



CLINICAL CHEMISTRY

Learning Guide



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The Intended Audience

This learning guide is intended to serve the basic educational needs of health care professionals who are involved in the field of laboratory medicine. Anyone associated with the specialty of clinical chemistry will find this monograph of special interest.

Laboratorians, those who use the laboratory's services, and those who service the laboratory will find this guide most useful. This includes laboratory technicians and technologists, laboratory supervisors and managers, nurses, laboratory suppliers, and other physician office and laboratory support personnel.

How to Use This Learning Guide

To offer you the most benefit from this learning guide, each section begins with a *Section Overview* so you can quickly review its goal and content. Next you will find a set of *Learning Objectives*. These will help you focus on the key concepts presented in each section. There is a short *Section Review* quiz at the end of each section designed to help you recall the concepts introduced. If you answer a question incorrectly, review the appropriate portions of the text before moving to the next section.

A glossary and an explanation of acronyms are included at the end of this learning guide for quick reference. There is also a bibliography devoted to other recommended reading if you wish to further your studies.

This learning guide ends with a questionnaire about its effectiveness. You may wish to complete and return it to Abbott Diagnostics. With your feedback, we will be able to ensure that future editions of this guide will be as beneficial as possible.

I. CLINICAL CHEMISTRY: BASIC TECHNOLOGY

Section Overview

This section discusses photometry and potentiometry to measure the concentrations of many analytes in human specimens.

Learning Objectives

After completing this section, you should be able to:

1. Describe the methodology of photometry and potentiometry.
2. Differentiate between endpoint and rate reactions.
3. Explain the application of endpoint and rate measurements to chemistry analyzers.
4. Explain the principle of sample blanking and how it is used to minimize sample interferences.

Terms in **red** are defined in glossary on page 62.

Key Concepts

1. Chemical reactions can be used to measure analytes in clinical specimens.
2. Chemical reactions are based upon the specimen analyte reacting with one or more reagent(s) which then produces a measureable change in detection response.
3. The photometry measures the change in color of a liquid solution.
4. The potentiometry measures the change in electrical potential of an ion sensor.

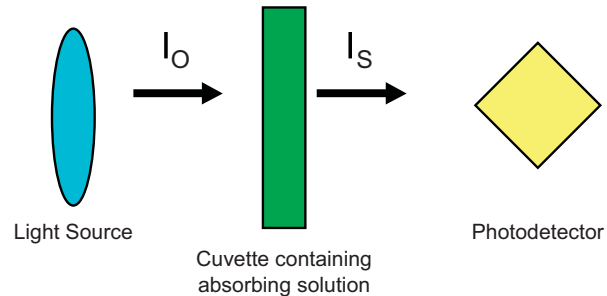
A. Photometry

The quantification of routine chemistry analytes is generally achieved using one of two measurement technologies, **photometry** or **potentiometry**.

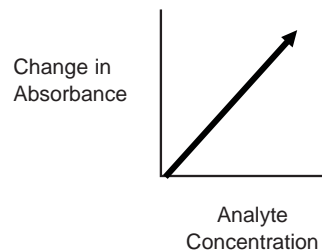
In photometry, an aliquot of sample containing analyte is mixed in a cuvette with a liquid reagent. The reagent reacts with analyte producing a change in absorbance (color) within the reaction solution. The absorbance is measured using a photometry system.

Photometry measures transmitted light to determine reaction absorbance.

This is achieved by comparing the amount of transmitted (I_s) light to the amount of light entering (I_o) the cuvette.



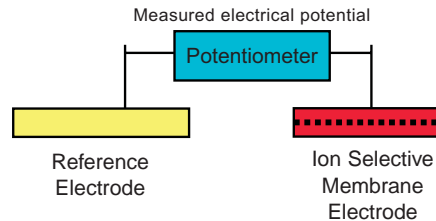
The change in absorbance is proportional to the concentration of analyte in the sample. Typically, more analyte in the sample generates a darker colored solution in the cuvette. Thus, less light gets through to the detector.



B. Potentiometry

Potentiometry used to measure electrolytes.

Potentiometry is based on electrochemical reactions and is the measurement of the electrical potential between two electrodes in an electrochemical cell. Examples of analytes that typically utilize potentiometry for their measurement are the electrolytes sodium (Na^+), potassium (K^+) and chloride (Cl^-).



Ion-selective membrane electrodes (ISE) are utilized with specific permeability to selected anions and cations (e.g., Valinomycin membrane to measure K^+). Sample containing analyte is brought into contact with the ion specific membrane. Concentrations are calculated from the measured potential through the Nernst equation.

C. Analytical Considerations

Reading Principles: Automated photometers use different methods for mixing of reagents and reading of absorbance signals.

1. **Endpoint:** This method utilizes signal development as a function of time. The reaction between analyte and reagent needs a period of time to reach endpoint; the analyte concentration can then be calculated.

These reactions are described as either **endpoint-up** or **endpoint-down** depending on whether the endpoint signal is greater or less than the initial reaction signal. Examples of analytes which typically utilize the endpoint-up reaction are glucose, calcium, phosphorus, and albumin. Urea is an example of an analyte that utilizes the endpoint-down reaction.

Endpoint-Up
↑ absorbance signal

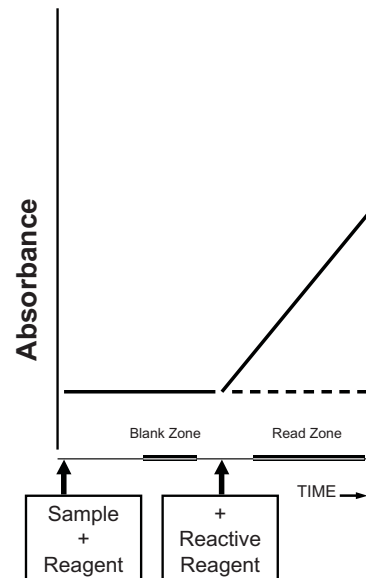
Endpoint-Down
↓ absorbance signal

Rate Reaction
= $\frac{\text{change in absorbance}}{\text{Time}}$

Enzymes measured by
rate reaction

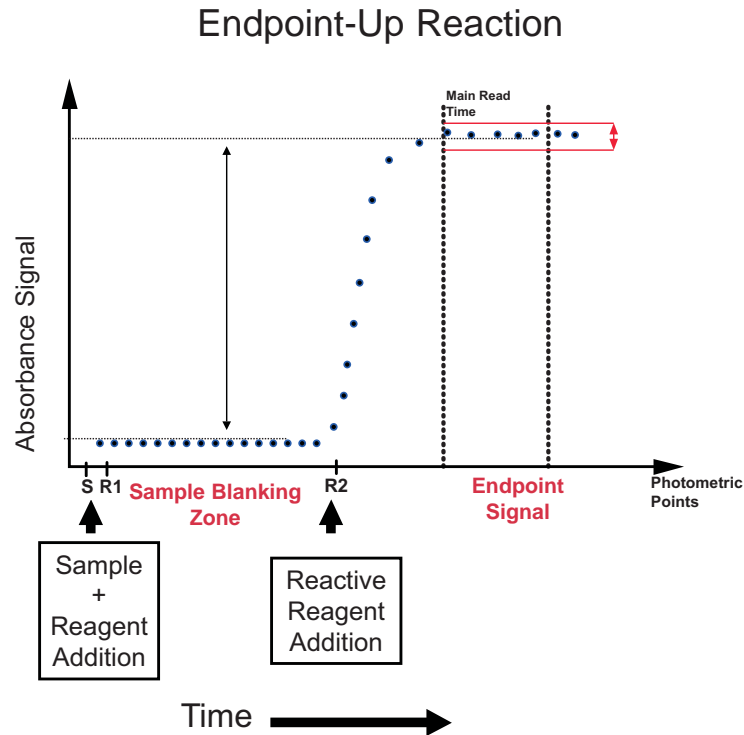
- Rate Reaction (reaction change as a function of time):** Using this principle, a result is calculated from the change in signal per unit of time. The rate of the signal change is measured. These reactions can also be described as either **up** or **down**. Enzymes are measured using the rate reaction. Examples of rate-up are CK and LDH. Examples of rate-down are ALT and AST.

Rate-Up Reaction:



Sample Blanking
↓ Endogenous Assay
Interferences

3. **Sample Blanking:** Interferences from hemolyzed, icteric and lipemic specimens may be minimized by subtracting the sample signal obtained prior to addition of reactive reagent from the endpoint signal.



Summary

The measurement of chemistry analytes utilize two common detection technologies, photometry and potentiometry.

Photometry commonly utilizes liquid reagents which interact with the specimen analyte producing a measurable change in the reaction solution's color or turbidity.

Potentiometry represents an electrochemistry measurement technology in which specimen is brought into contact with an electrochemical cell and the change in electrode potential is measured.

Photometry and potentiometry have been applied to laboratory instrumentation to provide high quality assay results with easy to use formats. Common detection schemes utilize either endpoint or rate measurements to quantify the amount of analyte in specimen.

Review Questions (I)

1. Assay interferences may be minimized using which of the following:
 - a. Photometry
 - b. Sample Blanking
 - c. Rate-Up Reaction
 - d. Potentiometry

2. Photometry measures the amount of:
 - a. Transmitted Light
 - b. Membrane Electrode Potential
 - c. Sample Interference
 - d. Reflected Light

3. The following analytes typically use potentiometry for their measurement:
 - a. Lipid Profile
 - b. Electrolytes
 - c. Enzymes
 - d. Triglycerides

Click on this link to go to the Answers page.

[Check Your Responses](#)

II. ROUTINE CLINICAL CHEMISTRIES

Section Overview

The Clinical Chemistry laboratory measures chemical changes in the body for diagnosis, therapy, and prognosis of disease. Primarily, testing is performed using body fluids such as serum, plasma, and urine to determine the chemical components. This section discusses the tests that are considered “routine” in the clinical chemistry laboratory, including electrolytes, enzymes, and products of metabolism.

Learning Objectives

After completing this section, you should be able to:

1. Differentiate the tests used to diagnose a disease from those used to evaluate a disease process.
2. Describe the use for certain chemical tests.
3. Describe some possible causes for error in testing.
4. Identify some profiles and panels used in diagnosing a disease process.

Key Concepts

1. Many tests are not specific for a certain disease process.
2. Many times a panel of tests is used in diagnosing a disease process.
3. Some tests are very specific for a disease and can be used for diagnosis.
4. Maintaining a sample properly can eliminate result errors.

A. Typical Tests and Panels

The following table lists many of the most common routine clinical chemistry analytes run on clinical chemistry analyzers.

TABLE 1

Common routine clinical chemistry analytes

Enzymes

- Acid Phosphatase
- Alkaline Phosphatase
- ALT
- AST
- Amylase
- Cholinesterase
- Creatine Kinase
- GGT
- LD
- Lipase

Metabolites

- Ammonia
- Bilirubin, Total
- Bilirubin, Direct
- Bilirubin, Neonatal
- Creatinine
- Urea Nitrogen
- Uric Acid

Electrolytes

- Sodium
- Potassium
- Chloride
- Carbon Dioxide

Lipids/Lipoproteins

- Cholesterol
- HDL, Direct
- LDL, Direct
- Triglycerides

Metals

- Calcium
- Iron
- Total Iron Binding Capacity (TIBC)
- Unsaturated Iron Binding Capacity (UIBC)
- Magnesium
- Phosphorus

Carbohydrates

- Glucose
- Lactic Acid
- Glycated Hemoglobin

Proteins

- Albumin
- Apolipoprotein A1
- Apolipoprotein B
- ASO
- C3
- C4
- C-Reactive Protein (CRP)
- Hs-CRP (high sensitivity)
- Haptoglobin
- IgA
- IgG
- IgM
- Microalbumin
- Prealbumin
- Total Protein
- Transferrin
- Rheumatoid Factor (RF)

An individual chemistry test often lacks sufficient sensitivity and specificity to categorically identify a specific disease state. Thus, multiple tests are frequently requested as a small group of tests in a panel, which when used together give the physician results that aid the clinical diagnosis. Physicians may add individual routine chemistry or immunoassay analytes to these panel requests to provide further focus on a particular suspected disease state.

Common Panels

Name	Assays
Electrolyte	Na ⁺ , K ⁺ , Cl ⁻ , CO ₂
Hepatic (Liver) Function	Alb, AlkP, ALT, AST, Tbili
Renal (Kidney) Function	Urea (serum and urine), Crea (serum and urine), Urine Na ⁺
Basic Metabolic (Chem 7)	Urea, Crea, Glu, Na ⁺ , K ⁺ , Cl ⁻ , CO ₂
Comprehensive Metabolic	Alb, AlkP, ALT, TBili, Urea, Ca, Crea, Glu, TP, Na ⁺ , K ⁺ , Cl ⁻
Cardiac Risk Assessment	Chol, LDL-Chol, HDL-Chol, Trig, Glucose

B. Enzymes

Enzymes are metabolic catalysts.

High levels of acid phosphatase are found in the prostate gland.

Prostate cancer ↑ acid phosphatase levels.

Metabolic reactions in the body are regulated by biological **catalysts** called enzymes. Enzymes are present in all body cells, and each has a specific purpose. Table 2 on the next page summarizes the most clinically important enzymes.

1. Acid Phosphatase (ACP). Acid phosphatase is an enzyme that is distributed in the bone, liver, spleen, kidney, red blood cells, and platelets. The largest pool of acid phosphatase is found in the prostate gland.

Increased values for acid phosphatase are found in metastatic carcinoma of the prostate, **Gaucher's disease**, and in some bone diseases.

TABLE 2

Enzymes: these substances are important indicators of many disease states.

Enzyme	Major Source	Application
Acid phosphatase	Prostate	Prostate cancer
Alkaline phosphatase	Bone Intestine Liver	Bone diseases Liver diseases
Amylase	Salivary gland Pancreas	Pancreatic disorder
Cholinesterase	Liver	Insecticide poisoning, suxamethonium sensitivity, liver disease
Creatine kinase (CK)	Bone Heart Brain	Muscle damage Brain damage (rarely) Myocardial infarction
Aspartate amino-transferase (AST)	Heart Bone Liver	Liver disease Muscle damage
Alanine aminotransferase (ALT)	Liver Bone Heart	Liver disease
Gamma-glutamyl-transferase (GGT)	Kidney Pancreas Liver	Liver disorders Alcoholism
Lactate dehydrogenase (LD)	Liver Heart Bone RBCs	Heart disease Hemolysis Myocardial infarction Liver disease
Lipase	Pancreas	Pancreatic disorder

Alkaline phosphatase is found in bone and liver.

Liver disease ↑ alkaline phosphatase levels.

Amylase digests starches.

Pancreatitis ↑ amylase levels.



2. Alkaline Phosphatase (ALP). Alkaline phosphatase is widely distributed in the body and is present in high concentrations in bone, intestinal mucosa, and renal tubule cells. Lower concentrations appear in the liver, leukocytes, and placenta.

Increased values for alkaline phosphatase are found in all bone disorders, liver disease, and during the third trimester of pregnancy. Decreased values are found in hypophosphatasemia, hypothyroidism, pernicious anemia, and in dwarfs.

3. Amylase. Amylase is an enzyme that is secreted by the salivary and pancreatic glands. It is important for the digestion of starches and is rapidly cleared by the kidneys.

Increased values of amylase are found in acute pancreatitis, obstruction of the pancreatic ducts, and (mildly) in obstruction of the parotid gland. Decreased values are found in acute or chronic hepatocellular damage.



Creatine kinase is found in muscle and brain.

Myocardial infarction ↑
creatinase kinase-MB, ...

...it returns to normal in
24–48 hours.

4. Total Creatine Kinase (CK). Creatine kinase is present in high concentration in skeletal muscle, cardiac muscle, thyroid, prostate, and brain tissue.

Increased values for creatine kinase are found when skeletal muscle, myocardium, and (rarely) brain tissue have been damaged.

Which enzyme is increased in CO poisoning?

processes can also give positive creatine kinase-MB results but these levels will stay constant. With a myocardial infarction the creatine kinase-MB levels will typically return to normal within 24 to 48 hours. Figure 1 on the next page shows the different timelines of some enzymes important in monitoring heart disease.

What are some differences between CK and CK-MB?

5. Creatine Kinase-MB (CK Isoenzyme). Creatine kinase-MB usually can be found in patients' samples about 4–6 hours after the onset of chest pain in acute myocardial infarction. It is important to note that some disease

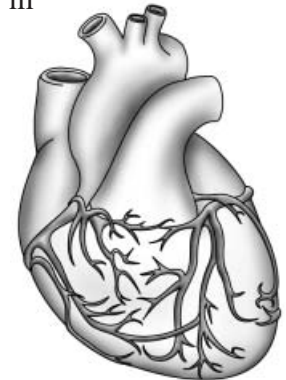
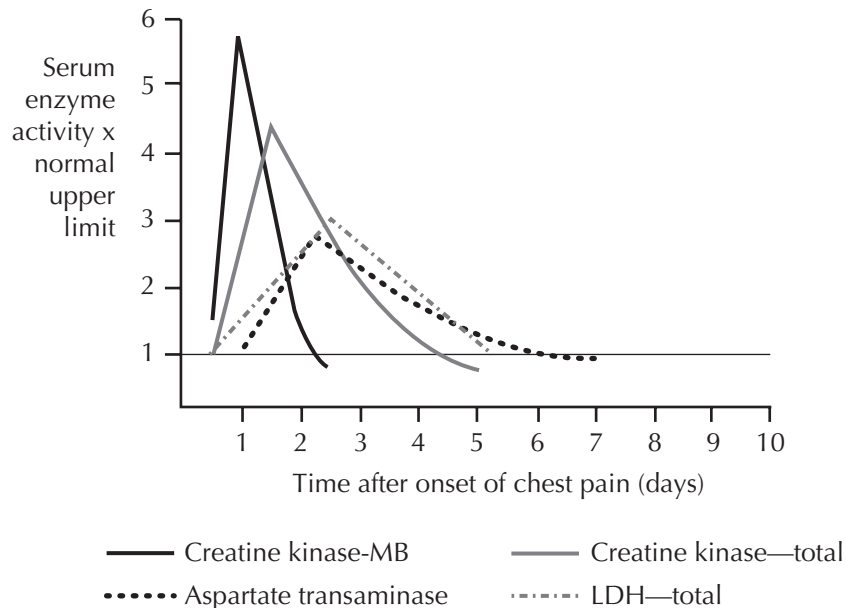


FIGURE 1

Serum enzyme levels:
monitors of heart disease.



Severe angina ↑ CK-MB levels.

Increased values for creatine kinase-MB can be found in acute myocardial infarction, severe angina, pericarditis, carbon monoxide poisoning, muscular dystrophy, **polymyositis**, malignancy, and open-heart surgery.

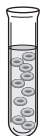
Liver disease ↑ AST levels.

Myocardial infarction ↑
AST levels.

ALT is more specific for
liver disease than AST.

6. Aspartate Aminotransferase (AST). AST is present in heart, skeletal muscle, and liver in equal amounts. Measurement of AST is valuable in the diagnosis of liver disease.

AST and ALT usually rise and fall together when the patient has hepatic cell damage. Increased values for AST are found in myocardial infarction, liver disorders, trauma or diseases affecting skeletal muscle, after renal infarction, and in various hemolytic conditions.



7. Alanine Aminotransferase (ALT). The highest ALT levels are found in liver tissue and the primary use of this test is to diagnosis liver disease. ALT is more specific for liver malfunction than AST.

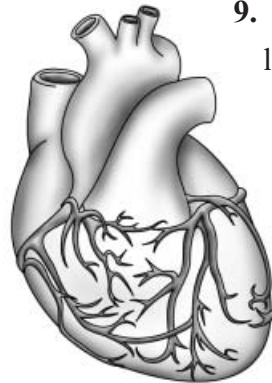
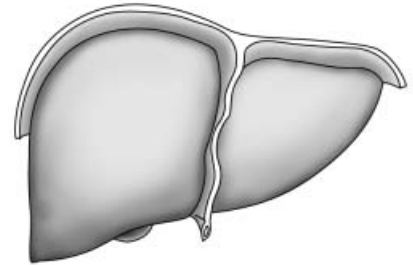
Increased values for ALT are found in acute hepatitis, alcoholic hepatitis, cirrhosis, **Reye's syndrome**, hepatomas, and cholestatic disease.

Liver disease and alcoholism
↑ GGT levels.

Myocardial infarction
↑ LDH levels.

LDH stays elevated longer
than CK-MB.

8. Gamma-glutamyltransferase (GGT). GGT is present in the kidney, pancreas, liver, and prostate. It is a sensitive indicator of liver disease, is very helpful in diagnosing hepatobiliary obstruction, and is elevated in all forms of liver disease and alcoholism.



9. Lactate Dehydrogenase (LDH). LD is distributed in the liver, cardiac muscle, kidney, skeletal muscle, erythrocytes, and other tissues.



Pancreatitis ↑ lipase levels.

10. Lipase. Lipase is primarily produced in the pancreas. Rapidly elevated in acute pancreatitis, it remains elevated longer than amylase.

In pancreatitis, which is elevated longer, lipase or amylase?



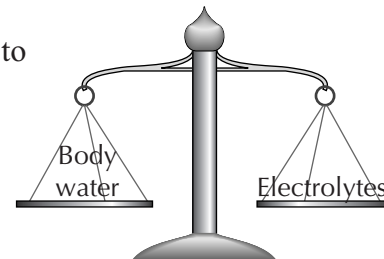
Which enzyme reacts with a common muscle relaxant?



11. Pseudocholinesterase. Pseudocholinesterase is a serum enzyme that reacts with succinylcholine, a short-acting muscle relaxant that is used when patients are going to surgery. Some people have a genetic deficiency of the enzyme pseudocholinesterase and, when injected with the succinylcholine, may have an extended reaction to the drug. Cholinesterase levels in serum are also useful as an indicator of possible insecticide poisoning.

C. Electrolytes

The term electrolytes in the medical usage is applied to sodium, potassium, chloride, and carbon dioxide. Electrolytes help regulate water balance and acid-base balance in the body. These **analytes** are primarily used to measure kidney function.



1. Carbon Dioxide (CO₂ / Bicarbonate). Fats, proteins, and carbohydrates are broken down in the body to create energy, and the carbon atoms are converted to carbon dioxide. During the process of respiration, the lungs rapidly eliminate carbon dioxide. The kidneys can also eliminate excess carbon dioxide through the urine.



Samples for carbon dioxide should be maintained in a stoppered tube until analyzed as the analyte will evaporate and give falsely decreased values.

An increased carbon dioxide level is found in metabolic **alkalosis**, compensated respiratory **acidosis**, and frequently in alkalosis when there is a large deficiency of potassium. Decreased carbon dioxide levels are found in metabolic acidosis and compensated respiratory alkalosis.

CO₂ is a byproduct of food.

CO₂ is eliminated by the lungs and the kidneys.

Metabolic alkalosis ↑ CO₂ levels.

Metabolic acidosis ↓ CO₂ levels.

Cl^- is the #1 extracellular element.

$\uparrow \text{Cl}^-$ is found with $\uparrow \text{Na}^+$.
Dehydration $\uparrow \text{Cl}^-$ levels.

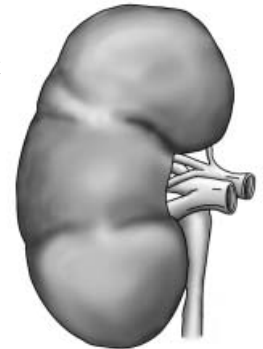
K^+ is the #1 element in cells.

The kidneys excrete K^+ .

2. Chloride (Cl^-). Chloride is the element that has the highest **extracellular** concentration in the **serum**. Chloride plays an important role in maintaining electrolyte balance, hydration, and **osmotic pressure**. It is ingested through a normal diet, absorbed in the intestine, and removed from the body by **excretion** in urine and sweat. Excessive amounts of chloride can be lost during periods of intense perspiration.

Normally elevations of chloride will be accompanied by elevations of sodium. Increased chloride level is found in dehydration, certain types of **renal** tubular acidosis, and hyperventilation. Decreased levels are found in uncontrolled diabetes, metabolic acidosis, and **Addison's disease**.

3. Potassium (K^+). Potassium is the element that has the highest concentration within cells. It is ingested through a normal diet and absorbed through the intestines. The kidney excretes excess potassium through urine. Elevated levels of potassium may cause serious problems with muscle irritability. Potassium also plays an important role in nerve conduction.



Vomiting and diarrhea ↓ K^+ levels.

Na^+ is the #1 cation in the blood.
↑ Na^+ is accompanied by
↑ Cl^- .

Dehydration ↑ Na^+ levels.

Diarrhea ↓ Na^+ levels.



Potassium samples should not have **hemolysis**, which can give falsely elevated results. Increased levels are found in shock, circulatory failure, and in both metabolic and renal tubular acidosis. Decreased levels can be caused by vomiting, diarrhea, diuretics, and some carcinomas.

4. Sodium (Na^+). Through excretion and reabsorption in the kidneys, the body attempts to keep sodium levels constant. Sodium helps to maintain osmotic pressure, acid-base balance, and nerve impulses.

Increased levels are found in severe dehydration, **Cushing's syndrome**, comatose diabetics, and diabetes insipidus. Decreased levels are found following a large loss of gastrointestinal secretions. Additional causes include renal disease and Addison's disease.

D. Other Routine Analytes

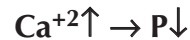
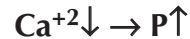
Ca^{+2} is vital to blood clotting.

Ca^{+2} and P deposits are linked.

$\downarrow \text{Ca}^{+2}$ levels are accompanied by \downarrow vitamin D levels.

1. Calcium (Ca^{+2}). Calcium, a mineral present in the body that is a vital component in the skeleton, bones, and teeth, is involved in the coagulation process.

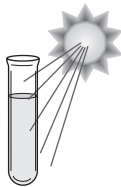
Calcium and phosphorus have a reciprocal relationship.



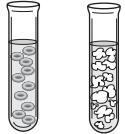
Increased calcium levels are found in hyperparathyroidism, some malignancies, multiple myeloma, and **Paget's disease**. Decreased values are found in hypoparathyroidism, pseudohypoparathyroidism, vitamin D deficiency, chronic renal disease, and acute pancreatitis.



2. Bilirubin (Conjugated, Total and Neonatal). Bilirubin, a breakdown product of **hemoglobin** in the red blood cells, is a by-product of hemolysis and is removed by the liver. Conjugated bilirubin circulates freely in the blood until it reaches the liver where it is excreted into the bile.



Samples to be analyzed for bilirubin should be protected from light and heat and, for best results, stored in the dark at low temperatures. Lipemia and hemolysis should also be avoided.



Total bilirubin checks for impairment of the excretory function of the liver and for excessive hemolysis of red cells. Conjugated bilirubin (**Direct Bilirubin**) checks only for the impairment of the excretory function of the liver, such as blockage.

Increased values of total bilirubin are found in viral hepatitis, cirrhosis, and infectious mononucleosis. Increased values for conjugated bilirubin are found in hepatobiliary disease, biliary tract obstruction, cancer of the head of the pancreas, choledocholithiasis, and **Dubin-Johnson syndrome**.

Handle bilirubin samples carefully.

Total bilirubin checks liver function.

Hepatitis and cirrhosis ↑ bilirubin levels.

Neonatal bilirubin is unconjugated bilirubin.

Increased neonatal bilirubin can cause CNS problems.

Neonatal bilirubin refers to the unconjugated or indirect bilirubin. Under normal conditions this bilirubin is bound to albumin and causes no problem. However, if the unconjugated bilirubin levels exceed the binding capacity, the bilirubin can pass into an infant's central nervous system and cause mental retardation, hearing deficits, or cerebral palsy. Figure 2 below illustrates unbound bilirubin crossing the blood-brain barrier.

Samples for bilirubin determination must be protected from light until analysis.

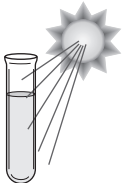
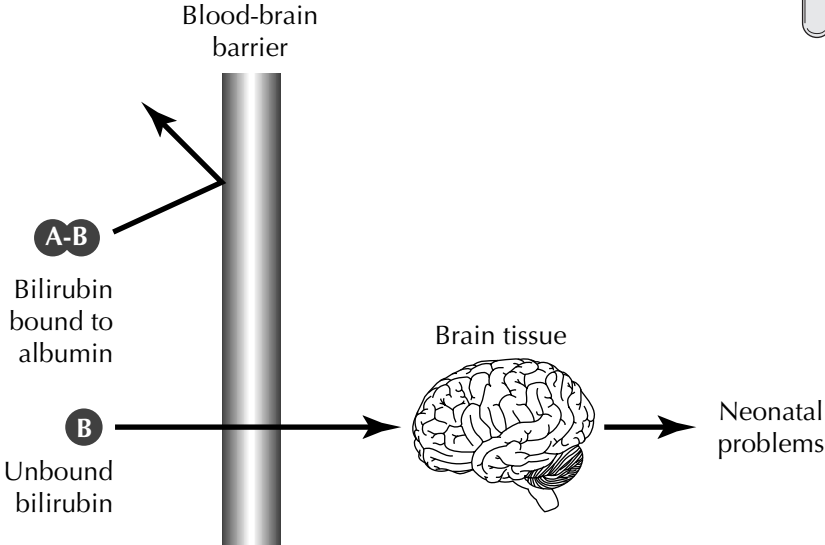


FIGURE 2

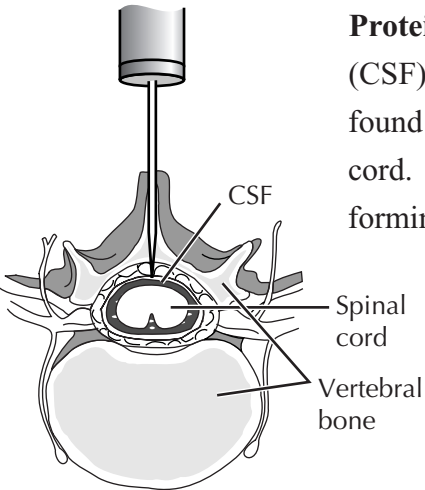
Bilirubin: unconjugated can present problems in neonates.



CSF bathes brain and spinal cord.

Do not test bloody CSF.

Red blood cells may use up CSF glucose.



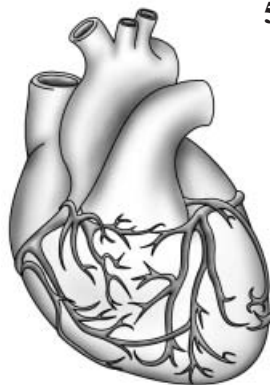
3. Cerebrospinal Fluid

Protein. Cerebrospinal fluid (CSF) is a clear, colorless liquid found in the brain and the spinal cord. Fluid is obtained by performing a spinal tap.



Test results for CSF protein are not valid if the sample is bloody. Increased values for CSF protein are found in meningitis, neuro-syphilis, some cases of encephalitis, and frequently after cerebral hemorrhage.

4. Cerebrospinal Fluid Glucose. Decreased values of CSF glucose may indicate bacterial meningitis.



5. Cholesterol. Cholesterol is a complex alcohol that is converted by the adrenals and the gonads into steroid hormones. Elevated cholesterol has been implicated as one of the risk factors in coronary artery disease.

Increased values for cholesterol also suggest hypothyroidism, uncontrolled diabetes mellitus, and **nephrotic** syndrome.

Decreased values for cholesterol are found in hyperthyroidism, hepatocellular disease, anemias, starvation, and certain genetic defects.

6. Creatinine and Urea Nitrogen. Creatinine is a waste product formed in muscle tissue after energy production and is excreted in the urine.

Increased values for creatinine are found in congestive heart failure, shock, vomiting, diarrhea, diabetes insipidus, uncontrolled diabetes mellitus, and excessive use of diuretics.

Blood urea nitrogen (BUN), usually correlates with creatinine.

Nephrotic syndrome
↑ cholesterol levels.

Vomiting and diarrhea
↑ creatinine levels.

BUN↑ → Creatinine↑

BUN↓ → Creatinine↓

Blood urea nitrogen is the end product of protein breakdown. BUN levels are influenced by factors not connected with renal function or urine excretion. Creatinine is a better indicator of kidney function even though BUN and creatinine usually rise and fall together.

Increased values for BUN are found in high protein diets, administration of cortisol-like steroids, and stressful situations. Decreased values for BUN are found in late pregnancy, starvation, and in patients whose diet is grossly deficient in proteins.

7. Glucose. Glucose testing is the screening procedure used to detect disorders of metabolism. Two hormones directly regulate glucose—glucagon and insulin.

Increased values for glucose are found in diabetes mellitus, Cushing's disease, acute stress, hyperthyroidism, pancreatitis, chronic liver disease, and brain trauma. Decreased values are found in insulin overdose, Addison's disease, bacterial sepsis, hepatic necrosis, hypothyroidism, and glycogen storage disease.

What two hormones regulate glucose?



High protein diets
↑ BUN levels.

Diabetes ↑ glucose levels.

Insulin overdose ↓ glucose
levels.

Glycated hemoglobin is a less sensitive glucose measure.

FIGURE 3

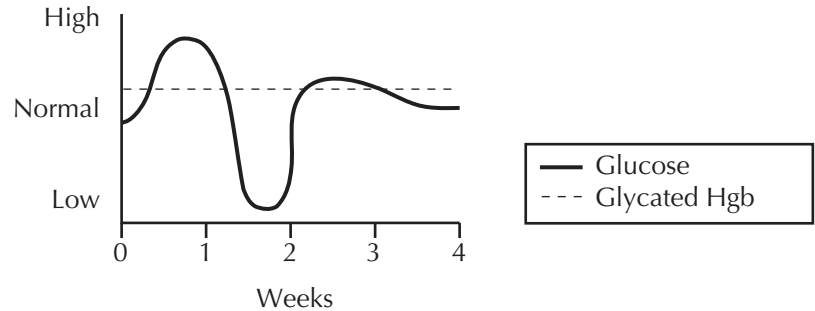
Glycated hemoglobin: used to check control of diabetes.

HDL and LDL transport cholesterol.

↓ HDL } ↑ risk coronary heart disease
↑ LDL }

8. Glycated Hemoglobin. Glycated hemoglobin indicates the average blood glucose concentrations over the preceding 8 to 12 weeks. Values are not as subject to day-to-day fluctuations as are glucose levels.

Increased values for glycated hemoglobin indicate that the glucose values have varied widely (poor control).



9. High-density and Low-density Lipoprotein Cholesterol. High-density lipoprotein (HDL) removes cholesterol from tissues and carries it to the liver for disposal.

Low-density lipoproteins (LDL) move cholesterol to the peripheral tissues.

When this process is halted, plaque begins to form and clog arteries.

What is one difference between HDL and LDL? (with a large blue question mark icon)

Increased values for high-density lipoprotein are found in nephrotic patients, and patients on a high carbohydrate diet. Decreased values for HDL lead to an increased risk of coronary heart disease.

Fe^{+2} is recycled in the body.

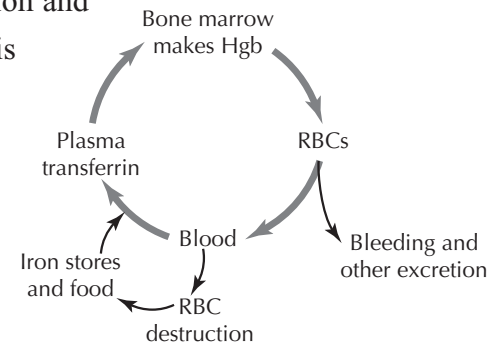
Transferrin regulates Fe^{+2} stores.

10. Iron (Fe^{+2})/TIBC/UIBC/Transferrin. Iron is an essential component of heme proteins that function in oxygen transport. Most of the body's iron is contained in hemoglobin. Iron stores are recycled in the body.

What is the difference between TIBC and UIBC?

UIBC is the serum unsaturated iron binding capacity—the reserve iron binding capacity of serum transferrin. Normally, only about one third of the iron binding sites of transferrin are occupied by iron.

Transferrin is a protein that regulates iron absorption and transport in the body. The quantity of transferrin is measured by the amount of iron with which it can bind, referred to as the total iron binding capacity (TIBC). Table 3 catalogs the main tests which monitor iron stores in the body.



What are the ways the body loses Fe^{+2} ?

TABLE 3

Iron and iron stores:
important in assessing
anemias.

Test	Increased	Decreased
Iron	Hemolytic anemia, pernicious anemia, lead poisoning, acute hepatic cell necrosis	Dietary deficiency, acute blood loss, neoplasia, and rheumatoid arthritis
TIBC	Late pregnancy, iron deficiency anemia, after acute hemorrhage or destruction of liver cells	Infection, neoplasia, uremia, nephrosis
Transferrin	Hemolytic anemia, acute hepatitis, and pernicious anemia	Iron deficiency anemia, late pregnancy, infection, neoplasia, and after acute hemorrhage

Mg⁺² is the #4 intracellular cation.

11. Magnesium (Mg⁺²). Magnesium is absorbed in the upper intestines, and is needed for blood clotting. Along with sodium, potassium, and calcium it regulates neuromuscular irritability. Decreases in calcium sometimes lead to decreases in magnesium; decreased potassium also accompanies decreased magnesium.

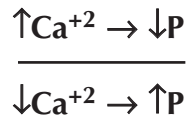


Chronic renal disease ↑
Mg⁺² levels.

Increased values of magnesium are found in chronic renal disease, severe dehydration, and adrenal insufficiency. Decreased values are found in malabsorption, prolonged diarrhea, acute pancreatitis, acute alcoholism, and with the use of some diuretics.

Ca²⁺ and P deposits are linked.

12. Phosphorus. Most phosphorus is found in the body in the bone matrix. Phosphorus is excreted in the urine. Levels of calcium and phosphorus are closely linked because they are both deposited in the bone together.



↑ vitamin D accompanies
↑ P.

Increased values of phosphorus are found in advanced renal insufficiency, pseudohypoparathyroidism, hypervitaminosis D, and with patients who have hypersecretion of growth hormone. Decreased values are found in hyperparathyroidism, rickets, steatorrhea, and in some renal diseases.

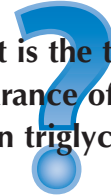
Triglycerides are fatty acids.

Acute alcoholism
↑ triglyceride levels.

13. Triglycerides. Most of the fatty acids in the body are components of triglycerides and stored in the **adipose** tissue as fat. Cells must also contain glucose for triglyceride formation. An overnight fasting specimen is required when testing for triglycerides.

Increased values of triglycerides are found in hypothyroidism, nephrotic syndrome, acute alcoholism, obstructive liver disease, acute pancreatitis, uncontrolled diabetes, and glycogen storage disease. When triglycerides are high, the serum or plasma is usually turbid or milky, and this is called lipemia. Decreased values are found in abetalipoproteinemia.

What is the typical appearance of serum high in triglycerides?



14. Uric Acid. Uric acid, the result of the breakdown or destruction of cells, circulates in plasma and is excreted by the kidney. This test is used to diagnose or follow the treatment of gout. It can also be used to evaluate renal failure and leukemia.

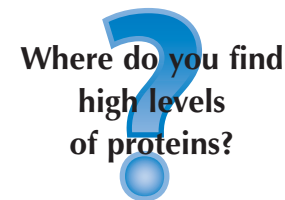
Increased values of uric acid are found in gout, renal disease, leukemia, polycythemia, toxemia, and resolving pneumonia. Decreased values are found in patients on certain medications including steroids, aspirin, allopurinol (a gout medicine), and penicillamine. Values can also be decreased when renal tubular absorption is defective.

Renal disease and leukemia
↑ uric acid levels.

E. Proteins: General

Proteins can be antibodies, clotting factors, or enzymes.

Proteins are present in all **body fluids**. Their concentration is normally high only in blood, serum, **plasma**, lymph fluid, and some **exudates**. There is a small amount of protein in spinal fluid and a trace of protein in urine.



Proteins have many purposes. They function as **antibodies**, form part of the endocrine system, and provide a complex blood-clotting system. Additionally, they are carriers for other compounds, provide tissue nutrients, and function as **enzymes**. To determine disease processes it is important to compare levels for each fraction of the proteins to normal values.

Table 4 on pages 41 and 42 summarizes the different protein fractions and the effects when levels are abnormal.

TABLE 4

Proteins: total protein and fractions are important in many disease states.

(continued)

Proteins	Increased	Decreased
Total Protein	Dehydration; monoclonal disease; some chronic polyclonal diseases, eg, liver cirrhosis, sarcoidosis, systemic lupus erythematosus (LE), and chronic infections	Inadvertent overhydration, protein loss through the kidneys, severe burns, starvation, and severe nonviral liver cell damage
Albumin	Rare and temporary, in acute dehydration or shock	Same conditions as total protein
Prealbumin	Hodgkin's disease	Inflammation, malignant liver disease
Microalbumin	Indicates an increased risk of diabetic neuropathy, end-stage renal disease, and proliferative retinopathy in the diabetic patient	Not significant
α 1 (e.g. α_1 -acid glycoprotein)	Infections and inflammations	Acute hepatitis
α 2 (e.g. α_2 -macroglobulin)	Rheumatoid arthritis, LE, and myocardial infarction (MI)	Acute hepatocellular disease
β (e.g. ApoB) lipoprotein	Hyperlipemias	Not significant

TABLE 4 (CONT'D)

Proteins: total protein and fractions are important in many disease states.

Proteins	Increased	Decreased
γ (e.g. Immunoglobins)	Viral hepatitis, sarcoidosis, rheumatoid arthritis, chronic infections, and some leukemias	Terminal stages of Hodgkin's disease and in congenital conditions
C3 and C4 Complement proteins that function with antigen-antibody complexes to destroy viruses, bacteria, and host cells	Acute phase reactions such as surgery, MI, infections, and tumors	C3 and C4—LE, subacute bacterial endocarditis, and gram-positive bacteremia C3—rheumatoid vasculitis, streptococcal glomerulonephritis, and gram-negative bacteremic shock
Haptoglobin Transports free hemoglobin from destroyed red cells	Acute phase reactions	Chronic intravascular hemolysis

F. Proteins: Immunoglobulins and Immunity

Infection ↑ immunoglobulin levels.

Multiple myeloma is a monoclonal disease.

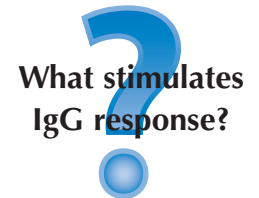
IgA is found in milk and other secretions.

IgG is the predominant immunoglobulin.

Immunoglobulins are circulating antibodies essential for defense against foreign proteins of any sort. Increased levels of immunoglobulins are found in chronic infection. In some conditions known as monoclonal diseases, only one of the immunoglobulins may increase. Monoclonal diseases include multiple myeloma, **Waldenstrom's macroglobulinemia**, **cryoglobulinemia**, and some cases of lymphomatous diseases.

1. Immunoglobulin A (IgA). The IgA class of immunoglobulins protects mucous membrane surfaces from bacterial or viral attack. IgA is in various fluids like **colostrum**, milk, saliva, tears, and sweat. About 10% to 15% of the circulating immunoglobulins are IgA.

2. Immunoglobulin G (IgG). IgGs make up about 75% to 80% of the total immunoglobulins. Production of IgGs is stimulated by an invasion of bacteria or viruses. The IgGs attach to the pathogen and serve as places for other cells to attach and destroy the foreign body. IgG immunoglobulins also cross the placenta and give passive immunity to a fetus.



IgM is the largest immunoglobulin and the first to form.

3. Immunoglobulin M (IgM). The largest immunoglobulins in size, IgMs are the first of the immunoglobulins to be formed. They make up about 5% to 10% of the immunoglobulins and work to eliminate foreign bodies by activating **complement**. In response to an infection, the immune system produces IgM antibodies first, followed later by IgG antibodies.

G. Proteins: Other

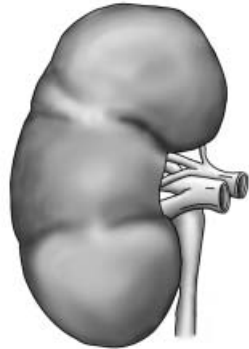
This table represents other proteins that are used to monitor the body's response to certain disease states.

TABLE 5

Serological protein assays: important in inflammatory processes.

Proteins	Increased	Decreased
C-reactive protein-CRP Method for evaluating the severity and progress of inflammatory diseases. Detected 18–24 hrs after onset of tissue damage in acute disease	Cardiovascular disease, rheumatic fever, rheumatoid arthritis, LE, MI, malignancy, bacterial and viral infections	Not significant
Anti-streptolysin O (ASLO/ ASO) Used to detect a recent streptococcal infection	A two-dilution-step rise in titer is a good indication of infection	Not significant after it returns to normal
Rheumatoid factor	Rheumatoid arthritis, LE, endocarditis, tuberculosis, syphilis, cancer, viral infections, diseases affecting the liver, lung, and/or kidney	Not significant

Urine Proteins. Urine protein is usually tested to evaluate some renal diseases. Most often a urine sample is tested using a sample that has been collected for 24 hours.



Increased values for urinary protein are found in nephrotic syndrome and in other diseases (e.g., Diabetes) that produce renal lesions. The measurement of urinary albumin (often referred to as microalbumin) is utilized to detect and monitor Diabetes.

What is the typical sample for urinary protein?

Nephrotic syndrome ↑
urinary protein levels.

Summary

Chemistry testing is a vital part of laboratory testing and is an aid to physicians diagnosing and treating patients. It is important to understand the use of each of the tests and the proper testing procedures for each.

- Electrolytes help the physician monitor the patient's acid-base and fluid balance.
- Chemistry profiles or panels are a group of tests which are usually accompanied by other specialized tests to monitor or aid in diagnosing a patient.
- Enzymes tests monitor patients' reactions to a disease process.
- Proteins represent a major class of analytes and reflect a patient's nutritional status and immune response.

Review Questions (II)

1. The test used primarily to diagnose liver disease is _____.
 - a. calcium
 - b. CO₂
 - c. potassium
 - d. total bilirubin
2. Which is the best method to monitor diabetic glucose control over an 8-12-week period?
 - a. glucose
 - b. glycated hemoglobin
 - c. haptoglobin
 - d. phosphorus
3. The test primarily performed to evaluate the patient for gout is _____.
 - a. iron
 - b. magnesium
 - c. sodium
 - d. uric acid

4. Which test requires that the sample be kept in a sealed tube because of a problem with evaporation?
 - a. carbon dioxide
 - b. chloride
 - c. sodium
 - d. potassium

5. What tests are generally considered part of a basic metabolic panel (BMP)?
 - a. Sodium, Potassium, Chloride
 - b. Glucose, Creatinine, Urea, Sodium, Potassium, Chloride, CO₂
 - c. Cholesterol, Triglycerides, HDL
 - d. Alb, AlkP, ALT, AST, Tbili

[Check Your Responses](#)

Click on this link to go to the Answers page. When you are finished, click the BACK button to return to the Review Questions.

III. SPECIALIZED TESTS

Section Overview

This section briefly discusses other specialized tests sometimes run in the clinical chemistry laboratory.

Learning Objectives

After reviewing this section, you should be able to:

1. Differentiate between drugs of abuse and therapeutic drugs.
2. Identify the use of other selected “special chemistries.”

Key Concepts

1. Testing a patient for a therapeutic level of a drug is very important to treatment.
2. Sample handling is critical to successful ammonia testing; lactic acid can indicate muscle damage.

A. TDMs and Toxicology

Therapeutic drug monitoring is an important lab function.

A key goal of today's clinical laboratory is to monitor therapeutic drugs. Physicians monitor medication levels in the patient and determine if the level of drug present is meeting the patient's needs. Therapeutic Drug Monitoring (TDM) also helps the physician control medications and avoid overmedication and its resulting problems. The following table summarizes the most common drugs that are routinely monitored.

TABLE 6

TDMs: some of these drugs can be toxic and even lethal.

Common Name	Drug Name	Condition Treated
Acetaminophen	Tylenol®	Pain and fever
Digoxin	Lanoxin®	Heart failure
Lithium	—	Manic-depressive disorders
Phenytoin	Dilantin®	Ventricular arrhythmias Seizures
Phenobarbital	Luminal®	Sedation Epilepsy
Theophylline (aminophylline)	—	Acute and chronic bronchial asthma
Tobramycin	Tobrex®	External ocular infections
Gentamicin	Garamycin®	Serious infections
Carbamazepine	Tegretol®	Seizures
Valproic acid	Depakene®, Depakote®	Seizures

Drugs of abuse have very limited or no therapeutic value.

Some drugs are not routinely prescribed for therapeutic purposes but are considered drugs of abuse. Some of the most common drugs of abuse are listed in the following table.

TABLE 7

Drugs of abuse: instances of abuse can be important in legal considerations.

Common Name	Common Brands/Names	Uses of Drug
Amphetamines	Dexedrine®, Ritalin®, Nodoz®	Central nervous system stimulants (“uppers”)
Barbiturates	Pentobarbital, Talbutal, Barbital, Triclofos	Sedatives and hypnotics
Benzodiazepines	Xanax®, Tranxene®, Vistaril®, Valium®	Antianxiety agents
Cannabinoids (eg, marijuana)	—	Hallucinogens
Cocaine	—	Stimulants
Methadone	Dolophine®	Analgesic—severe pain narcotic abstinence
Opiates	Percocet®, Fiorinal®, Tylox®, heroin	Analgesic—moderate to severe pain
PCP	phencyclidine, “Angel Dust”	Hallucinogen
Propoxyphene	Darvon®, Darvocet®	Analgesic

Three of the most common toxicology tests available on clinical chemistry analyses are discussed in detail below.

1. Acetaminophen. Acetaminophen is the active ingredient of many non-aspirin containing analgesics. Severe hepatic toxicity is associated with overdose (15g) but is not evident until 3-5 days after injection. Therefore, measurement of serum acetaminophen becomes critical for proper clinical assessment.

2. Ethanol. Serum is the sample of choice. This test is most often used to determine if the patient is impaired according to legal limits set in each state. Physicians will also use this information to determine treatment. Never use alcohol for cleansing the skin, as the sample could become contaminated.

3. Salicylate. The most common salicylate is aspirin; salicylates are found in many over-the-counter medications. Aspirin is used to reduce fever, pain, and inflammation. No salicylates should appear in the serum of people who are not taking the drug.

Increased levels of salicylates are found in patients who are taking this medication for therapy in certain disease processes like rheumatoid arthritis, or in cases of overdose.

Ethanol or alcohol levels can be important legally.

Toxic levels of aspirin or salicylates can occur by accident or by suicidal intent.

Patients with what disease take high levels of aspirin?



B. Specific Chemistry

Some tests do not fit easily into a category but provide valuable pieces of the diagnostic puzzle. Only two are mentioned here.

1. Ammonia. Ammonia is one of the end products of protein metabolism. Measurement of ammonia levels is used to evaluate metabolism and to follow severe liver disease.



Ammonia should be collected in a heparin tube and placed on ice immediately. Specimens should be analyzed as quickly as possible. If rapid analysis is a problem, the sample should be centrifuged, separated, and frozen. Probing for a vein, use of a heparin lock, drawing blood into a syringe and transferring it to a tube containing anti-coagulant, or only filling the evacuated tube partially are all causes of an increased ammonia level. Smoking by the patient or the phlebotomist is a source of ammonia contamination.

What are some pre-analytical concerns with ammonia levels?

Ammonia levels monitor liver function.

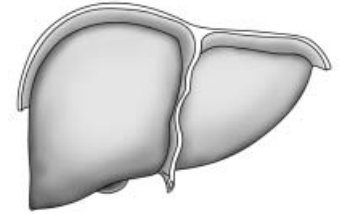
Cirrhosis ↑ ammonia levels.

Lactic acid comes from muscles.

Increased levels for ammonia are found in liver disease, cirrhosis, severe hepatitis, severe heart failure, acute bronchitis, and pericarditis.

2. Lactic Acid. Lactic acid is found in muscle tissue and is released into the circulation when there is muscle tissue damage.

Increased levels of lactic acid are found in cases of shock, muscle fatigue, diabetic ketoacidosis, and tissue hypoxia.



Summary

Monitoring of medications used to treat disease is vital to treatment. Drug levels help to establish whether a medication is working at a maximum level or whether excess levels may be contributing to symptoms. Also, they can monitor for drugs of abuse to determine if there is an induced problem.

Finally, there are an assortment of other specialized tests to help the physician diagnose, monitor, and treat various conditions.

Review Questions (III)

1. Which of the following drugs is used to treat seizures?
 - a. acetaminophen
 - b. gentamicin
 - c. tobramycin
 - d. valproic acid

2. A drug used to treat anxiety that has a high potential for abuse is _____.
 - a. barbiturate
 - b. benzodiazepine
 - c. cannabinoid
 - d. cocaine

3. What is a common form of salicylate?
 - a. aspirin
 - b. digoxin
 - c. lithium
 - d. Tylenol®

[Check Your Responses](#)

Click on this link to go to the Answers page. When you are finished, click the BACK button to return to the Review Questions.

Answers to Review Questions

*Click the BACK button
to return to the
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- I. 1. b
2. a
3. b

BACK

- II. 1. d
2. b
3. d
4. a
5. b

BACK

- III. 1. d
2. b
3. a

BACK

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Glossary

Absorbance: refers to the amount of light which is absorbed by a solution; directly proportional to concentration of analyte.

Acidosis: state of decrease of alkali and an accumulation of acid metabolites in blood or body fluids.

Addison's disease: chronic adrenocortical insufficiency.

Adipose: of or relating to fat in blood or body fluids.

Alkalosis: state of excess of base or loss of acid in blood or body fluids.

Amino acid: organic acid used to form proteins.

Analyte: substance that is being measured, eg, glucose, sodium.

Antibody: protein formed as the result of antigenic stimulation.

Antigen: foreign substance that results in antibody production.

Body fluid: fluid in body cavities or spaces, eg, pleural, abdominal, pericardial.

Catalyst: substance that accelerates a chemical reaction.

Cation: ion carrying a positive charge.

Colostrum: first milk secreted at the termination of pregnancy.

Complement: group of serum proteins that produce inflammatory effects and lysis of cells when activated.

Cryoglobulinemia: presence of cryoglobulin, an abnormal plasma protein, in the blood plasma.

Cushing's syndrome: adrenal hyperplasia caused by an adenoma of the pituitary gland.

Dubin-Johnson syndrome: inherited defect in hepatic excretory function, characterized by abnormally high levels of conjugated bilirubin.

Enzyme: protein in the body that acts as a catalyst.

Excretion: process by which undigested food and waste products are separated from the blood and cast out.

Extracellular: outside the cell.

Exudate: fluid which has leaked out of a tissue or capillary, usually in response to inflammation or injury.

Gaucher's disease: lysosomal storage disease resulting from a genetic deficiency, most commonly seen in infants.

Hemoglobin: protein of red blood cells that transports oxygen from the lungs to tissues.

Hemolysis: rupture of red blood cells and release of hemoglobin into plasma or serum.

Hemostasis: state of balance in the body, between blood clotting and clot lysis.

Hodgkin's disease: malignant neoplasia of the lymphoid cells, of uncertain origin.

Homeostasis: state of balance in the body.

Icterus: yellow discoloration of plasma caused by bilirubin accumulation.

Immunoassay: assay which relies on an antigen-antibody reaction.

Lipemia: milky coloration of plasma caused by increased lipoproteins accumulation.

Neonatal: referring to the period immediately following birth.

Nephrotic: relating to diseases of renal tubules.

Osmotic pressure: force that moves water or another solvent across a membrane separating a solution. Usually, the movement is from the lower to the higher concentration.

Paget's disease: skeletal disease, frequently familial, leads to softening of bones.

Panel: a group of related tests ordered together.

Photometry: process of measuring light intensity at various wavelengths.

Plaque: lipid deposits in arteries.

Plasma: the clear, yellow fluid obtained when blood is drawn into a tube containing anticoagulant (usually a purple, green, or light blue tube) and is centrifuged.

Polymyositis: inflammation of a number of voluntary muscles.

Potentiometry: measurement of electrical potential difference between two electrodes in an electrochemical cell.

Reaction Velocity: describes the speed at which a detection measurement changes over time.

Renal: relating to the kidney.

Reye's syndrome: a rare, acute, and often fatal encephalopathy of childhood marked by acute brain swelling; most often occurs as a consequence of influenza and URT infections.

Serum: liquid portion of plasma that remains after clot is removed.

Titer: the amount of a known or unknown analyte determined by volumetric means.

Waldenstrom's macroglobulinemia: hyperglobulinemia with peak in γ or β_2 globulins, frequently exhibits mucosal bleeding.

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