MUST TO KNOW IN CLINICAL CHEMISTRY (From CC by Rodriguez)	
Quality Control	
Practicability	Method is easily repeated
Reliability	Maintain accuracy and precision
Intralab/Interlab QC	Daily monitoring of accuracy and precision
Interlab/External QC	Proficiency testing (Reference lab) Long-term accuracy Difference of >2: not in agreement w/ other lab
QC materials	Available for a min. of 1 yr
Bovine control materials	Preferred (Human: biohazard) Not for immunochem, dye-binding and bilirubin
Matrix effect	Improper product manufacturing Unpurified analyte Altered protein
Precision study	First step in method evaluation
Nonlab. personnel	29% of errors (lab results)
SD	Dispersion of values from the mean
CV	Index of precision Relative magnitude of variability (%)
Variance	SD^2 Measure of variability
Inferential statistics	Compare means or SD of 2 groups of data
T-test	Means of 2 groups of data
F-test	SD of 2 groups of data
Cumulative Sum Graph (CUSUM)	V-mask Earliest indication of systematic errors (trend)
Youden/Twin Plot	Compare results obtained from diff. lab
Shewhart Levey Jennings Chart	Graphic representation of the acceptable limits of variation
Trend	Gradual loss of reliability Cause: Deterioration of reagents (Systematic error)
Shift	Values: one side or either side of the mean Cause: Improper calibration (Systematic error)
Outliers	Values: far from the main set of values Highly deviating values Random or systematic errors
Kurtosis	Degree of flatness or sharpness
Precision	Random error
Accuracy	Systematic error
Random error (Imprecision; Indeterminate)	Causes: -Mislabeling -Pipetting error -Improper mixing of sample and reagents -Voltage/Temperature fluctuation -Dirty optics Parameters: SD and CV
Systematic error (Inaccuracy/Determinate)	Causes: -Improper calibration -Deterioration of reagents -Contaminated solution -Sample instability/unstable reagent blanks

Isoelectric focusing	Molecules migrate through a pH gradient pH = pI For isoenzymes: same size, different charge
Densitometry	Scan & quantitate electrophoretic pattern
Capillary electrophoresis	Electro-osmotic flow
Southern blot	DNA
Northern blot	RNA
Western blot	Proteins
Chromatography	Separation by specific differences in physical-chemical characteristics of the different constituents
Paper chromatography	Fractionation of sugar and amino acid Sorbent: Whatman paper
TLC	Screening: Drugs
Retention factor (R_f) value	Relative distance of migration from the point of application R_f = Distance leading edge of component moves / Total distance solvent front moves
Gas chromatography	Separation of steroids, barbiturates, blood, alcohol, and lipids Volatile compounds Specimens → vaporized Mobile phase: Inert gases
Gas Solid chromatography	Differences in absorption at the solid phase surfaces
Gas Liquid chromatography	Differences in solute partitioning between the gaseous mobile phase and the liquid stationary phase
Mass Spectrometry	Fragmentation and ionization
GC-MS	Gold standard for drug testing
MS/MS	Detect 20 inborn errors of metabolism from a single blood spot
HPLC	Most widely used liquid chromatography Fractionation of drugs, hormones, lipids, carbohydrates and proteins
Hydrophilic gel	Gel filtration Separation of enzymes, antibodies and proteins Ex: Dextran and agarose
Hydrophobic gel	Gel permeation Separation of triglyceride and fatty acid Ex: Sephadex
Ion exchange chromatography	Separation depends on the sign and ionic charge density
Partition chromatography	Based on relative solubility in an organic solvent (nonpolar) and an aqueous solvent (polar)
Affinity chromatography	For lipoproteins, CHO and glycated hemoglobins
Adsorption chromatography	Based on differences between the adsorption and desorption of solutes at the surfaces of a solid particle
Fluorometry/Molecular Luminescence Spectro.	Det. amt. of lt. emitted by a molecule after excitation by electromagnetic radiation Lt. sources: Mercury arc and Xenon lamp (UV) Lt. detector: Photomultiplier tubes 2 monochromators: Primary filter – selects wavelength absorbed by the solution to be measured Secondary filter – prevents incident light from striking the photodetector Sensitivity: 1000x than spectro
Quenching	Major disadvantage of fluorometry pH and temperature changes, chemical contaminants, UVL changes

	Increased: "P(u)BLIC" Proteins BUN Lipids Iron Calcium
Standing → Supine	Hemodilution Decreased: "TLC" Triglycerides Lipoproteins Cholesterol
Prolonged standing	Increased: K^+ (muscles)
Prolonged bedrest	Decreased: Albumin (Fluid retention)
Tourniquet	Recommended: 1 minute application
Prolonged tourniquet app.	Hemoconcentration Anaerobiosis Increased: "C_2LEA_2K" Calcium Cholesterol Lactate Enzymes Ammonia Albumin K^+
Tobacco smoking (Nicotine)	Increased: "$TUNG_2C_3$" Triglycerides Urea Nonesterified fatty acid Glucose GH Catecholamines Cortisol Cholesterol
Alcohol ingestion	Increased: "THUG" Triglycerides Hypoglycemia (chronic alcoholism) Uric acid/Urates GGT
Ammonia	Increases by 100-200µg/L/cigar
Stress (anxiety)	Increased: "LAGIC" Lactate Albumin Glucose Insulin Cholesterol
Drugs	Medications affecting plasma volume can affect protein, BUN, iron, calcium Hepatotoxic drugs: increased liver function enzymes Diuretics: decreased sodium and potassium
Diurnal variation	"CA_3PI_2TG" Cortisol ACTH ACP

	For ESR
Respinning gel tubes	Increases potassium
Thixotropic gel	Gel separator (SG: 1.04) Serum: (SG: 1.03) RBC: (SG: 1.05)
Laboratory Mathematics	
% w/v	Grams of solute = $\frac{\%\text{ solution desired} \times \text{total volume desired}}{100}$
% v/v	mL of solute = $\frac{\%\text{ solution desired} \times \text{total volume desired}}{100}$
% w/w	Grams of solute = $\frac{\%\text{ solution desired} \times \text{grams of the total solution}}{100}$
Molarity	$M = \frac{\text{grams of solute}}{\text{GMW} \times \text{volume of solution}}$
Moles	$\text{Mol} = \frac{\text{weight (grams)}}{\text{GMW}}$
To prepare a molar solution	Grams of solute = Molarity x GMW of the solute x Volume (L) desired
To convert % w/v to Molarity	$M = \frac{\%\text{ w/v} \bullet 10}{\text{GMW}}$
Normality	$N = \frac{\text{Grams of solute}}{\text{EW} \times \text{volume (L)}}$
Equivalent weight (EW)	$EW = \frac{MW}{\text{valence}}$
To prepare a normal solution of solids	Grams of solute = Normality x EW x volume (L)
To convert % w/v to Normality	$N = \frac{\text{w/v} \bullet 10}{EW}$
Normality	N = Molarity x valence
Molarity	$M = \frac{\text{Normality}}{\text{valence}}$
Molality	$m = \frac{\text{Grams of solute}}{\text{MW} \times \text{kg of solvent}}$
Milliequivalents	$\text{mEq/L} = \frac{\text{mg/dL} \bullet 10 \bullet \text{valence}}{\text{MW}}$
Millimoles	$\text{mmol/L} = \frac{\text{mg/dL} \bullet 10}{\text{MW}}$
Ratio	$\text{Ratio} = \frac{\text{Volume of solute}}{\text{Volume of solvent}}$
Dilution	$\text{Dilution} = \frac{\text{Volume of solute}}{\text{Volume of solution}}$
0.179	Conversion factor for iron (mg/dL → µmol/L)
0.01	Conversion factor for phospholipid (g/dL to g/L)
2.27	Conversion factor for folate
Analytical reagent (AR) grade	For qualitative and quantitative analyses For accuracy Established by American Chemical Society (ACS) Uses: Trace metal analysis and preparation of standard solutions
Ultrapure reagents	Additional purification steps Ex: Spectrograde, nanograde, HPLC grade Uses: Chromatography, atomic absorption, immunoassays
Chemically Pure (CP) or	Indicates that the impurity limitations are not stated

	1-Hr = 20-50 mg/dL above fasting 2-Hr = 5-15 mg/dL above fasting 3-Hr = fasting level or below
Lipids	
Phospholipids	Most abundant lipid Amphipathic: polar (hydrophilic head) and nonpolar (hydrophobic side chain)
Sphingomyelin	Reference material during 3rd trimester of pregnancy Concentration is constant as opposed to lecithin Not derived from glycerol but from sphingosine (amino alcohol)
Forms of phospholipids	70% Lecithin/Phosphatidyl choline 20% Sphingomyelin 10% Cephalin
TLC + Densitometric quantitation	Method for L/S ratio
Microviscosity	Measured by fluorescence polarization
Cholesterol	Not a source of fuel Not affected by fasting 70% Cholesterol ester (plasma/serum) 30% Free cholesterol (plasma/serum and RBC)
LCAT	Esterification of cholesterol
Apo A-1	Activator of LCAT
Cholesterol increases after the age of 50	2 mg/dL/year between 50 and 60 years old
Liebermann Burchardt	Cholestadienyl Monosulfonic acid Green end color
Salkowski	Cholestadienyl Disulfonic acid Red end color
Color developer mixture (Cholesterol)	Glacial acetic acid Acetic anhydride Conc. H_2SO_4
One-step method	Colorimetry (Pearson, Stern and Mac Gavack)
Two-step method	Color. + Extraction (Bloor's)
Three-step method	Color. + Extract. + Saponification (Abell-Kendall)
Four-step method	Color. +Extract. + Sapon. + Precipitation (Schaenheimer Sperry, Parekh and Jung)
Abell, Levy and Brodie mtd (Chemical method)	CDC reference method for cholesterol: -Hydrolysis/saponification (Alc. KOH) -Hexane extraction -Colorimetry (Liebermann-Burchardt)
Triglycerides	Most insoluble lipid Main storage lipid in man (adipose tissue) – 95% Fasting: 12 hours
Triglyceride increases after the age of 50	2 mg/dL/year between 50 and 60 years old
Van Handel & Zilversmith (Colorimetric)	Chromotropic acid (+) Blue color compound
Hantzsch Condensation (Fluorometric)	Diacetyl acetone (+) Diacetyl lutidine compound
Modified Van Handel and Zilversmith (Chemical method)	CDC reference method for triglycerides: -Alkaline hydrolysis -Chloroform extraction → extract treated w/ silicic acid -Color reaction w/ chromotropic acid – meas. HCHO

	RBC: 150x LDH than in serum Sources: LD_1 (α-HBD) and LD_2 = Heart, RBC, Kidneys LD_3 = pancreas, lungs, spleen LD_4 an LD_5 = liver and muscle LD_6 = alcohol dehydrogenase
Methods (LDH)	1. Wacker method (forward/direct) = pH 8.8, 340 nm, most commonly used 2. Wrobleuski LaDue (reverse/indirect) = pH 7.2, 2x faster 3. Wrobleuski Cabaud 4. Berger Broida
10-fold increase (LDH)	Hepatic carcinoma and toxic hepatitis
2-3x URL	Viral hepatitis and cirrhosis
Creatine Kinase	Isoenzymes: CK-BB = most anodal, brain CK-MB = myocardium (20%) CK-MM = least anodal, skeletal and smooth muscles (Major, 94-100%)
Duchenne's muscular dystrophy	Total CK: 50x URL (highest)
CK-MB	Most specific indicator of myocardial damage (AMI) Not elevated in angina
Methods (CK)	1. Tanzer-Gilbarg (forward/direct) = pH 9.0, 340nm 2. Oliver-Rosalki/ Rosalki & Hess (reverse/indirect) = most commonly used method, faster reaction; pH 6.8, 340nm
Adenylate kinase	Inside RBCs Interferes w/ CK assay Inhibited by adenosine monophosphate
N-acetylcysteine	Activate CK
Liver cells and RBC	Do not contain CK
Cleland's reagent and glutathione	Partially restore lost activity of CK
Electrophoresis	Reference method for CK
CK relative index (CKI)	CKI (%) = CK-MB/Total CK x 100
Aldolase	Isoenzymes: Aldolase A = Skeletal muscles Aldolase B = WBC, liver, kidney Aldolase C = brain tissue
5' Nucleotidase	Marker for hepatobiliary diseases and infiltrative lesions of the liver Methods: 1. Dixon and Purdon 2. Campbell, Belfield and Goldberg
GGT	Located in the canaliculi of the hepatic cells Differentates the source of an elevated ALP level Sensitive indicator of occult alcoholism Increased: Obstructive jaundice Alcoholic hepatitis (most sensitive)
Methods (GGT)	Substrate: gamma-glutamyl-p-nitroanilide 1. Szass 2. Rosalki and Tarrow 3. Orlowski
Cholinesterase/	Monitor effects of relaxants (succinylcholine) after surgery

contractility Neuromuscular excitability	Calcium Magnesium
Cofactors (enzyme)	Calcium Magnesium (CK) Zinc Chloride (AMS) Potassium
ATPase ion pump	Magnesium
Production and use of ATP from glucose	Magnesium Phosphate
Acid-base balance	Bicarbonate
Replication of DNA and translation of mRNA	Magnesium
Sodium	Major contributor of osmolality (92%, together w/ Chloride and Bicarbonate) ↑100 mg/dL glucose = ↓1.6 mmol/L sodium
Aldosterone	↑Sodium ↓Potassium = ↓ Magnesium
Atrial natriuretic factor	↓ Sodium
Hypernatremia	Excess water loss Decreased water intake Hyperaldosteronism (Conn's disease) Hypothalamic disease (Chronic hypernatremia)
Hyponatremia	Renal failure SIADH (increased water retention) Marked hemolysis (dilutional effect) <125 mmol/L = severe neuropsychiatric symptoms
Thirst	Major defense against hyperosmolality and hypernatremia 1-2% water deficit = severe thirst 150-160 mEq/L Na^+ = Moderate deficit of water >165 mEq/L Na^+ = Severe water deficit
Pseudohyponatremia (artifactual)	Hyperlipidemia (turbidity) Hyperproteinemia
Methods (Na^+)	1. FEP 2. AAS 3. ISE = Glass aluminum silicate 4. Colorimetry = Albanese Lein
Potassium	Concentration in RBC is 105 mmol/L Reciprocal relationship with H^+
Specimen Considerations (K^+)	0.5% hemolysis = ↑ 0.5 mmol/L Gross hemolysis = ↑ 30% Serum K^+ > Plasma K^+ by 0.1-0.7 mmol/L because of platelets (clot) ↑10-20% in muscle activity ↑0.3-1.2 mmol/L = mild to moderate exercise ↑2-3 mmol/L = vigorous exercise; fist clenching
Hyperkalemia	Decreased resting membrane potential → incr. contractility → lack of muscle excitability Decreased renal excretion (Dehydration, renal failure, Addison's disease) Acidosis (DM) Muscle injury Spironolactone
Hypokalemia	Increased resting membrane potential → arrhythmia

	Cancer Hyperthyroidism Milk-alkali syndrome
Hypocalcemia	Tetany Alkalosis (Ca^{2+}: from Blood → Bones) Acute pancreatitis (Ca^{2+}: binds to damage pancreatic tissues)
Primary hypocalcemia	Low PTH Parathyroid gland disease
Secondary hypocalcemia	High PTH Renal failure (↑ excretion)
Methods (Ca^{2+})	1. Clark Collip precipitation method -(+) Oxalic acid -Renal calculi 2. Ferro Ham Chloranilic acid precipitation method -(+)Chloranilic acid 3. Colorimetric = Ortho-Cresolphthalein complexone dyes -Dye: Arzeno III -8-hydroxyquinoline = chelates (inhibits) Mg^{2+} 4. EDTA titration method (Bachra, Dawer and Sobel) 5. AAS = Reference method 6. ISE = Liquid membrane 7. FEP
Inorganic Phosphorus	85% → Bones 15% → ECF (iPO_4) Maximally absorbed in the jejunum (Ca^{2+}: duodenum) Trancellular shift: Once absorbed inside cells, it no longer comes out → used for energy production Diurnal variation: ↑ late morning, ↓ evening Organic phosphate = principal anion within cells Inorganic phosphate = part of the blood buffer (Measured in the clin.lab.)
3 Forms of Inorganic Phosphorus	55% = Free 35% = Complexed with ions 10% = Protein-bound
PTH	↓ PO_4 = ↑ Ca^{2+}
Calcitonin	↑ PO_4 = ↓ Ca^{2+}
Growth hormone	↑ PO_4 (renal reabsorption)
Practical considerations	Fasting is required (Nonfasting: ↓ PO_4)
Hyperphosphatemia	Hypoparathyroidism Renal failure Hypervitaminosis D
Hypophosphatemia	Alcohol abuse = most common cause Primary hyperparathyroidism Avitaminosis D (Rickets, Osteomalacia)
Methods (iPO_4)	Most accurate: unreduced phosphomolybdate formation (340nm) 1. Fiske Subbarow Method (Ammonium molybdate method) -Reducing agents: Pictol, Elon, Senidine, Ascorbic acid -(+) Phosphomolybdenum blue
Magnesium	53% → Bones 46% → Muscles and soft tissues 1% → Serum and RBC Vasodilator
3 Forms of Magnesium	55% = Free/Ionized/Physiologically active

Regulation of Acid-Base balance	Lungs and Kidneys $CO_2 + H_2O$ <--(Carbonic anhydrase)--> H_2CO_3 H_2CO_3 <-------(Carbonic anhydrase)--> $H^+ + HCO_3^-$
20:1	HCO_3^-: H_2CO_3 ratio
4:1	HPO_4: H_2PO_4 ratio
Expanded Henderson-Hasselbalch equation	$pH = 6.1 + \log \frac{[\text{Total } CO_2 - (pCO_2 \times 0.03)]}{pCO_2 \times 0.03}$
Chloride-isohydric shift	Buffering effect of hemoglobin
pCO_2	Index of efficiency of gas exchange Increased: Barbiturates, morphine, alcohol, heparin (↑12-15%)
pO_2	Reflects the availability of the gas in blood but not its content Excessive O_2 supply → acidosis
Metabolic Acidosis	Causes: -Bicarbonate deficiency -DKA (normochloremic acidosis) -Renal failure -Diarrhea (↓HCO_3^-) Compensation: Hyperventilation Compensated: ↓ HCO_3^- + ↓pCO_2 + pH <7.4
Metabolic Alkalosis	Causes: -Bicarbonate excess -Vomiting (↓Cl^-) -Hypochloremia -Hypokalemia Compensation: Hypoventilation Compensated: ↑ HCO_3^- + ↑ pCO_2 + pH >7.4
Respiratory Acidosis	Causes -CO_2 excess (Hypoventilation) -COPD -Drug overdose (morphine, barbiturates, opiates) Compensation: Bicarbonate retention Compensated: ↑ HCO_3^- + ↓ pCO_2 + pH <7.4
Respiratory Alkalosis	Causes -CO_2 loss (Hyperventilation) Compensation: Bicarbonate excretion Compensated: ↓ HCO_3^- + ↓ pCO_2 + pH >7.4
Full compensation	pH → normal range
Partial compensation	pH → near normal
Buffer base	All forms of base that will titrate hydrogen ions
Methods for Blood Gases and pH	Specimen: Arterial blood Blood gas analyzers: meas. pH, pCO_2, pO_2
Factors affecting Blood gases & pH measurements	For every ↑1°C above 37°C: ↓ pH by 0.015 ↓ pO_2 by 7% ↑ pCO_2 by 3% Bacterial contamination: consume O_2 (↓pO_2) Excess heparin (acid MPS) = ↓pH Air exposure (bubbles): ↑pO_2 = 4 mmHg/2mins ↓pCO_2 = 4 mmHg/2mins
Methods	1. Gasometer