speech)
0.27 – 0.40	Unable to stand or walk, vomiting, impaired consciousness
0.35 – 0.50	Coma and possible death

≥0.10 – PRESUMPTIVE EVIDENCE OF DRIVING UNDER ALCOHOL INFLUENCE

- Cyanide
  - ✓ Odor of **bitter almonds**
- Arsenic
  - ✓ Odor of **garlic**; keratinophilic
- Carbon monoxide
  - ✓ Odorless, colorless and tasteless gas
  - ✓ Binds to hemoglobin 250 times (in terms of affinity) as compared to oxygen
  - ✓ Makes blood **cherry-red** in color
- Mercury
  - ✓ Nephrotoxic and can bind myelin (in neurons)
- Lead
  - ✓ Specimen of choice: **whole blood**
  - ✓ Inhibits enzymes D-ALA synthetase & pyrimidine-5'-nucleotidase
- Organophosphates
  - ✓ Found in insecticides and pesticides
  - ✓ Hepatotoxic
  - ✓ Can inhibit enzyme **acetylcholinesterase**

DRUGS OF ABUSE	
DRUG	**METABOLITE**
Amitriptyline	Nortryltyline
Cocaine	Benzoylecgonine
Heroin	Morphine
Marijuana	Tetrahydrocannabinol
Primodine	Phenobarbital
Procainamide	NAPA

TOXIC DRUG MONITORING

DEFINITION OF TERMS:

- Pharmacodynamics: what the drugs do to the body
- Pharmacokinetics: what the body does to the drug (biotransformation, distribution, metabolism and elimination)
- First pass metabolism: drugs enter the hepatic route first before entering the general circulation
- Half-life: time needed for a drug's concentration in serum to decrease into half
- Peak specimen: collection of this is done 30-60 mins **after** the administration of drug
- Trough specimen: this is collected **before** administration of the succeeding dose

CLASSIFICATION OF DRUGS	REPRESENTATIVE DRUGS
Antibiotics	Aminoglycosides, chloramphenicol, vancomycin
Anticonvulsants	Ethosuximide, Carbamazepine, Phenytoin, Phenobarbital, Valproic acid
Antidepressants	Lithium, Fluoxetine and tricyclic antidepressants
Anti-inflammatory/analgesics	Aspirin, acetaminophen
Anti-neoplastic	Busulfan, methotrexate
Bronchodilators	Theohylline
Cardioactive	Digoxin, Procainamide, Lidocaine, Propanolol, Quinidine
Immunosuppressives	Tacrolimus (FK-506), Prednisone, Cyclosporine

GOOD TO KNOW FOR THE DRUGS:

- ✓ Aspirin: drug that inhibits **cyclooxygenase**
- ✓ Acetaminophen: hepatotoxic drug
- ✓ Lithium: for treatment of bipolar disorder or manic depression
- ✓ Phenobarbital: used for treatment of grand-mal
- ✓ Valproic acid: for treatment of petit mal
- ✓ Vancomycin: cause of **red man syndrome**

CONVERSION FACTORS
(derived from Clinical Chemistry Handbook of Dean Maria Teresa T. Rodriguez, RMT, MAEd, MSMT)

ANALYTES	CONVENTIONAL UNITS TO SI UNITS	CONVERSION FACTOR
ALBUMIN	g/dL to g/L	10
PHOSPHOLIPID		0.01
TOTAL PROTEIN		10
AMMONIA	µg/dL to µmol/L	0.587
THYROXINE	µg/dL to nmol/L	12.9
BICARBONATE	mEq/L to mmol/L	1.0
CHLORIDE		1.0
MAGNESIUM		0.5
POTASSIUM		1.0
SODIUM		1.0
LITHIUM	mEq/L to µmol/L	1.0
BUN	mg/dL to mmol/L	0.357
CALCIUM		0.25
CHOLESTEROL		0.026
GLUCOSE		0.0555
PHOSPHORUS		0.323
TRIGLYCERIDE		0.0113
URIC ACID		0.0595
BILIRUBIN	mg/dL to µmol/L	17.1
CREATININE		88.4
IRON		0.179
pCO_2	mm/Hg to kPa	0.133

pO_2		0.133

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