MEDLAB BASICS · STUDY SHEET

Chemistry Methods and Instrumentation

Beer's law and photometers, optical methods, immunoassay formats and their interferences, electrodes, osmometry, electrophoresis, chromatography and mass spectrometry, automation, point of care, and specimen interferences

45 sources + more detail

medlabbasics.com/sheets/lab-chemistry-methods.html

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1 Spectrophotometry and Beer's law

- Beer's law: A = εbc. Absorbance (A) is proportional to concentration (c) when the path length (b, cm) and molar absorptivity (ε) are fixed.
- Absorbance = log (I0 / I), where I0 is light reaching the detector through the blank and I through the sample. From percent transmittance: A = 2 − log %T.
- Unknown concentration = (A unknown ÷ A standard) × concentration of the standard, valid only inside the method's linear range. Above it, dilute, re-read and multiply by the dilution factor (see the Beer's Law and Dilution Factor calculators).
- Zero the photometer with a reagent blank so that color from the reagent itself is not counted as analyte.

COMPONENT	WHAT IT DOES	EXAM POINTS
Light source	Deuterium lamp for ultraviolet, tungsten-halogen lamp for visible light	Wrong or aging lamp: low energy, noisy readings in its range
Monochromator	Prism or diffraction grating with slits isolates a narrow band of wavelengths	Narrow slit: better resolution but less light and more noise
Cuvette	Holds the sample in a fixed light path (usually 1 cm)	Quartz for ultraviolet (glass absorbs it); scratches and fingerprints add absorbance
Detector	Photodiode or photomultiplier tube turns light into an electrical signal	Photomultiplier: most sensitive, for low light
Double-beam design	Measures reference and sample paths at the same time	Cancels lamp drift and fluctuations

CHECK	MATERIAL	A FAILURE MEANS
Wavelength accuracy	Holmium oxide glass or solution: sharp bands at certified wavelengths	Every band shifted by the same amount: recalibrate the wavelength scale
Photometric accuracy and linearity	Neutral-density (nearly neutral absorbing) glass filters or certified absorbance solutions	Absorbance values wrong even at the right wavelength
Stray light	Cutoff solutions or filters that transmit essentially no light at the test wavelength (light still detected there is stray light)	Light outside the band reaches the detector; high absorbances read low and the curve bends

EXAM TIP Absorbance above the linear range underestimates the concentration. Never report it: dilute until the reading falls in range, then multiply by the total parts (1 part sample + 4 parts diluent = a factor of 5).

2 Other optical and atomic methods

METHOD	WHAT IS MEASURED	TYPICAL USES	WATCH FOR
Flame emission photometry	Light emitted at the element's own wavelength by atoms excited in a flame	Sodium, potassium, lithium (largely replaced by ISE)	Sample is diluted, so lipemia and high protein lower the result (volume displacement)
Atomic absorption (flame or graphite furnace)	Light from a hollow cathode lamp of the element, absorbed by ground-state atoms	Lithium, trace metals; graphite furnace for very low levels such as lead	One element per lamp; being replaced by ICP-MS
Fluorometry	Light emitted when a molecule excited at one wavelength returns to ground state; detector at 90 degrees	Fluorescent immunoassays, time-resolved fluorescence	Self-quenching and inner-filter effects at high concentration; background fluorescence (read a blank)
Chemiluminescence	Light from a chemical reaction; no excitation lamp needed	Most automated immunoassays (acridinium or ruthenium labels, or enzyme labels with luminol or dioxetane substrates)	Very wide dynamic range and high sensitivity
Turbidimetry	Loss of transmitted light (absorbance) through a suspension of immune complexes	CRP, immunoglobulins, latex-enhanced assays on general chemistry analyzers	Lipemia; antigen excess (prozone) reads falsely low
Nephelometry	Light scattered by immune complexes at a fixed angle (for example 13 to 24 degrees)	Immunoglobulins, complement, free light chains	Lipemia; antigen excess; kinetic readings help detect excess
ICP-MS	Elements ionized in an argon plasma and counted by mass	Trace and toxic elements, many in one run	Costly; spectral interferences must be controlled

3 Immunoassay formats and their interferences

FEATURE	COMPETITIVE (REAGENT-LIMITED)	SANDWICH, TWO-SITE (REAGENT EXCESS)
Design	Labeled analyte competes with the patient's analyte for a limited amount of antibody	Capture antibody and labeled detection antibody bind two sites on the analyte
Signal vs concentration	Inverse: more analyte, less signal	Direct: more analyte, more signal
Typical analytes	Small molecules: drugs, steroids, free T4 and free T3	Large molecules: TSH, troponin, hCG, ferritin, prolactin
High-dose biotin (streptavidin-biotin capture)	Falsely HIGH	Falsely LOW
Heterophile antibodies, HAMA, rheumatoid factor	Can bind the conjugate or label: error in either direction	Bridge capture to detection antibody: usually falsely high
High-dose hook effect	Not the classic problem	Antigen excess, especially in one-step assays: falsely low; dilution reveals the true value

- Heterogeneous assays separate bound from free label (a wash step) before reading; homogeneous assays read without separation because binding itself changes the signal.
- Cross-reactivity: structurally similar compounds or metabolites are read as the analyte (cortisol assays and fludrocortisone, cyclosporine metabolites, digoxin-like factors). Drug screens detect a class by cross-reactivity, so positives are confirmed by GC-MS or LC-MS/MS.
- Immunoassays are generally unaffected by hemolysis and icterus; lipemia matters most for nephelometric and turbidimetric methods.

SAFETY Biotin above 1 mg a day (hair and nail supplements up to 10 mg, much more for multiple sclerosis) can give a falsely LOW troponin on affected sandwich assays and a Graves-like thyroid pattern (low TSH, high free T4). An undetectable troponin in a patient with a proven infarction must be questioned before release.

EXAM TIP Clues to interference: results that do not fit the patient, disagreement between platforms, and a corrected result that changes with dilution. Rising corrected results on dilution = hook effect; falling corrected results on dilution or a large drop after blocking reagent = heterophile antibody.

4 Electrochemistry

METHOD	PRINCIPLE	CLINICAL EXAMPLES
Potentiometry (ion-selective electrodes)	Voltage between an indicator and a reference electrode at almost zero current; voltage changes with the log of ion activity (Nernst equation)	Na, K, Cl, Li, ionized Ca; pH (glass electrode); pCO2 (Severinghaus electrode)
Severinghaus pCO2 electrode	CO2 diffuses through a membrane into a bicarbonate solution and changes its pH, read by a glass pH electrode	Blood gas pCO2
Amperometry	Current at a fixed applied voltage, proportional to the amount of substance oxidized or reduced	pO2 (Clark electrode), glucose biosensors and meters
Clark pO2 electrode	Oxygen crosses a permeable membrane and is reduced at a platinum cathode held at about −0.8 V; silver/silver chloride anode	Blood gas pO2
Coulometry	A silver anode releases silver ions that precipitate chloride as AgCl; when chloride is used up, free silver ions raise the current and stop the titration; charge (time) is proportional to chloride	Chloridometer: sweat chloride, serum or urine chloride
Conductometry	Electrical conductivity of the whole solution, not one ion	Sweat conductivity: screening only, never diagnostic for cystic fibrosis

FEATURE	DIRECT ISE	INDIRECT ISE
Sample	Undiluted	Diluted (about 1:5 to 1:46) before measurement
Measures	Ion activity in plasma water	Ion in the diluted whole sample, assuming plasma is about 93% water
Where	Blood gas and point-of-care analyzers, some dry-slide systems	High-throughput automated chemistry analyzers
Very high lipids or protein	Unaffected	Falsely LOW Na, K and Cl (electrolyte exclusion): pseudohyponatremia

EXAM TIP Low sodium on the chemistry analyzer with severe lipemia or a paraprotein, a normal measured osmolality, and a normal sodium on the blood gas analyzer = pseudohyponatremia from indirect ISE. Bromide is read as chloride: very high chloride and a negative anion gap.

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5 Osmometry, electrophoresis and separation methods

METHOD	PRINCIPLE	EXAM POINTS
Freezing point osmometer	Dissolved particles lower the freezing point of water in proportion to osmolality (colligative property)	The usual clinical method; detects ethanol, methanol and ethylene glycol, so it is the one used for the osmolal gap
Vapor pressure osmometer	Dissolved particles lower the vapor pressure (dew point)	Misses volatile alcohols, so an osmolal gap from it is unreliable for toxic alcohols
Electrophoresis (agarose gel)	Proteins move in an electric field by charge and size (usually in barbital buffer near pH 8.6), are stained and scanned	Fractions: albumin, alpha-1, alpha-2, beta (beta-1, beta-2), gamma; abnormal band typed by immunofixation
Capillary zone electrophoresis	Separation in a narrow capillary filled with buffer; proteins are detected, not stained	Automated, fast; abnormal peak typed by immunosubtraction
HPLC	Liquid mobile phase pumped through a packed column; compounds separate by how strongly they partition into the stationary phase (normal or reverse phase)	Reference method for drugs such as cyclosporine; detectors include UV and mass spectrometry
Gas chromatography	Gas mobile phase carries volatile, heat-stable compounds through a column; identified by retention time	Volatile alcohols; GC-MS confirms drugs of abuse with library-matched spectra
LC-MS/MS (tandem mass spectrometry)	LC separation, electrospray ionization, then two mass filters select a precursor ion and its product ion	Most specific routine method: steroids, vitamin D, drugs, newborn screening; stable isotope internal standards correct ion suppression
MALDI-TOF MS	Sample mixed with matrix, hit by a laser; ions fly down a tube and lighter ions arrive first; ribosomal protein spectrum is matched to a database	Identifies bacteria and yeast from colonies in minutes; E. coli and Shigella are not separated

EXAM TIP SPEP is run on serum. Plasma, or serum from a tube that did not clot fully, keeps fibrinogen, which forms a narrow band at the border of beta and gamma that can be mistaken for a monoclonal protein; immunofixation settles it.

6 Hemolysis, icterus and lipemia

INTERFERENT	HOW IT INTERFERES	TESTS MOST AFFECTED	WHAT TO DO
Hemolysis	Release of cell contents; hemoglobin color; chemical reaction with reagents	Potassium, LD, AST raised; bilirubin can be lowered	Check the H-index; recollect for affected tests; report with a comment if policy allows
Icterus	Bilirubin color (spectral) and reaction with reagents	Peroxidase-coupled methods (cholesterol, triglyceride, uric acid) and creatinine read low; co-oximetry	Check the I-index; use assay limits; dilution or another method
Lipemia	Light scatter and absorption (worst at short wavelengths); volume displacement; uneven layering	NADH methods read at 340 nm (ALT, AST, glucose); Na, K, Cl by indirect ISE; nephelometry	Check the L-index (visual grading is unreliable); high-speed or ultracentrifugation, then test the infranatant

SAFETY Lipid removal is not acceptable for triglycerides or for hydrophobic analytes (some drugs and hormones) that move into the lipid layer: run those on the original specimen first, or recollect after the patient has fasted.

7 Tubes, additives and order of draw

TUBE (USUAL STOPPER)	ADDITIVE	EFFECTS TO KNOW
Light blue	3.2% (0.109 M) sodium citrate, 1 part to 9 parts blood	Coagulation; chelation can inhibit AST and ALP
Red, gold (SST)	Clot activator, with or without separator gel	Gel can absorb hydrophobic drugs (phenytoin, phenobarbital, carbamazepine, lidocaine): 20 to 50% lower after 24 h at 4 °C; very dense serum may not float above gel
Green	Sodium or lithium heparin	Heparin binds ions, so balanced heparin is used for ionized calcium; no lithium heparin for lithium levels
Lavender	K2 or K3 EDTA	Hematology; chelates Ca, Mg and Zn: carried into other tubes it gives falsely high K and low Ca and Mg
Gray	Sodium fluoride with potassium oxalate (or EDTA)	Fluoride blocks enolase, but glycolysis goes on for 1 to 4 hours; oxalate shrinks cells and inhibits several enzymes

1. Blood culture bottles (sterile first)
2. Non-additive tube (or discard tube)
3. Sodium citrate (coagulation)
4. Serum tubes: clot activator, then serum separator
5. Heparin, including plasma separator tubes
6. EDTA
7. Fluoride/oxalate

EXAM TIP A very high potassium with a very low calcium in a patient who looks well points to EDTA contamination, not hyperkalemia. Ask for a recollection; do not report the potassium as a critical value without checking.

8 Automation, autoverification and point of care

AREA	WHAT IT COVERS	EXAM POINTS
Total laboratory automation	Preanalytical: tracks, barcode reception, sorting, centrifugation, aliquoting. Analytical: analyzers linked by middleware. Postanalytical: autoverification, result transfer, storage	Fewer manual steps, shorter and more predictable turnaround
Random access analyzer	Any test on any specimen in any order	Unlike batch analyzers that run one test on a group of specimens
Autoverification	Rules in middleware or the LIS release results without a person	Rules use instrument flags, QC status, serum indices, critical values, delta checks, measuring range and moving averages; each laboratory sets and validates its own limits (CLSI AUTO10 and AUTO15)
Point-of-care testing	Handheld strips and lateral flow devices, cartridges, small bench analyzers near the patient	Waived tests must follow the manufacturer's instructions (42 CFR 493.15); operator training and QC records matter most
Glucose meters	Amperometric strips with glucose oxidase or glucose dehydrogenase on whole blood	High hematocrit reads low, anemia reads high; oxygen therapy lowers glucose oxidase results; maltose (icodextrin dialysate) falsely raises some GDH strips

SAFETY A meter reading that does not fit the patient (for example a high reading in a sweaty, confused patient on icodextrin dialysate) is confirmed at once with a laboratory plasma glucose by a glucose-specific method, and the laboratory value is acted on.

MORE Beer's law worked examples

1. Within range. A 200 mg/dL standard reads A = 0.400 and a patient reads A = 0.300 against the same reagent blank. Concentration = 0.300 ÷ 0.400 × 200 = 150 mg/dL.
2. Above range. The method is linear to A = 0.800 and a patient reads 1.250. The sample is diluted 1 part plus 4 parts saline (factor 5) and reads 0.250. 0.250 ÷ 0.400 × 200 = 125 mg/dL; × 5 = 625 mg/dL. The first reading (1.250) would have given 625 only if the curve stayed straight, which it does not.
3. Transmittance. A = 2 − log %T, so 100%T gives A = 0, 10%T gives A = 1.0 and 1%T gives A = 2.0. Half the light through (50%T) is A = 0.301. Absorbance, not %T, is proportional to concentration.
4. Molar absorptivity. If **ε** is known, c = A ÷ (**ε** × b). A compound with **ε** = 6,220 L/mol/cm (the usual NADH value at 340 nm) giving A = 0.311 in a 1 cm cuvette is at 0.311 ÷ 6,220 = 5.0 × 10^{-5} mol/L (50 µmol/L).

Why curves bend at the top: at high concentration molecules interact and scatter light, and stray light (light outside the selected band) is a larger share of the little light that still reaches the detector, so absorbance rises less than concentration. Lipemia, turbidity and bubbles add apparent absorbance that is not analyte. Wavelength error moves the reading off the absorbance peak, lowering sensitivity and linearity.

EXAM TIP Endpoint vs kinetic: an endpoint method reads once after the reaction is complete; a kinetic (rate) method reads the change in absorbance per minute, which is how enzyme activities and many NADH-linked assays are measured. A falling absorbance at 340 nm means NADH is being used up.

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