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 _____ that is used for QC purposes only
- _____: a colorless solution with **known** concentration of substances used for calibration
- _____: 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 _____
- **Accuracy:** nearness of measured value to that of the _____
- _____: nearness of measured values to each other
- **Diagnostic specificity:** defined as the ability of a method to detect a population of individuals _____
- **Diagnostic sensitivity:** defined as the ability of a method to detect a population of individuals having the _____
- **Intralab QC (internal QC):** control samples are run simultaneously with a patient to ensure reliability of methods and result
- **Interlab QC (external QC):** laboratories are given samples with unknown concentrations for them to test and results are compared with other laboratories thus maintaining "_____" to methods utilized.
- _____: average of a set of values (**mean = Σx/n**)
- **Median:** _____ of a set of values
- **Mode:** the most frequent among all values/data
- **Standard Deviation:** it is defined as the measure of dispersion of values to that of the mean

$$SD = \sqrt{\frac{\Sigma(\mathbf{x}-\mathbf{mean})^2}{n-1}}$$

- **Coefficient of variation:** mean expression in percentile

$$CV = \frac{SD}{mean} x\ 100$$

- **T-test:** this is used to assess if there is a statistical difference between the _____ of 2 groups of data
- **F-test:** this is used to assess if there is a statistical difference between the _____ of 2 groups of data
- _____: most widely used QC chart
- **Trend:** six or more consecutive values that _____ gradually **(will cross the mean) – main cause:** _____
- _____: six or more consecutive values that are distributed on one side or other side of the mean **(does NOT cross the mean) – main cause:** instrument calibration is improper

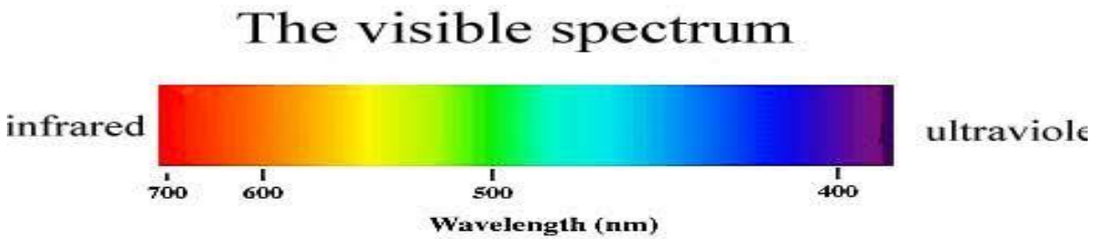
WESTGARD RULES		
TYPE OF ERROR	**RULES**	**SOURCES OF ERROR**
RANDOM - **Tests for** _____	1_{2s} (warning rule), 1_{3s} and R_{4s}	**By chance errors:** mislabeling, pipetting error, fluctuations in temperature & voltage
SYSTEMATIC - **Tests for** _____	2_{2s}, 4_{1s} and 10x	Improper calibration, reagent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

- _____: 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 _____

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** _____ **and inversely proportional to** _____.
  - ✓ Absorbance is proportional to the inverse log of transmittance

$$A = -\log T = \log 1/T$$

SINGLE-BEAM SPECTROPHOTOMETER

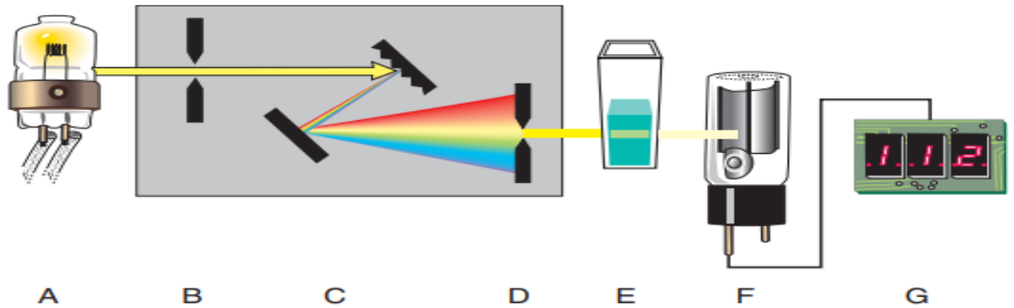


Figure 4-5 Components of a single-beam spectrophotometer. **A,** Exciter lamp; **B,** entrance slit; **C,** monochromator; **D,** exit slit; **E,** cuvet; **F,** photodetector; **G,** light-emitting diode (LED) display.

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
2. **ENTRANCE SLIT** – minimizes the entry of stray light to the monochromator
3. _____ – isolates specific wavelength
   - ✓ Prisms: light is _____
   - ✓ _____: 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)
5. **CUVETTE –** contains the solution
6. **PHOTODETECTOR –** aids in the conversion of light transmitted to photoelectric energy
   - ✓ Barrier layer cell: simplest
   - ✓ Photodiode: has excellent linearity
   - ✓ _____: most commonly used
   - ✓ Phototube
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.
- _____ **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 _____
  - ✓ 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: _____

TURBIDIMETRY

- Measures _____ by molecules
- Used for immunoglobulins, immune complexes and complement

NEPHELOMETRY

- Measures _____ by molecules
- Used for measuring amount of _____

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: _____
  - ✓ 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
  - ✓ _____ – separation by charge and molecular size

ELECTROPHORETIC PATTERN OF CERTAIN CONDITIONS	
Alpha$_1$-globulin flat curve	Juvenile cirrhosis
Alpha$_2$-globulin band spike	
Beta-gamma bridging	Hepatic cirrhosis
Monoclonal gammopathy (gamma spike)	
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)
  - ✓ 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
  - ✓ **Hyperglycemic**
    - Glucagon – released by a cells of islet of Langerhans
    - Somatostatin – released by delta cells of islet of Langerhans
    - Cortisol
    - Epinephrine
    - Growth hormone
    - Thyroxine
- MUST KNOW FOR SPECIMEN FOR GLUCOSE DETERMINATION
  - ✓ FBS should be obtained from an 8-10 hours fasting sample
  - ✓ In terms of glucose levels: _____**>** _____ **but <** _____
  - ✓ Glucose is metabolized at:
    - Room temperature: _____
    - _____: 2 mg/dL/hr
  - ✓ Tube of choice: Gray top **(anticoagulant:** _____**; anti-glycolytic agent:** _____**)**

GLUCOSE DETERMINATION

METHOD	PRINCIPLE	REAGENTS	END PRODUCT/ COLOR REACTION
Folin-Wu - **Modification: Benedict's Test**	Copper Reduction	Alk. Copper reagent _____	Molybdenum – **BUE**
Nelson- Somogyi	Copper Reduction	Alk. Copper reagent _____	Molybdenum – **BLUE**
Neocuproine	Copper Reduction	Cuprous ions Neocuproine	Cuprous-Neocuproine Complex – **YELLOW/ YELLOW ORANGE**
Ortho-Toluidine	Dubowski reaction; Condensation Method	O-toluidine Glacial Acetic Acid	Glycosylamine – **BLUE GREEN**
Autoanalyzer (Hagedorn-Jensen)	Ferricyanide reduction (Inverse Colorimetry)	$K_3Fe(CN)_6$	$K_3Fe(CN)_6^{-4}$
Glucose Oxidase - **Saifer Gernstenfield** - **Clarke electrode**	Enzymatic - Colorimetric - Polarographic	Glucose Oxidase Peroxidase O-dianisidine	Oxidized o-dianisidine – **ORANGE BROWN**
_____ **(Reference Method)**	Enzymatic	Hexokinase G6PD	$NADPH^+$

LABORATORY TESTS

- Screening Tests
  - ✓ **Fasting Blood Sugar** – _____
    - 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 _____ **CHO diet per day for** _____ **prior to procedure**
    - Patient should be ambulatory
    - The patient should be finished **within 5 minutes**
    - Glucose loads: **adult (_____), pregnant (100g) and children (1.75g/kg)**
    - Normal: <140 mg/dL
    - Impaired: 140-199 mg/dL
    - Diabetic: ≥ 200 mg/dL
- Monitoring Test
  - ✓ _____ – 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!**
- _____ – 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	
	Insulin-dependent DM	Non-insulin dependent 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	
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 (_____)
  - ✓ **CHOLESTEROL**
    - Not readily catabolized = **not a source of fuel**
    - No fasting is required

- Two forms: esterified (60-70%) and free cholesterol (30-40%)

✓ **PHOSPHOLIPIDS**
- Structure: 2 fatty acids + phospholipid attached to glycerol
- Can also be found as **surfactants** in lungs
- Forms: _____ (major, 70-75%), sphingomyelin (18-20%), phosphatidylserine and phosphatidylethanolamine (3-6%) and lysophosphatidylcholine (4-9%)

✓ **LIPOPROTEINS**
- Carrier proteins for lipids

	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	B	C	A, B, C, E

- Minor lipoproteins: IDL, **Lp(a)** aka _____, linked to _____
- Abnormal lipoproteins: _____ – linked to **obstructive jaundice**, β-VLDL aka _____

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 _____
- ✓ _____**:** 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:
         - o **Bloor's method** – extraction of cholesterol by Bloor's, L-B reaction
         - o **Abell-Kendall method** – extraction of cholesterol by Zeolite, L-B reaction

3. **HDL**
   Methods: Electrophoresis & Modified Bloor's

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 a2 band
TYPE V	Creamy layer – Cloudy plasma	Increased a2 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

Put here your own mnemonic for the classification

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		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	

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

ALBUMIN

- Most abundant protein
- Acts as a transport protein
- _____ **acute phase reactant**
- Analbuminemia: albumin absence
- Bisalbuminemia: there are 2 bands seen in the albumin region
- Hypoalbuminemia: low levels of albumin

PREALBUMIN

- Aka transthyretin
- **Marker for** _____
- 2nd most predominant protein in the CSF

GLOBULIN

- Alpha-1-acid-glycoprotein: carrier proteins for steroid hormones
- AFP: a tumor marker for hepatocellular carcinoma
- Transferrin: transports iron
- Haptoglobin: transports free hemoglobin
- Ceruloplasmin: transports copper; _____**:** disease associated with low levels of ceruoplasmin

FIBRINOGEN

- Protein present in plasma but not in serum
- Method for measurement: **Parfentjev method**

OTHERS

- Bence-Jones protein: protein found in patients with _____
  - ✓ **Unique feature:** Coagulates at _____ and dissolves at _____
  - ✓ **Method for measurement:** Immunofixation
  - ✓ **Electrophoretic pattern:** "tall spike" or "monoclonal peak"

FRACTIONS	SPECIFIC PROTEINS
Prealbumin	Prealbumin
Albumin	Albumin
Alpha$_1$ globulin	Alpha$_1$ antitrypsin, AFP, AAG, Alpha$_1$-anti-chymotrypsin, Gc-globulin
Alpha$_2$ globulin	Ceruloplasmin, haptoglobin, alpha$_2$ macroglobulin
Beta globulin	Beta$_2$ microglobulin, complement system, CRP, fibrinogen, LDL, VLDL, hemopexin, transferrin
Gamma globulin	Immunoglobulins, CRP (in other references)

METHODS FOR ALBUMIN QUANTITATION

- Electrophoresis
- Biuret Method
  - ✓ Principle: measurement of atleast 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
  - ✓ _____
  - ✓ 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

Non-protein nitrogen

UREA

- Most abundant (45-50%) NPN
- **Major end product of** _____
- 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
  - ✓ _____
    - Reference method

CREATININE

- **Major end product of** _____
- 100% is excreted
- Creatine: 100% is reabsorbed by kidney
- Methods:
  - ✓ Jaffe reaction
    - **Color reagent:** Alkaline picrate
    - Lloyd's reagent: sodium aluminum silicate
    - Fuller's Earth: aluminum magnesium silicate

AMINO ACIDS

- Building blocks of proteins

AMMONIUM

- Used to monitor _____
- Important indicator of _____

URIC ACID

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

LIVER FUNCTION TEST

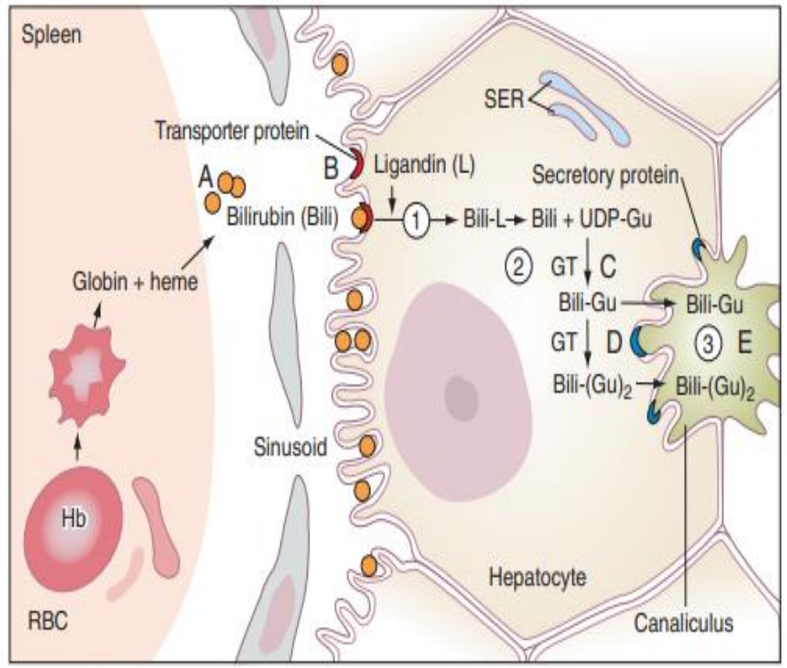


Figure 21-2 Schematic summary of the pathway of bilirubin *(Bili, in brown circles)* transport and metabolism. Bilirubin is produced from metabolism of heme, primarily in the spleen, and is transported to the liver bound to albumin. It enters the hepatocyte by binding to a transporter protein *(red crescents)* and crosses the cell membrane *(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, bilirubin is conjugated to glucuronic acid by UDP-glucuronyl transferase 1 *(circled 2 and labeled GT)*, producing monoglucuronides and diglucuronides of bilirubin—Bili-Gu and Bili-$(Gu)_2$. Conjugated bilirubin is then secreted into the canaliculi *(circled 3)* by the adenosine triphosphate-binding cassette transporter protein MRP2/cMOAT/ABCC2 *(shown 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, leading 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 transferase *(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 defect 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 conjugated bilirubin in hepatocytes and, ultimately, in serum, resulting in the Dubin-Johnson syndrome **(D),** an autosomal recessive disease. Conjugated hyperbilirubinemia is also 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-occupying 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

- _____: color reaction for bilirubin
  - ✓ Color reagent: Diazo reagent
  - ✓ Product: _____
  - ✓ Evelyn-Malloy
    - **Medium: ACID**
    - **Dissociating agent:** _____
    - **End color: red/reddish purple**
  - ✓ Jendrassik-Grof
    - **Medium: ALKALINE**
    - **Dissociating agent:** _____
    - **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. _____
   - ✓ 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: _____
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 a-keto	_____ 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 upto 24 hours
GGT **Canaliculi of hepatic cells, Kidney, Prostate and Pancreas**	SZAZ	Gammaglutamyl p-nitroanilide	Most sensitive marker for _____
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: _____ Light and pH sensitive
AST (SGOT) **Liver, heart, skeletal muscle**	Karmen Method (Ph 7.5; 340 nm)	Aspartate a-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: _____ 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			
_____ **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	_____/Triolein (pure form of TAG)	End product: Fatty Acids _____ 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 Tartrate	Organophosphates	Very labile (add 5M acetate buffer/citrate tablet to preserve)
PSA			Most useful for tumor marker for prostate cancer RR: 0-4ng/mL

ACUTE MYOCARDIAL INFARCTION MARKERS (Bishop, Rodriguez, Coderes)			
Marker	**Onset (hrs)**	**Peak (hrs)**	**Duration (hrs)**
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		48-72
AST	6-8	24	5 days
LDH		48-72	10-14 days

ELECTROLYTES

ELECTRONEUTRALITY

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

_____: 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

<table>
<tr><th>ELECTROLYTES</th><th>INFORMATION</th></tr>
<tr><td>Sodium</td><td>Most abundant cation in the ECF
Has the greatest influence in serum osmolality
Aldosterone: responsible for the reabsorption in tubules
_____: 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
<table>
<tr><th>Hypernatremia</th><th>Hyponatremia</th></tr>
<tr><td>Excessive water loss</td><td>Increase water retention</td></tr>
<tr><td>Water intake is decreased</td><td>Water imbalance</td></tr>
<tr><td>Increase Na+ intake/retention</td><td>Sodium loss</td></tr>
</table>
Methods:
Flame Emission Photometry (FEP) - yellow
ISE – glass aluminum silicate
AAS
Colorimetry - Albanese Lein</td></tr>
<tr><td></td><td>Major intracellular cation
Regulates ICF volume regulation and H+ concentration, contraction of the heart and excitability of mucles
<table>
<tr><th>Hyperkalemia</th><th>Hypokalemia</th></tr>
<tr><td>Extracellular shift</td><td>Renal loss</td></tr>
<tr><td>Increased intake</td><td>GI loss</td></tr>
<tr><td>Renal excretion is decreaed</td><td>Intracellular shift</td></tr>
<tr><td>Artifactual (eg. Hemolysis, thrombocytosis)</td><td>Intake is decreased</td></tr>
</table></td></tr>
<tr><td></td><td>Methods:
FEP – violet
ISE – valinomycin gel
AAS
Colorimetry – Lockhead and Purcell</td></tr>
<tr><td>Chloride</td><td>Major extracellular anion
Only anion that serves as an _____
Sweat chloride: diagnosis for _____
<table>
<tr><th>Hyperchloremia</th><th>Hypochloremia</th></tr>
<tr><td>GI loss</td><td>Hyperparathyroidism</td></tr>
<tr><td>Diabetic ketoacidosis</td><td>Low reabsorption of HCO_3</td></tr>
<tr><td>Low Na+ levels</td><td></td></tr>
<tr><td>Mineralocorticoid excess & deficiency</td><td></td></tr>
</table>
Methods:
Mercurimetric method: Schales and Schales (indicator: diphenylcarbazone)
Coulometric amperometric titration: Cotlove chloridometer
Colorimetry
ISE – electrodes with AgCl membranes</td></tr>
<tr><td>Calcium</td><td>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</td></tr>
<tr><td>Magnesium</td><td>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</td></tr>
<tr><td>Bicarbonate</td><td>2nd most abundant ECF anion</td></tr>
</table>

	Acts as _____ 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	
Ca, Mg	Blood coagulation
	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 _____ a H+ ion
- **Base**: a compound that could _____ a H+ ion
- _____**:** 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 _____ & excrete hydrogen	Pneumonia, emphysema
Respiratory Alkalosis	↑	↓	↓	N	Reverse of respiratory acidosis	Hyperventilation, _____ _____
Metabolic Acidosis	↓	N	N	↓	Hyperventilate (CO_2 blew off)	Diabetic ketoacidosis, renal disease and prolonged diarrhea
Metabolic Alkalosis	↑	N	N	↑	_____ (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 =** _____
   - ✓ **If HCO_3 is in the** _____ **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, _____ happened.

NORMAL VALUES

- pH: **7.35 – 7.45**
- pCO_2: **35-45 mmHg**
- pO_2: **81-100 mmHg**
- HCO_3: _____
- 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, _____	↓	↑	↑	**Alkalosis**
Hyperventilation				**Alkalosis**
				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

HORMONESe

- 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 _____ ● 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
		Inhibitor of GH and TSH production
	Dopamine	Prolactin release inhibitor
Anterior pituitary		Most abundant pituitary hormone **Gigantism**: increase (excess) in GH _____ the closure of epiphyseal plate **Acromegaly**: increase (excess) in GH _____ the closure of epiphyseal plate _____: a deficiency of GH
	Prolactin	Initiates and maintains lactation Highest levels at 4am, 8am, 8pm and 10pm _____**:** most common type of functional pituitary tumor

	TSH	Stimulation for the production of _____ and _____
	LH	For secretion of _____; for ovulation
	ACTH	Stimulation for the production of adrenocortical steroid formation and secretion
	FSH	For secretion of _____ For development of seminiferous tubules; spermatogenesis
Posterior pituitary ~ only releases hormones (doesn't produce)		Stimulates contraction of the uterine "Fergusson Reflex" Also acts in parturition and in transport of sperm Also for milk ejection (suckling as stimulator)
	ADH	Aka _____ For water balance and blood pressure elevation Deficiency: _____
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 _____ **:** manifestation of Hashimoto's disease
	Calcitonin: produced by parafollicular cells	A calcium and phosphate regulator
_____: 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 _____ 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: _____ ✓ Confirmatory: low dose dexamethasone suppression test and CRH stimulation test **Cushing's disease:** increased levels of ACTH due to _____ on the pituitary gland Methods: **Porter-Silber reaction** (corticosteroids); + reaction = yellow pigment **Zimmerman reaction** (ketosteroids) + reaction = reddish purple color
	Aldosterone	Secreted by _____ 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 _____: tumor that results to overproduction of catecholamines
Reproductive glands		Principal androgen in the blood Most potent male androgen **Synthesized by the** _____
	Estrogen	**Estrone:** most abundant in _____ **Estradiol:** most potent; most abundant in pre-menopausal women _____**:** major estrogen detected during pregnancy; produced by placenta; marker for _____ _____**:** 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
  - ✓ _____ (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
	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	

≥0.10 – PRESUMPTIVE EVIDENCE OF DRIVING UNDER ALCOHOL INFLUENCE

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

DRUGS OF ABUSE

DRUG	METABOLITE
Amitriptyline	Nortryltyline
Cocaine	
Heroin	Morphine
	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)
- _____: 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 _____ the administration of drug
- Trough specimen: collected _____ 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
	Theohylline
Cardioactive	Digoxin, Procainamide, Lidocaine, Propanolol, Quinidine
Immunosuppressives	Tacrolimus (FK-506), Prednisone, Cyclosporine

GOOD TO KNOW FOR THE DRUGS:

- ✓ Aspirin: drug that inhibits _____
- ✓ Acetaminophen: hepatotoxic drug
- ✓ _____: for treatment of bipolar disorder or manic depression
- ✓ Phenobarbital: used for treatment of grand mal
- ✓ _____: for treatment of petit mal
- ✓ Vancomycin: cause of _____

CONVERSION FACTORS (derived from Clinical Chemitry Handbook of Dean Maria Teresa T. Rodriguez, RMT, MAEd, MSMT)		
ANALYTES	**CONVENTIONAL UNITS TO SI UNITS**	**CONVERSION FACTOR**
ALBUMIN	g/dL to g/L	
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		
PHOSPHORUS		0.323
TRIGLYCERIDE		
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

REFERENCES:

Henry's Clinical Diagnosis and Management by Laboratory Methods

Clinical Chemistry: Principles, Procedures and Correlations by Michael Bishop

Clinical Chemistry Review Handbook for Medical Technologists by Dean Maria Teresa T. Rodriguez, RMT, MAEd, MSMT

Clinical Chemistry Notes of Mr. Errol E. Coderes RMT, IMLT, MLS (ASCPi)CM

Clinical Chemistry Checkpoint notes of Ms. Judea Marie Policarpio, RMT

Intensive Review Notes of University of the Immaculate Conception – Medical Laboratory Science Program