Clinical Chemistry
Lecture Guide

Basic Principles

I. Basic Principles

★ Safety - A review of safety rules and regulations is in order.

1. Laboratory general safety guidelines based on

   a. Good personal behavior & habits such as professional conduct and appearance, frequent hand washing, no eating, smoking, drinking, or application of makeup, etc. in lab. Should also make yourself aware of emergency procedures / location and proper use of emergency equipment and fire escape route.

   b. Good housekeeping - Laboratory cleanliness, properly labeled containers, immediate appropriate cleanup of any spills, warning signs of potential danger posted, etc.

   c. Good laboratory technique - carefully read all instructions and labels, do not operate equipment until you are instructed and authorized to do so, always respect the power of acids, bases and corrosive chemicals. Know the carcinogens list provided in MLAB 1201. Protect yourself with PPDs and use proper eyewear protection devices. Never recap needles, etc.

2. Infection prevention/control - Role of OSHA, and later the development and implementation of CDC's Universal Precautions

3. Radioactivity - Beta or gamma emitters are used in radioimmunoassay procedures. Review precautions taken in storage, handling, and disposal of radioactive materials.

4. Fire safety

★ Specimen Collection and Processing

1. Medical ethics in specimen collection

2. Special collection procedures

   a. Fasting specimens: overnight for most tests, 12 hours for lipid studies

   b. Timed interval specimens

      1) Examples include glucose tolerance, therapeutic drug monitoring, and hormone stimulation testing

      2) In some cases urine collection also required

   c. Legal chain of evidence

d. Others

3. Specimen processing

   a. Determining specimen acceptability

   b. Specimen accessioning

   c. Serum separators

      1) Gel barrier
      2) Beads, crystals or fibers
      3) Plastic tube device

★ Quality Assurance

1. Review of Quality Control objectives

   a. Record of **precision**

   b. Early warning of **shifts** and **trends**

   c. Permits a valid judgment on the **accuracy** of a measurement

   d. Facilitates comparison of test methods

   e. Monitor equipment performance

   f. Indicates the analytical abilities of a tech

   g. Accumulate a body of knowledge to satisfy outside accrediting agencies

2. Classification of error

   1) Clerical error

   2) Analytical error

      a) Random or indeterminant

      b) Systematic or determinant

3. Use of assayed and unassayed controls in clinical chemistry

4. Establishment of a QC system

   a. Collecting data

      1) Charting techniques

a) Levey Jennings
b) Yoden plot

2) A minimum number of determinations (per control level) is required to establish an acceptable mean and practical SD

b. Terms

1) **Standard Deviation (SD)** – a measure of the scatter around the mean ($\bar{x}$) in a Gaussian distribution (Bell curve, or normal frequency distribution)

2) **95% confidence limit** – 95% of all the results in a Gaussian distribution, also ± 2 SD

3) **Coefficient of variation (CV)** – the % of variance from the mean

$$CV = \frac{SD}{mean} \times 100 = \_\_\_\_\_\_\_\%$$

(the smaller the cv, the more reproducible the results (more values closer to mean)

4) Measures of central tenancy

a) **Mean** – arithematic average

b) **Median** – the middle value of a group of data

c) **Mode** – the most frequently occurring variable

c. Data interpretation and evaluation

1) Must be able to calculate standard deviation, range (95% confidence limits), CV, etc.

2) Use of SD

a) Most labs make use of ± 2 SD or 95% confidence limit. To put this into a workable form, you must establish the **range** of the ± 2 SDs

b) Using the 95% confidence limit, how many values can be expected to be out of range?

3) Corrective methods

4) Comparison of chemistry methods

★ Laboratory Mathematics and Chemistry Calculations

1. Basic SI quantities and units

Quantity	Metric	Symbol	SI Basic Unit
length	meter	m	meter
mass	gram	g	kilogram
volume	liter	L	not recognized
amount of substance	mole	mol	mole

2. Metric or SI prefixes

Prefix	Factor	Symbol
atto	10^{-18}	a
femto	10^{-15}	f
pico	10^{-12}	p
nano	10^{-9}	n
micro	10^{-6}	μ
milli	10^{-3}	m
centi	10^{-2}	c
deci	10^{-1}	d
deka	10^{1}	da
hecto	10^{2}	h
kilo	10^{3}	k
mega	10^{6}	M
giga	10^{9}	G
Tera	10^{12}	T
Penta	10^{15}	P
exa	10^{18}	E

3. Converting like units

   a. Combine a prefix with a basic unit results in a statement of a *specific* length, weight or volume

   b. Most conversions within the metric system occur in units of TEN where changing a unit of measure to a higher or lower designation requires moving the decimal one place either to the left or to the right. When converting measures in either the high end of the scale (example kilo to mega) or the low end of the scale (examples milli to micro, micro to nano, etc.) the decimal must be moved three places right or left as the prefix designations are assigned only to every third unit in the extreme ends.

femto	pico	nano	micro	milli	centi	deci	unit	deka	hecto	kilo	mega
10^{-15}	10^{-12}	10^{-9}	10^{-6}	10^{-3}	10^{-2}	10^{-1}	10^{0}	10^{1}	10^{2}	10^{3}	10^{6}

c. Rules for converting units of measure in the range between milli and kilo

1) To change a unit of measure to the *next larger unit*; multiply by 0.1 **OR** move the decimal one place to the left

2) To change a unit of measure to the *next smaller unit*; multiply by 10 **OR** move the decimal one place to the right

4. Scientific notation

5. Chemistry calculations

a. Temperature

1) Centigrade to Fahrenheit (9/5 C) + 32 = F
2) Fahrenheit to Centigrade 5/9(F - 32) = C

b. Expressing concentration

1) The concentration of a solution refers to the *weight or volume of the solute* present *in a specified amount of the solvent or solution*

2) Examples

a) Percent solution (parts/100)

(1) % w/w
(a) weight to weight
(b) solid mixed with a solid
(c) example units: gm/gm, mg/gm

(2) % w/v
(a) weight to volume
(b) solid in a liquid
(c) example units: mg/dL, gm/L

(3) % v/v
(a) volume into a volume
(b) liquid diluted in another liquid
(c) example units: ml/L
(d) Note: volumes of liquids are not necessarily additive

* b) Molar solutions

(1) number of moles of solute in 1 liter of solution

(2) a mole is obtained from the molecular or formula weight

* c) Molal solution

(1) rarely used
(2) number of moles added to 1,000 grams of solution

d) Normal solutions

(1) number of equivalent weights in 1 liter of solution
(2) equivalent weight is obtained from the molecular weight divided by the positive valence of the compound
(3) working with normality is most important when dealing with acids or bases in neutralization reactions

c. Titration – Method of measuring concentration of one solution by comparing it with a measured volume of a solution whose concentration is known.

General formula:

d. Dilution – The ratio of the concentrate to the total (final) volume

Example 1: Procedure result exceeds linearity.

1 part serum	$\frac{1 \text{ serum}}{4 \text{ total}} = \frac{1}{X}$	X = 4
+ 3 parts diluent		
4 parts total		

After running the diluted specimen (and determining its concentration) multiply results X 4

e. Hydrate – When a chemical (reagent) comes in a hydrated form (i.e., $CuSO_4 \bullet 5H_2O$), the weight of the water must be taken into account when figuring the gram molecular weight

f. Density – An expression in terms (usually) of a mass per unit of volume

★ Chemical Grades

1. Analytical reagent grade or reagent grade or American Chemical Society (ACS) grade

a. Very pure – guaranteed to meet specified standards
b. Label must state actual impurities
c. Best choice for lab work

2. National Formulary (NF) or US Pharmacopeia (USP)

   a. Used for drugs
   b. May or may not be suitable for lab use

3. Pure grade or CP

   a. Do not list impurities
   b. Not recommended for lab use

4. Technical or commercial grade
   a. Used in manufacturing
   b. Never used in lab

★ Water

1. Quality levels

   a. Tap – water not suitable for lab use

   b. Distilled – water is purified by a distillation process to remove most *organic* materials

   c. Deionized – water has some or all of the ions removed

2. CAP recommends routine culture, pH, and resistivity tests be done

★ Standards

1. Primary – very pure, exact composition known

2. Secondary – may not be so pure, sometimes made from a solution of a primary standard

★ Blanks

1. Water blank
2. Reagent blank
3. Patient blank

★ Controls

★ Labware

1. Types of glass
   a. High thermal borosilicate
      1) Can take long periods of high temperatures
      2) Scratches easily
      3) Acceptable for chemistry work
      4) Examples: Pyrex, Kimax

   b. Aluminosilicate

1) Can withstand heat as long as not in contact with acids or alkalis
2) Resists scratching
3) Acceptable for chemistry work
4) Examples: centrifuge tubes, thermometers

c. Soda lime – not suitable for lab use

2. Types of plastic resins

a. Polystyrene
   1) Clear, rigid
   2) Can withstand temperatures to 70°C
   3) Examples: many disposables

b. Polyethylene
   1) Translucent in appearance
   2) Two types
      a) One type can withstand temperatures up to 80°C, and is flexible, i.e., reagent wash bottles
      b) Other can withstand temperatures up to 120°C and is rigid, i.e., droppers

c. Polyvinyl chloride
   1) Translucent in appearance, but rigid
   2) Withstands temperatures to 135°C
   3) Examples: screw cap enclosures

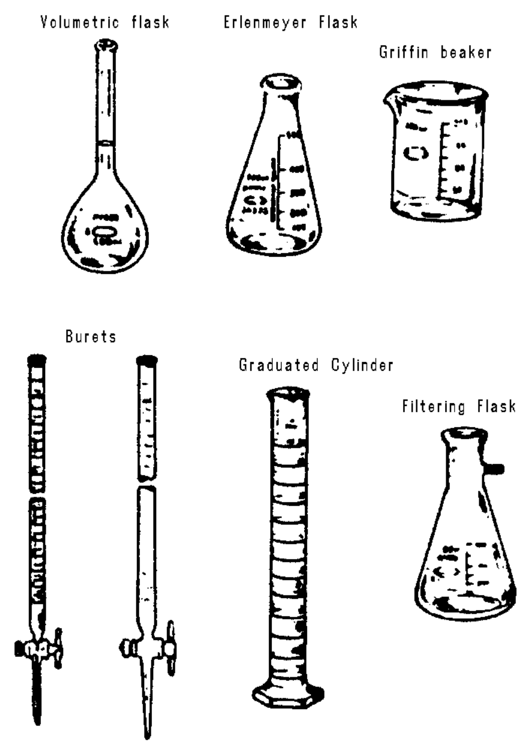
3. Types of glassware

a. Beakers
b. Flasks

   1) Volumetric

   2) Erlenmeyer

c. Graduated cylinders
d. Reagent bottles
e. Test tubes



4. Pipets – review the following as presented in MLT 1703

   a. Types

      1) Volumetric

   b. Ostwald-Folin

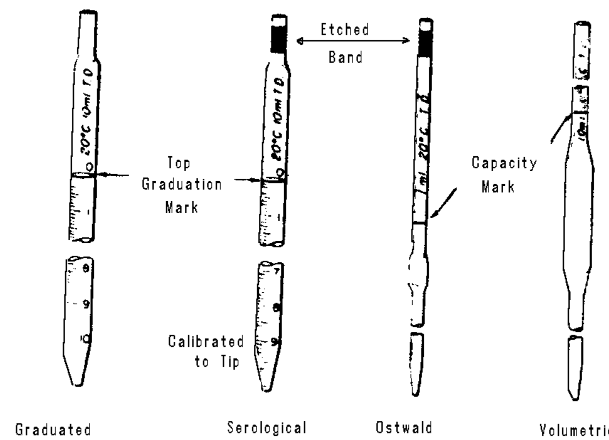
      1) Capillary

      2) Serologic

      3) Mohr

      4) Transfer

      5) Automatic and semiautomatic



Types of Pipets

   c. Style
      1) TD
      2) TC

   d. Proper use

      1) Use correct pipet for the job

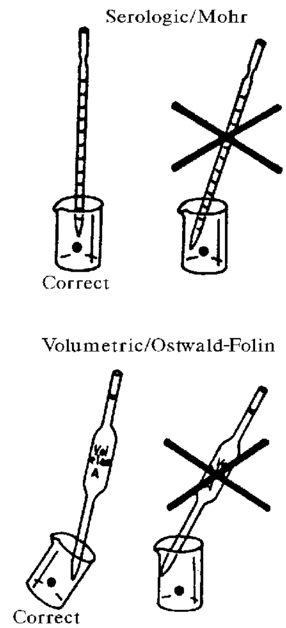
      2) Examine the pipet before use for cleanliness, chips, etc.

      3) **NEVER** pipet by mouth

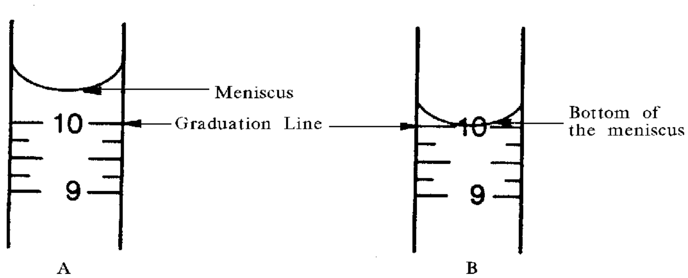
      4) Draw the solution slightly above the mark

      5) Wipe the tip with a Kimwipe

      6) Hold the pipet vertical, lower the meniscus to the line



Correct and incorrect pipet positions



Pipetting technique. (A) Meniscus is brought above the desired graduation line. (B) Liquid is allowed to drain until the bottom of the meniscus touches the desired calibration mark.

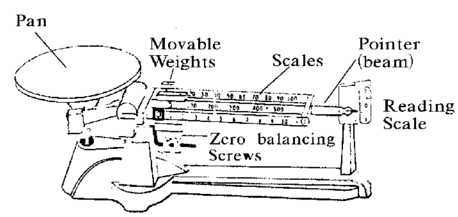
5. Buret – essentially an elaborate pipet mounted on a stand used in titration procedures

★ Cleaning of Lab Glassware

1. Majority of time can simply presoak, dishwash, and thoroughly rinse with tap and finally distilled/deionized water

2. *Chemically clean* glassware is required for certain chemistry procedures (enzymes, iron, heavy metals, etc.)
   dichromate acid

★ General Laboratory Equipment



Triple-beam balance

1. Balances

   a. The degree of accuracy needed usually determines the type of balance needed

   b. Types

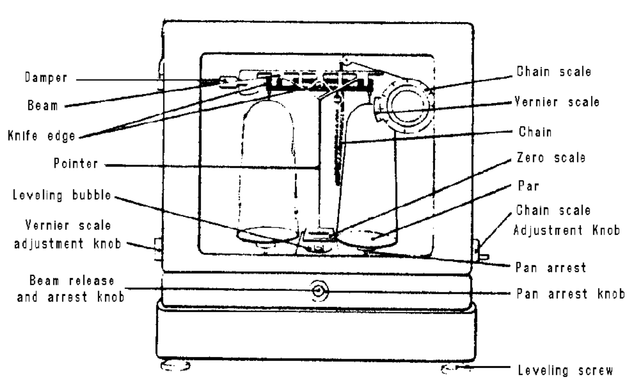
      1) Harvard Trip Balance

         a) A mechanical type with two pans
         b) Put desired weight on one end and desired substance on other

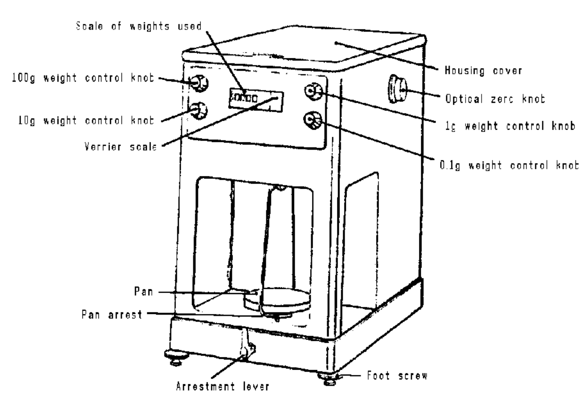
      2) Top-loading Balance – single pan, electronic readout

      3) Analytical Balance

         a) Very elaborate, accurate and most costly
         b) Has a single pan behind sliding doors and uses mechanical internal weights



Manual Analytical Balance



Automatic Analytical Balance

2. Centrifuge

1) Purpose – separating solids from a liquid suspension by means of centrifugal force

2) Types – vary according to the job being performed

3) Characteristics

a) Some have a fixed rotor while others use a swinging bucket (carrier)

b) All should have a closing lid to prevent aerosols and flying glass

4) Other ways of separating materials

a) Filtration – using filters of various materials and pore sizes

b) Dialysis – a method made popular by Technicon Corporation (early manufacturer of automated lab equipment). This method makes use of a semi-permeable membrane that allows separation of molecules using their size

★ Chemistry Techniques

1. Colorimetric
2. Enzymatic
3. Electrochemical
4. Electrophoretic separation
5. Chromatography
6. Titration

7. RIA (radioimmunoassay)

   a. Antigen-antibody reaction in which the antibody (or sometimes the antigen) is radioactively tagged

   b. Refinement of RIA procedures allowed measurement of substances present in the body in very small amounts

   c. Problems

8. RID (radial immunodiffusion)

9. IEP (immunoelectrophoresis)

   a. Electrophoretic separation of material

   b. Immunologic characterization

10. Nephelometry

a. Photometer-like instrument that measures light being scattered by particulate matter
b. Detector located at an angle

★ Preview of Chemistry Laboratory Instruments

1. **Photometers, colorimeters,** and **spectrophotometers**, **nephelometers,** and **densitometers** measure electromagnetic radiation (radiant energy or light).

The process of photometry deals with measurement of light transmitted through a solution to determine the concentration of light absorbing/blocking substances in the solution.

2. **Flame photometers** measure electromagnetic radiation (light) being produced by excited atoms returning to ground state.

3. **Atomic Absorption Spectrophotometers** are the instruments of choice to determine concentration of most metals (i.e., calcium, magnesium, lead or copper, etc.).

4. **Fluorometers** measure the concentration of substances that absorb light energy of a specific wavelength (short wavelength) then emit or give up the light energy at a longer wavelength.

5. **Gas chromatograph** (gas-liquid chromatography or GLC) can be used to separate mixtures of compounds that are volatile or can be made to be volatile.

6. **Osmometer** determines the concentration of solute particles in a solution by examining a **colligative property.** The four (4) colligative properties (freezing point, boiling point, vapor pressure, and osmotic pressure) depend on the number of particles in solution.

7. **Electrophoresis** – the process of separating mixtures of charged solute ions by their migration in an electric field.

8. **Automated chemistry instrumentation** is found in nearly every hospital clinic and clinic and doctor's office laboratory performing chemistry procedures.