BASIC PRINCIPLES OF CLINICAL CHEMISTRY

Course: Clinical Chemistry (PHAR 431)

Textbook:
Bishop ML, Fody EP, Schoeff LE (2013). Clinical Chemistry: principles, techniques and correlations, 7th ed.
Chapter 1

Aim of clinical chemistry lab

- To facilitate the correct performance of analytical procedures that yield accurate and precise information, aiding patient diagnosis and treatment
- How to achieve this aim?

Units of measure

- Any meaningful quantitative lab results consists of:
  - Test value (number) + units
- Units of measure: define the physical quantity or dimension such as mass, length, time or volume
- Not all lab tests have well-defined units, but whenever possible, it should be reported

"SI" system or SI units

- Adopted internationally in 1960
- Based on metric system
- There are several sub-classifications:
- 7 "***basic units***"
- ***Derived units***: derivative or mathematical function describing one of the basic units. Example: m/s (meter per seconds) is used to express velocity
- Non-SI units: some are widely used and are therefore accepted to use in clinical labs

TABLE 1-1 SI UNITS

BASE QUANTITY	NAME	SYMBOL
Length	Meter	m
Mass	Kilogram	kg
Time	Second	s
Electric current	Ampere	A
Thermodynamic temperature	Kelvin	K
Amount of substance	Mole	mol
Luminous intensity	Candela	cd
SELECTED DERIVED		
Frequency	Hertz	Hz
Force	Newton	N
Celsius temperature	Degree Celsius	°C
Catalytic activity	Katal	kat
SELECTED ACCEPTED NON-SI		
Minute (time)	(60 s)	min
Hour	(3,600 s)	h
Day	(86,400 s)	d
Liter (volume)	(1 $dm^3 = 10^{-3}$ m^3)	L
Angstrom	(0.1 nm = 10^{-10} m)	Å

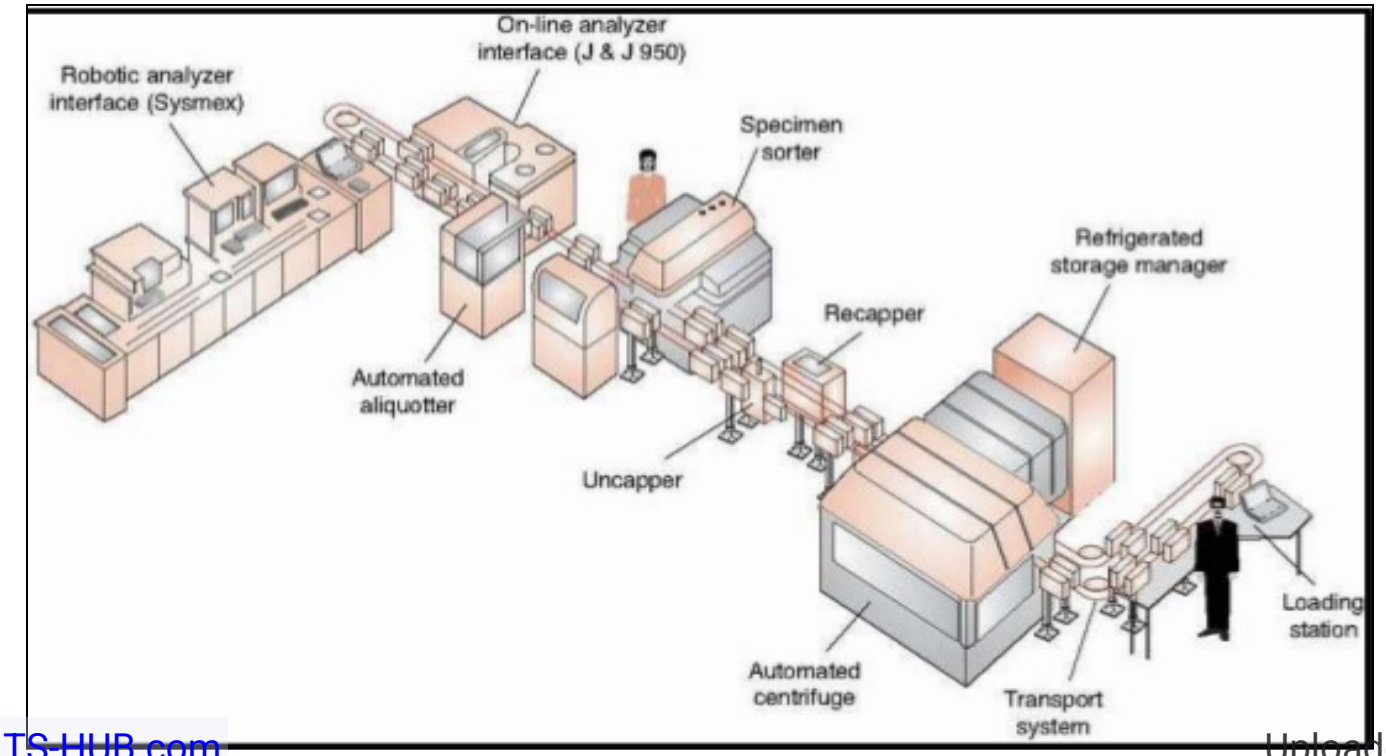
TABLE 1-2 PREFIXES USED WITH SI UNITS

FACTOR	PREFIX	SYMBOL	SELECT DECIMALS
10^{-18}	atto	a	—
10^{-15}	femto	f	—
10^{-12}	pico	p	—
10^{-9}	nano	n	—
10^{-6}	micro	μ	0.000001
10^{-3}	milli	m	0.001
10^{-2}	centi	c	0.01
10^{-1}	deci	d	0.1
10^{0}	Liter, meter, gram	Basic unit	1.0
10^{1}	deka	da	10.0
10^{2}	hecto	h	100.0
10^{3}	kilo	k	1,000.0
10^{4}	mega	M	—
10^{9}	giga	G	—
10^{12}	tera	T	—
10^{15}	peta	P	—
10^{18}	exa	E	—

Prefixes are used to indicate a subunit or multiple of a basic SI unit.

Reagents

- Clin chem labs are highly automated today
- Ready-to-use reagents or in a “kit” format
- Home-made reagents are still necessary in some cases



Reagents: chemicals

- Commercial grade (should never be used in clinical labs)
- Analytical grade reagent (AR)
- Ultrapure, chemically pure (CP)
- US Pharmacopia (USP), National Formulary (NF) are used to manufacture drugs therefore must not be injurious to humans… may or may not be used in clinical labs
- Chemicals suitable for use in most labs include: AR or ACS or labeled as "For Lab use" or "ACS standard-grade Reference Materials"
- CP: used for specific procedures such as chromatography and atomic absorption
- MSDS (material safety data sheets)

ACS: the chemical meets the specifications of the **American Chemical Society**. A certificate of analysis is available.

Reagents: **Reference materials**

- **Primary standard:** highly purified chemical that can be measured directly to produce a substance of *exact* known concentration and purity. ACS purity tolerances is 100± 0.02%.
- Analytical reagent of exceptional purity that is especially manufactured for standardizing volumetric solutions and preparing reference standards

Reagents: **Reference materials ///cont'd**

- Most biologic constituents are unavailable within these limits
- ***Standard reference materials (SRMs):*** used in clin chem instead of primary standard materials (approved by NIST)
- SRMs have assigned values after careful analysis, using state-of-the-art methods & equipment

NIST: National Institute of Standards and Technology

Reagents: **Reference materials ///cont'd**

- Many manufacturers use NIST SRMs for producing calibrator and standard materials and these materials are considered traceable to NIST
- Examples of SRMs: hormones, drugs, blood gases, …

- Secondary standard: a substance of lower purity, with its concentration determined by comparison with a primary standard. Its assigned value depends on its composition and on the analytic reference method

- There is no TRUE secondary standard
- Manufacturers should list the SRM or primary standard used for comparison. This info is needed for accreditation of the clinical lab

Reagents: **Water specifications**

- Distilled water: distillation
- DDW: double distittled
- Deionized water: ion exchange
- Reverse osmosis (RO) water
- Ultrafiltration
- **Reagent grade water:** six categories according to CLSI (clin & lab standard institute): deionized, 0.2 mm filter or more restrictive filter

- How to measure water purity?
- measurement of resistance, pH, colony counts, particulate matter, organics
- Three types of purity
- Type I, II, III
- Type I water has the most stringent requirements and is suitable for routine lab work (also used for test methods requiring minimum interference such as trace metal analysis)
- Type II water is also accepted for most analytic requirements

Solution properties

- Concentration
- **Percent solution (%):** parts per 100; w/w, w/v and v/v
- **Molarity (M):** mole/liter (influenced by temp or pressure)
- **Molality (m):** amount of solute per 1 kg of solvent (not influenced by temp or pressure)
- **Normality (N):** # of gram equivalent per 1 liter of solution. Equivalent weight = gmw divided by its valence

Colligative properties

- 4 repeatable properties based only on the relative number of each kind of molecule present
- **Osmotic pressure:** pressure that opposes osmosis when a solvent flows through a semipermeable membrane to establish equilibrium b/w compartme nts of differing conc
- **Vapor pressure:** pressure at which the liquid solvent is in equilibrium with water vapor
- **Boiling point:** temperature at which solvent vapor pressure reaches 1 atm
- **Freezing point:** temperature at which vapor pressure of solid and liquid phases are the same

Redox potential

- Oxidation-reduction potential
- Solution's ability to lose or gain electrons
- Oxidizing agents accept electrons
- Reducing agents donate electrons
- LEO (loss of electrons oxidation)
- GER (gain of electrons reduction)

Conductivity and resistivity

- Conductivity: how well electricity passes through a solution (ohm^{-1})
- Depends on the number and charges of the solute (ions)
- Resistivity: the opposite of conductivity (ohm)
- Used to check purity of water

pH and buffers

- Henderson-Hasselbalch equation
  - pH = pKa + log [A-]/[HA]
- When the ratio of $[A^-]$ to [HA] is 1, pH = pKa and the buffer has its greatest buffering capacity
- **Ionic strength** is the concentration or activity of ions in a solution or buffer
- $\mu = {}^1/_2 \Sigma\{(Ci)(Zi)^2\}$

Lab mathematics and calculations

- Read this section carefully and solve all problems. P19-28

Homework

- How are 50 ml of 20 mM NaOH prepared? gmw for NaOH is 40

- How you can prepare 1M HCl from a concentrated 37% HCl? HCl density is ~1.9 g/ml.

- You wish to prepare 2 Liters of 1M Sodium phosphate buffer, pH 8.0. You have 1M monobasic NaH_2PO_4 and 1M dibasic Na_2HPO_4. How much of each stock solution should be combined to make the desired buffer?

Specimen considerations

Specimen considerations

- Specimen collection, handling and processing remains the primary source of pre-analytical errors

- Phlebotomy or venipuncture: most frequent site is the antecubital vein of the arm
- Skin puncture: bottom of the foot (a heel stick) or finger prick (third or fourth finger) or earlobe

Types of specimens

- Whole blood, mostly venous blood
  - Anticoagulated: plasma & cells
  - No anticoagulant: serum
  - Most testing in clin chem is done on serum or plasma
  - Major difference between serum and plasma is that serum does not contain fibrinogen (total protein is less than in plasma)

- Arterial blood, mostly for analysis of blood gases and pH
- Urine: second most common fluid after serum. Either 24-hr or complete sample (in a specified time period) for quantitative analysis.
- Other fluids: Cerebrospinal fluid (CSF), paracentesis fluid (pleural, peritoneal or pericardial) & amniotic fluids
- CSF is an ultrafiltrate of plasma and therefore should reflect plasma levels of analytes

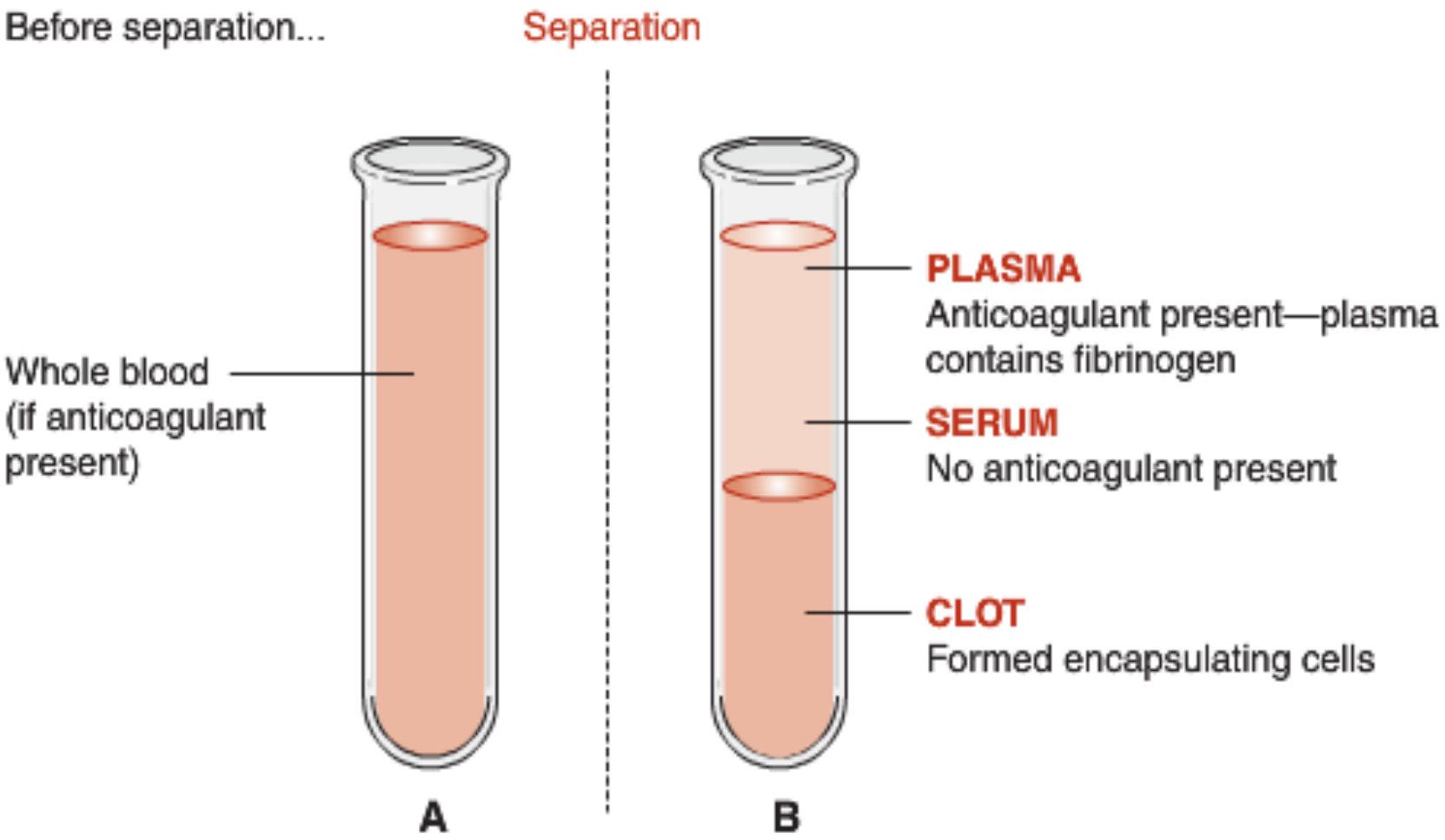


FIGURE 1-16 Blood sample.

Sample Processing

- Unless whole blood analysis is required, centrifugation must be performed.
- Allow blood samples to clot for ~20 minutes
- Centrifuge blood samples at 1000-2000xg for ~10 min, to separate serum from cells
- Separate serum from cells and store in new tube
- Patient identification & labeling correctly matching blood collection tube with the appropriate analyte request & patient ID labels. Bar codes are useful!
- Examine if sample is acceptable: volume, timing of samples, if sample is intact or deteriorated during transport (cooled, capped, …..)
- Note serum characteristics (hemolysis (⇧Hb), icterus (⇧bilirubin), turbidity (⇧lipids))
- Analyze within 4 hours or store appropriately (4 °C for 8 hours or -20 °C for longer storage)

Sample variables

- Physiologic considerations
  - Changes within the body
  - Cyclic changes- diurnal or circadian variation
  - Exercise, stress, age, gender, diseases, drugs or posture
- Proper patient preparation
- Problems in sample collection, processing and storage
- Drugs can affect different analytes
- Opiates cause increase in liver and pancreatic enzymes
- Oral contraceptives may affect many tests as well
- Smoking can increase glucose

TABLE 1-5 VARIABLES AFFECTING SELECT CHEMISTRY ANALYTES

FACTOR	EXAMPLES OF ANALYTES AFFECTED
Age	Albumin, ALP (↑ older), phosphorus (P), cholesterol
Gender	(↑ Males): Albumin, ALP, creatine, Ca^{2+}, uric acid, CK, AST, phosphate (PO_4), blood urea nitrogen, Mg^{2+}, bilirubin, cholesterol (↑ Females): Fe, cholesterol, γ-globulins, α-lipoproteins
Diurnal variation	↑ in AM: ACTH, cortisol, Fe, aldosterone ↑ in PM: ACP, growth hormone, PTH, TSH
Day-to-day variation	≥20% for ALT, bilirubin, Fe, TSH, triglycerides
Recent food ingestion	↑ Glucose, insulin, triglycerides, gastrin, ionized Ca^{2+} ↓ chloride, phosphorus, potassium, amylase, ALP
Posture	↑ When standing: albumin, cholesterol, aldosterone, Ca^{2+}
Activity	↑ In ambulatory patients: CK ↑ With exercise: lactic acid, creatine, protein, CK, AST, LD ↓ With exercise: cholesterol, triglycerides
Stress	↑ ACTH, cortisol, catecholamines
Race	TP ↑ (black), albumin ↓ (black); IgG 40% ↑, and IgA 20%↑ (black male vs. white male); → CK/LD ↑ black males; ↑ cholesterol and triglycerides > white >40 years old (glucose incidence diabetes in Asian, black, Native American, Hispanic)
Require fasting	Fasting blood sugar, glucose tolerance test, triglycerides, lipid panel, gastrin, insulin, aldosterone/renin
Anaerobic and require ICE slurry (immediate cooling)	Lactic acid, ammonia, blood gas (if not analyzed within 30 min = ↓ pH, and po_2), iCa^{+2} (heparinized whole blood if not analyzed within 30 min)
Hemolysis	↑ K^+, ammonia, PO_4, Fe, Mg^{2+}, ALT, AST, LD, ALP, ACP, catecholamines, CK (marked hemolysis)

ALP, alkaline phosphatase; CK, creatine kinase; AST, aspartate aminotransferase; ACTH, adrenocorticotropic hormone; ACP, acid phosphatase; PTH, parathyroid hormone; TSH, thyroid-stimulating hormone; ALT, alanine aminotransferase; LD, lactate dehydrogenase; TP, total protein.

Chain of custody

- Lab tests linked to a crime or accident, become forensic in nature
- Documented specimen identification and signature is needed at each step